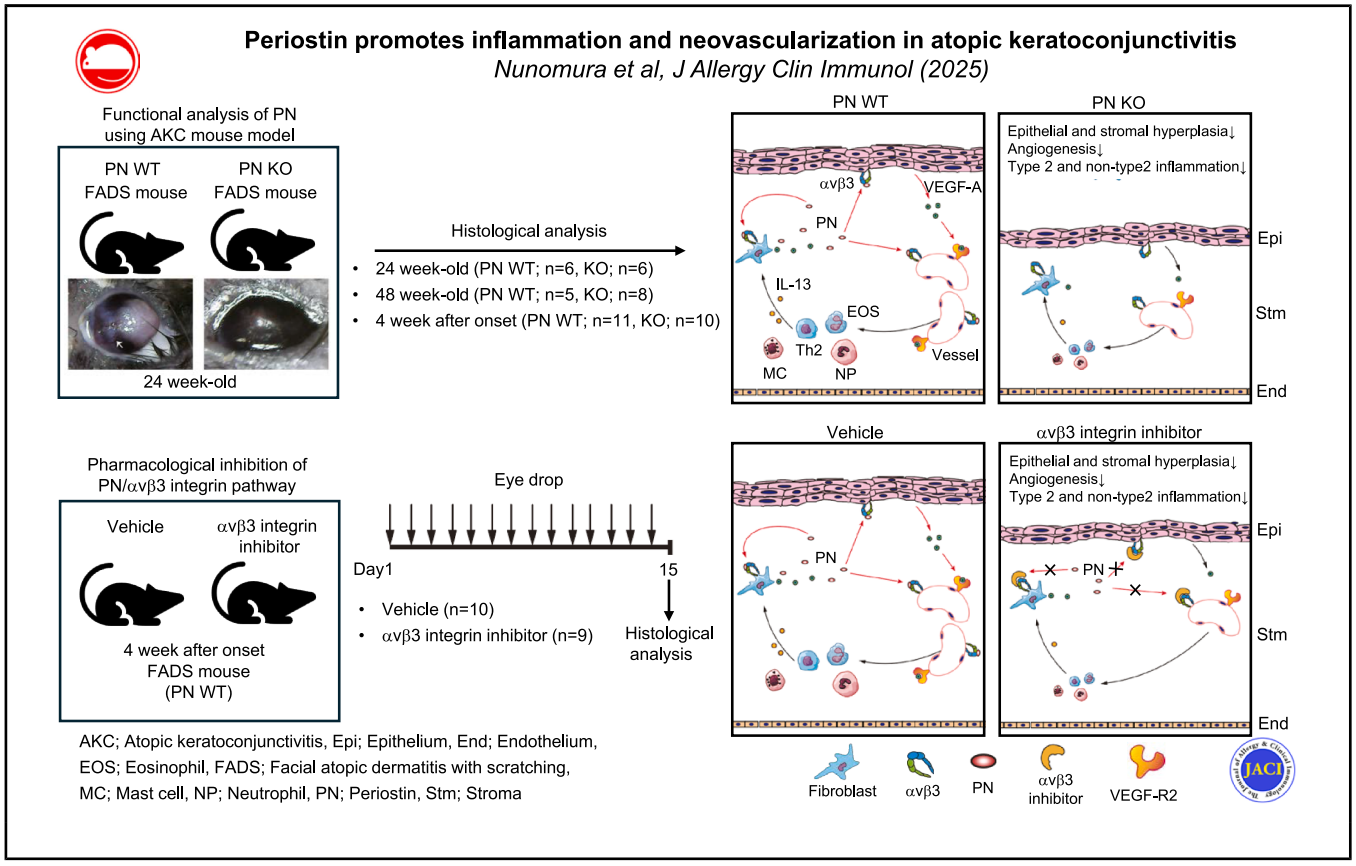
Periostin promotes inflammation and neovascularization in atopic keratoconjunctivitis

Satoshi Nunomura, PhD, Yasuhiro Nanri, PhD, Yuko Honda, PhD, Hironobu Takedomi, MD, PhD, Takashi Okada, PhD, Xin Tun, PhD, et al

GRAPHICAL ABSTRACT



Capsule summary: The periostin/$\alpha_V\beta_3$ integrin pathway promotes corneal neovascularization and atopic keratoconjunctivitis (AKC)-like ocular inflammation in mice, and $\alpha_V\beta_3$ integrin inhibitors targeting the periostin/$\alpha_V\beta_3$ integrin pathway could become novel therapeutic agents for treating AKC.

J Allergy Clin Immunol 2025;■■■:■■■-■■■.

https://doi.org/10.1016/j.jaci.2025.10.004

Periostin promotes inflammation and neovascularization in atopic keratoconjunctivitis

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Background: Atopic keratoconjunctivitis (AKC) is a chronic allergic conjunctival disease accompanied by corneal lesions, including epithelial damage, and neovascularization. Periostin, a downstream molecule of type 2 inflammation, is highly expressed in tears and conjunctiva of AKC patients and correlated with disease severity, but its involvement in the pathogenesis of AKC remains unclear. We have established facial atopic dermatitis with scratching (FADS) mice that spontaneously develop AKC-like ocular lesions with increased periostin expression and corneal neovascularization.

Objective: Using FADS mice, we sought to clarify the functional role of periostin in the pathogenesis of AKC with the eventual goal of developing novel therapeutic strategies targeting the periostin/integrin $\alpha_V\beta_3$ pathway.

Methods: We generated periostin-deficient FADS mice and analyzed AKC-like ocular lesions, including corneal neovascularization. Moreover, we examined the effects of CP4715, an $\alpha_V\beta_3$ integrin inhibitor, on the AKC-like ocular lesions in FADS mice.

Results: Genetic disruption and pharmacologic inhibition of the periostin/$\alpha_V\beta_3$ integrin pathway ameliorated corneal epithelium hyperplasia with NF-κB activation, infiltration of mast cells, T_H2 cells, or neutrophils associated with type 2 or non–type 2 inflammation in the corneal stroma and conjunctiva in FADS mice. Moreover, we found that inhibiting the periostin/$\alpha_V\beta_3$ integrin pathway decreased corneal neovascularization by reducing expression of VEGF-A/VEGF-R2 in corneal epithelium, stroma, and vascular endothelial cells.

Conclusions: Periostin plays an important role in corneal neovascularization and in type 2 and non–type 2 inflammation in AKC-like ocular lesions of FADS mice. Targeting the periostin/$\alpha_V\beta_3$ integrin pathway is a promising therapeutic strategy for treating AKC patients. (J Allergy Clin Immunol 2025;■■■:■■■-■■■.)

Key words: *Allergic conjunctival diseases, atopic keratoconjunctivitis, drug treatment, periostin, corneal neovascularization*

Atopic keratoconjunctivitis (AKC), a chronic allergic conjunctival disease (ACD) that occurs in patients with atopic dermatitis (AD), involves the facial skin and is often accompanied by fibrosis of the conjunctiva, neovascularization, and opacity of the cornea.[1] The prevalence of AKC is estimated to be approximately 5% in all patients with ACD, who are increasingly numerous worldwide.[2,3] Currently, 15-20% of the global population is affected by ACD. AKC can be accompanied by ocular pruritus, blepharitis, formation of giant papillae (GP), and corneal pathologic changes such as shield ulcer and corneal plaque, potentially leading to vision loss.[1,3-5] Although either immunosuppressive eye drops or topical corticosteroids are highly effective for AKC,[1] these treatments are ineffective for some patients[6] or cause various adverse effects such as glucocorticoid-induced glaucoma, susceptibility to infection, and burning or irritation of the eye.[3,7-9] Therefore, it is important to develop novel therapeutic agents with minimal or no adverse effects.

The notion that type 2 inflammation is dominant in the pathogenesis of AKC is widely supported.[10] It has been reported that IL-4 and IL-13, signature type 2 cytokines, are highly expressed in the GP of AKC patients.[11,12] Moreover, effector cells of type 2 inflammation, such as eosinophils and T_H2 cells, infiltrate the corneal and/or conjunctival tissues of ACD, including AKC.[13] Supporting these findings, tears from AKC patients contain elevated levels of IL-4, IL-5, IgE, eosinophil cationic protein, and eotaxin.[14-16] However, there are currently no therapeutic drugs targeting type 2 inflammation for AKC. Moreover, it is known that non–type 2 inflammation coexists with type 2 inflammation in the pathogenesis of AKC, which causes resistance to immunosuppressants (and topical glucocorticoids).[12] It has been reported that neutrophil infiltration in the conjunctiva is correlated with the severity of corneal damage.[17] We have previously shown that coexpression of type 2 and non–type 2 mediators in tears can predict resistance to tacrolimus, an immunosuppressive drug.[18] These findings suggest that the inflammatory processes involved in AKC pathogenesis are multifaceted and diverse, and that targeting both type 2 and non–type 2 inflammation would be a promising strategy for treating AKC cases resistant to immunosuppressants.

Periostin is a matricellular protein belonging to the fasciclin family that transduces signals in cells by interacting with integrins $\alpha_V\beta_3$ or $\alpha_V\beta_5$ on the cell surface downstream of IL-4 and IL-13

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Received for publication March 25, 2025; revised September 30, 2025; accepted for publication October 9, 2025.

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0091-6749

https://doi.org/10.1016/j.jaci.2025.10.004

Abbreviations used
ACD: Allergic conjunctival disease
AD: Atopic dermatitis
AKC: Atopic keratoconjunctivitis
EC: Endothelial cell
FADS: Facial AD with scratching
GP: Giant papillae
HUVEC: Human umbilical vein ECs
MC: Mast cell
NF-κB: Nuclear factor kappa–light-chain enhancer of activated B cells
NHCF: Normal human corneal fibroblasts
NHDF: Normal human dermal fibroblasts
NHLF: Normal human lung fibroblasts
PC: Palpebral conjunctiva
Tc2: Type 2 cytotoxic T
TNC: Tenascin C
VEC: Vascular ECs
VEGF: Vascular endothelial growth factor
VEGF-R2: VEGF receptor 2

signals.[19-22] We have previously shown that periostin is highly expressed in conjunctivas with GP in AKC patients and that tear periostin levels are significantly high in AKC patients with complications such as GP formation, corneal damage, and resistance to tacrolimus.[12,23] Notably, periostin produced by fibroblasts on stimulation of IL-4 and IL-13 binds to $\alpha_V\beta_3$ integrin on keratinocytes, activating p65/RelA and upregulating expression of nuclear factor kappa–light-chain enhancer of activated B cells (NF-κB)-related cytokines and chemokines—*Il1b, Il24, Il33, Ccl3, Ccl4,* and *Cxcl2*—in skin,[24] suggesting that periostin links NF-κB–related inflammation with type 2 inflammation. Additionally, evidence has accumulated showing that periostin acts as a proangiogenic mediator by promoting proliferation, migration, and tube formation of vascular endothelial cells (VEC) through enhancing expression of vascular endothelial growth factor (VEGF) receptor 2 (VEGF-R2).[25-27] A previous study reported that expression of VEGF-A and VEGF-R1/2 is increased in the vascularized corneal tissue of AKC patients.[28] Moreover, we have previously shown that genetic deficiency or knockdown of periostin decreases expression of VEGF-A mRNA in murine fibroblasts.[29] In mice, a genetic periostin deficiency reduced type 2 inflammation, followed by improving allergic conjunctivitis induced by ragweed.[30] All these findings suggest a potentially crucial role of periostin in the pathogenesis of AKC. However, this possibility had not been explored because of the lack of an appropriate animal model of AKC.

We have recently established as our mouse model of AKC facial AD with scratching (FADS) mice, in which *Ikk2,* a positive regulator of NF-κB signaling, is deleted in dermal fibroblasts controlled by nestin expression.[31] FADS mice spontaneously develop severe blepharitis, infiltration of mast cells (MCs), eosinophils, and T_H2/type 2 cytotoxic T (Tc2) cells in the conjunctiva and cornea, corneal epithelium defects, and neovascularization, all of which are similar to those of AKC patients with facial AD.[32] FADS mice exhibit increased serum IgE and periostin, systemic biomarkers reflecting type 2 inflammation, even before they develop AKC-like ocular lesions, as observed in AD patients.[31] In FADS mice, expression of periostin is upregulated in the conjunctival tissues and stroma area of the corneal lesions.[32] Additionally, FADS mice exhibit higher tear periostin levels, correlating significantly with tear IL-4 levels, suggesting that periostin is a downstream molecule of IL-4/IL-13 signals in ocular type 2 inflammation. Moreover, in FADS mice, glucocorticoid eye drops improved AKC-like lesions: high periostin in tears, swelling in the corneal epithelium and stroma, as well as infiltration of MCs, eosinophils, and T_H2/Tc2 cells in the corneal stroma. These findings suggest that FADS mice can help us first clarify the functional role of periostin in the pathogenesis of AKC and then estimate the efficacy of potential compounds as therapeutic agents against AKC.

We have shown that CP4715, an $\alpha_V\beta_3$ integrin inhibitor, potently prevents the binding of $\alpha_V\beta_3$ integrin to periostin and that administering CP4715 into FADS mice improves NF-κB–related skin inflammation and itching by suppressing the periostin/$\alpha_V\beta_3$ integrin pathway.[24,33] Because CP4715 is a hydrophobic small-molecule compound, it easily penetrates the cornea through the lipophilic corneal epithelium, benefiting the eyes.[34] Taking these findings together, we thought it would be of great interest to examine whether topically administering CP4715 attenuates type 2 inflammation, periostin-mediated NF-κB–related inflammation, and neovascularization in FADS mice. CP4715 could be potentially be developed as a therapeutic agent for AKC. Therefore, in this study, we sought to clarify the functional role of periostin in AKC pathogenesis and to predict the efficacy of CP4715 using FADS mice.

METHODS

All methods are described in the Methods section in this article's Online Repository available at www.jacionline.org.

RESULTS

FADS mice exhibit AKC-like ocular type 2 inflammation

FADS/*Postn*$^{+/+}$ mice spontaneously developed ocular inflammation, as previously demonstrated (Fig 1, *A*).[32] We examined whether gene expression of type 2 cytokines and $\alpha_V\beta_3$ integrin ligands is increased in the ocular lesions in FADS mice. We found that expression of *Il5* and *Il13,* but not *Il4,* was markedly increased in the cornea and palpebral conjunctiva (PC) of FADS/*Postn*$^{+/+}$ mice (Fig 1, *B*). Among integrin ligands, *Postn* and *Tnc*/tenascin C (TNC), downstream molecules of IL-4/IL-13 signaling, were significantly upregulated in both the cornea and PC (Fig 1, *C*). Moreover, FADS/*Postn*$^{+/+}$ mice showed increased serum periostin levels (Fig 1, *D*). These data suggest that FADS mouse is an AKC mouse model, showing extensive type 2 inflammation not only in ocular and dermal tissues but also systemically throughout the body. Similar to these observations in FADS mice, expression levels of *IL4, IL5, IL13, POSTN,* and *TNC* were elevated in the GP of AKC patients (Fig 1, *E*). Notably, periostin was the most abundant $\alpha_V\beta_3$ integrin ligand in AKC patients. Immunofluorescence microscopic analysis indicated that periostin was mainly localized around the $\alpha_V\beta_3$ integrin–positive regions in the stroma of the GP of AKC patients (Fig 1, *F*). These findings suggest that ocular type 2 inflammation in FADS mice closely resembles that of AKC and that the

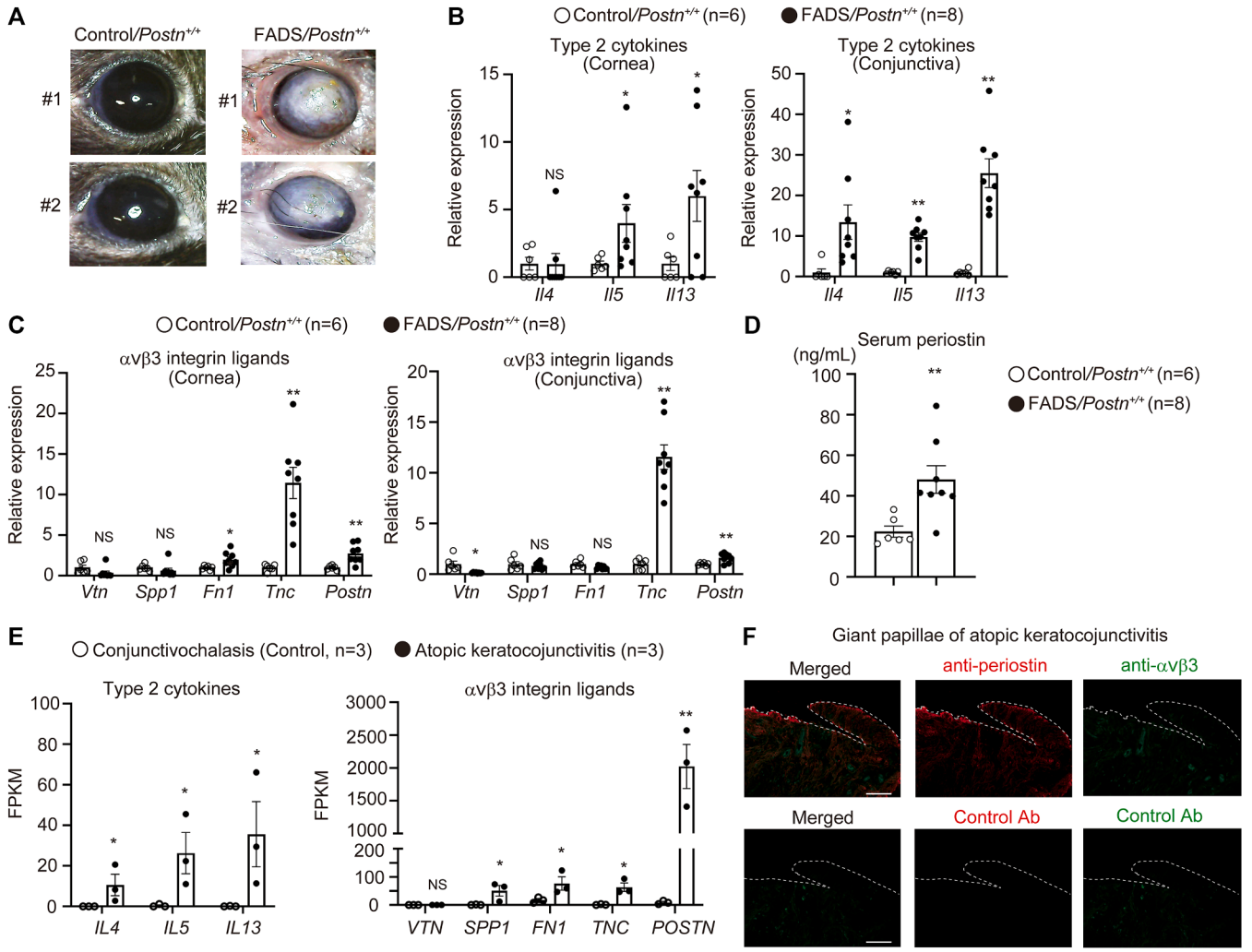

FIG 1. FADS mice exhibit AKC-like type 2 inflammation in cornea and PC. **(A)** Macroscopic features of control/*Postn*$^{+/+}$mice and FADS/*Postn*$^{+/+}$mice with corneal lesions. (**B** and **C**) Quantitative real-time PCR for expression of type 2 cytokines *(B)* and $\alpha_V\beta_3$ integrin ligands *(C)* in corneal and PC of control/*Postn*$^{+/+}$mice *(open circles)* and FADS/*Postn*$^{+/+}$mice *(solid circles).* **(D)** Serum periostin levels in control/*Postn*$^{+/+}$mice *(open circles)* and FADS/*Postn*$^{+/+}$mice *(solid circles).* **(E)** *In silico* analysis of RNA sequencing data for expression of type 2 cytokines *(left)* and $\alpha_V\beta_3$ integrin ligands *(right)* in control conjunctiva *(open circles)* and AKC GP *(solid circles). FPKM,* Fragments per kilobase of exon per million mapped reads. Data are shown as means ± SEMs. Statistical analysis was performed by 2-sided Mann-Whitney *U* test; **P* < .05, ***P* < .01. **(F)** Expression of $\alpha_V\beta_3$ integrin and periostin in GP of AKC. *Dashed lines* depict borders of epithelium and stroma. Scale bar, 100 μm.

periostin/$\alpha_V\beta_3$ integrin pathway may be involved in the ocular inflammation of AKC.

Periostin promotes ocular inflammation in FADS mice

To clarify the functional roles of periostin in the pathogenesis of AKC-like ocular lesions in FADS mice, we generated FADS/*Postn*$^{-/-}$ mice. Periostin deficiency significantly delayed the onset of corneal lesions (Fig 2, *A-C,* and see Fig E1, *A,* in the Online Repository available at www.jacionline.org). Moreover, approximately 30% of FADS/*Postn*$^{-/-}$ mice did not develop corneal lesions at all. Because of the delayed onset in FADS/*Postn*$^{-/-}$ mice, we compared the ocular lesions at 4 weeks after onset and at 24 and 48 weeks. Histologic analyses of the corneas showed that at all 3 time points, corneal epithelial thickness, corneal stromal thickness, and infiltration of neutrophils, eosinophils, MCs, and CD3$^+$Gata3$^+$ T cells (T_H2/Tc2) all decreased in FADS/*Postn*$^{-/-}$ mice compared to FADS/*Postn*$^{+/+}$ mice (Fig 2, *D* and *E*). Infiltration of neutrophils and MCs in the PC and eyelid dermis, as well as epidermal thickness of the eyelid skin, also decreased in FADS/*Postn*$^{-/-}$ compared to FADS/*Postn*$^{+/+}$ mice (Fig E1, *B,* and see Fig E2 in the Online Repository). We had previously demonstrated that in FADS mice, periostin is crucial for activation of NF-κB p65/RelA, followed by induction of several cytokines and chemokines related to keratinization and migration of neutrophils and MCs.[24] We therefore examined the activation of p65/RelA in the corneal epithelium, finding that both cytosolic expression and nuclear localization of p65/RelA were detected in corneal epithelial cells of FADS/*Postn*$^{+/+}$ mice, whereas these

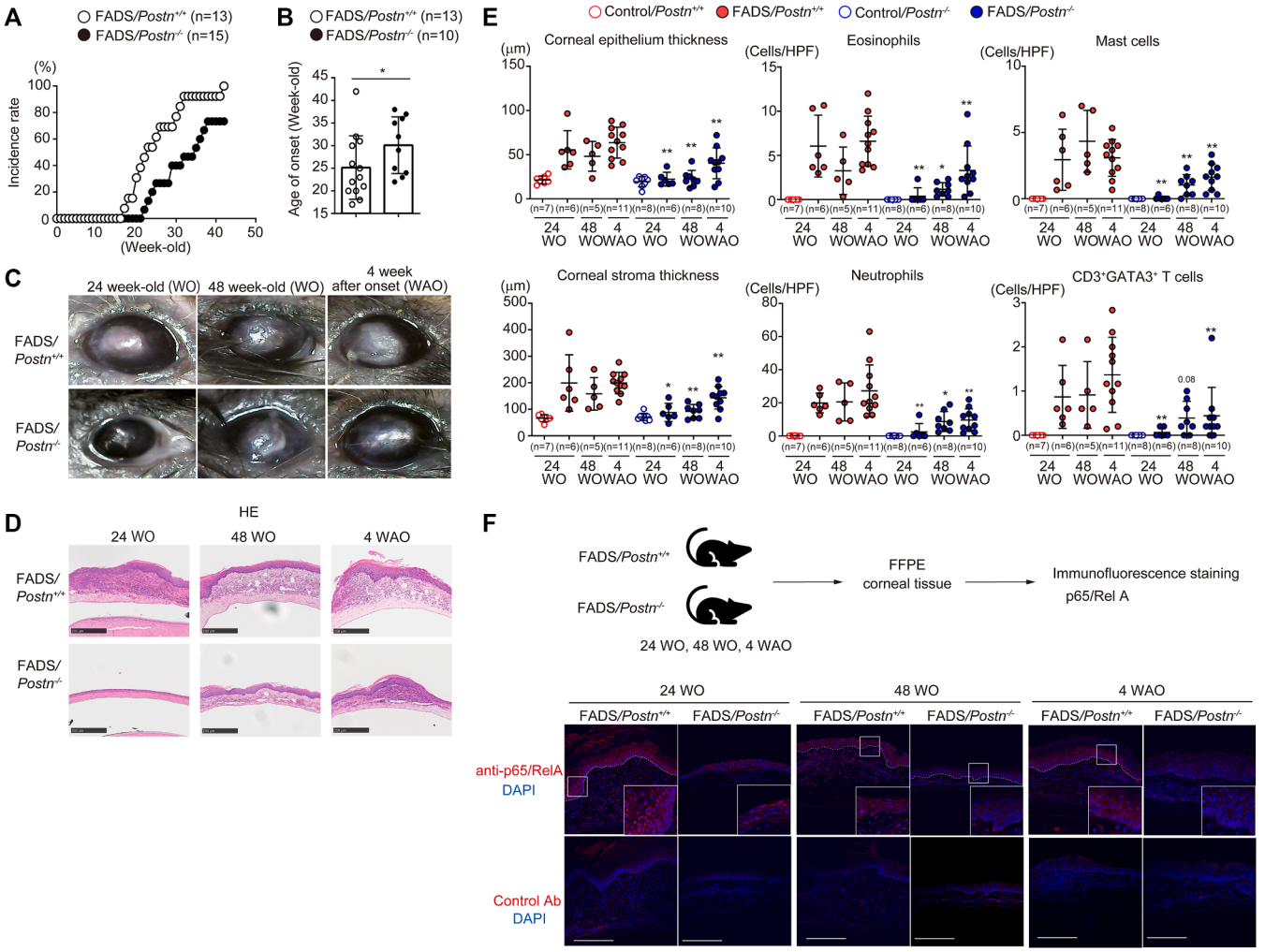

FIG 2. Periostin promotes corneal inflammation in FADS mice. (**A** and **B**) Incidence rate *(A)* and age at onset *(B)* of corneal lesions in FADS/*Postn*$^{+/+}$ *(open circles)* and FADS/*Postn*$^{-/-}$ *(solid circles)* mice. (**C** and **D**) Macroscopic features *(C)* and hematoxylin and eosin (HE) staining *(D)* for corneas of FADS/*Postn*$^{+/+}$ and FADS/*Postn*$^{-/-}$ mice at 24 weeks old (WO), 48 WO, and 4 weeks after onset (WAO) of corneal lesions. Scale bar, 250 μm. **(E)** Thicknesses of corneal epithelium and stroma, and numbers of eosinophils, neutrophils, MCs, and CD3^{+}Gata3^{+} T cells in corneal stroma. Control/*Postn*$^{+/+}$, *open red circles;* control/*Postn*$^{-/-}$, *open blue circles;* FADS/*Postn*$^{+/+}$, *solid red circles;* FADS/*Postn*$^{-/-}$ mice, *solid blue circles.* **(F)** NF-κB p65/Rel expression in corneal epithelium of 24 WO, 48 WO, and 4 WAO FADS/*Postn*$^{+/+}$ and FADS/*Postn*$^{-/-}$ mice. *Dashed lines* depict border of epithelium and stroma. Scale bar, 200 μm. Similar results were obtained from 3 separate experiments. Data are shown as means ± SDs. Statistical analysis between age- or condition-matched FADS/*Postn*$^{+/+}$ mice and FADS/*Postn*$^{-/-}$ mice was performed by 1-sided Mann-Whitney *U* test; **P* < .05, ***P* < .01.

both decreased in FADS/*Postn*$^{-/-}$ mice (Fig 2, *F*). These results demonstrate that in FADS mice, periostin is important for hyperplasia of epithelium and stroma, infiltration of inflammatory cells such as eosinophils, MCs, neutrophils, and T cells, as well as activation of NF-κB p65/RelA in corneas.

Periostin promotes corneal neovascularization in FADS mice

We had previously demonstrated that the corneal stroma of FADS mice clearly showed neovascularization,[32] so we decided to examine whether periostin was involved in corneal neovascularization in FADS mice. FADS/*Postn*$^{+/+}$ mice, but not FADS/*Postn*$^{-/-}$ mice, at 24 weeks showed apparent blood vessels extending from the conjunctiva to the cornea (Fig 3, *A*). The schema of the experiments is depicted in Fig 3, *B*. The numbers of CD31^{+} endothelial cells (ECs) in the corneal stroma significantly decreased in FADS/*Postn*$^{-/-}$ compared to FADS/*Postn*$^{+/+}$ mice at all 3 time points (Fig 3, *C* and *D*). Moreover, most CD31^{+} ECs were localized in the periostin-expressing corneal stroma of FADS/*Postn*$^{+/+}$ mice (Fig 3, *E*). These results suggest the possibility that periostin acts as a proangiogenic factor in the corneal stroma of FADS mice.

It is important to identify periostin-producing cells in the human cornea, not just mouse cornea. Thus, we next sought to determine which cells produced periostin in human corneal tissue. *In silico* analysis showed that *POSTN* is more highly expressed in corneal fibroblasts than in corneal epithelial cells and

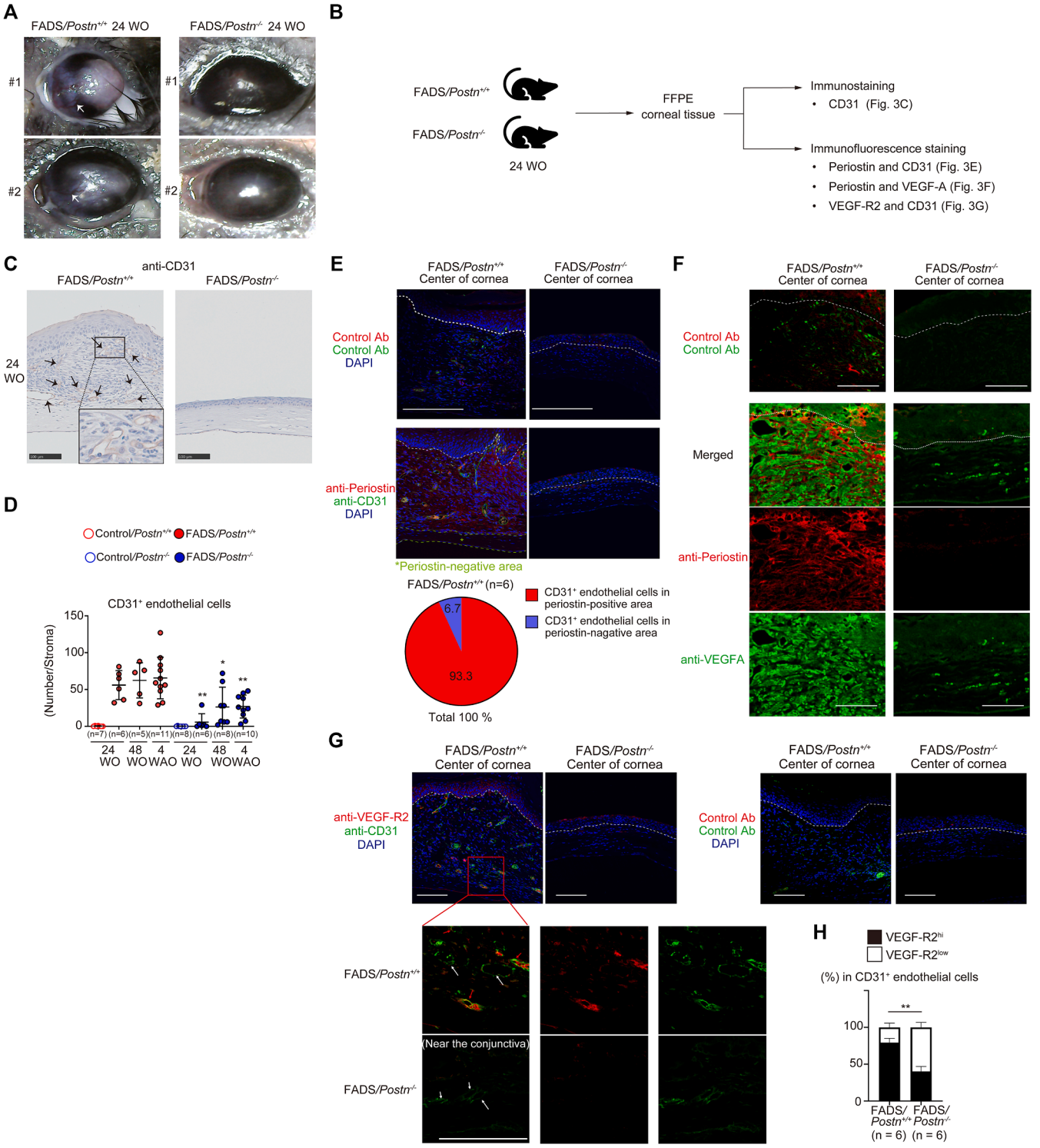

FIG 3. Periostin promotes corneal neovascularization in FADS mice. **(A)** Macroscopic features of cornea of 24-week-old (WO) FADS/*Postn*$^{+/+}$ and FADS/*Postn*$^{-/-}$ mice. *White arrows* indicate blood vessels. **(B)** Schematic summary of immunostaining for CD31 **(C)** or immunofluorescence staining for CD31 and periostin *(E),* VEGF-A and periostin *(F),* or VEGF-R2 and CD31 *(G)* in corneas of 24 WO FADS/*Postn*$^{+/+}$ and FADS/*Postn*$^{-/-}$ mice. *(C) Black arrows* indicate CD31^{+} endothelial cells in stroma. Scale bar, 100 μm. **(D)** Number of CD31^{+} endothelial cells in corneal stroma in control/*Postn*$^{+/+}$ *(open red circles),* control/*Postn*$^{-/-}$ *(open blue circles),* FADS/*Postn*$^{+/+}$ *(solid red circles),* and FADS/*Postn*$^{-/-}$ mice *(solid blue circles)* at 24 WO, 48 WO, and 4 weeks after onset (WAO) of corneal lesions. **(E-G)** *Dashed lines* depict border of epithelium and stroma. Scale bars, 200 μm (*E* and *F*) and 100 μm *(G).* Percentage of CD31^{+} endothelial cells in corneal stroma area with *(red)* or without *(blue)* periostin expression *(E)* and ratio of VEGF-R2^{hi} *(black)* and VEGF-R2^{low} *(white)* cells in CD31^{+} endothelial cells **(H)**. *Red* and *white arrows* indicate VEGF-R2^{hi}CD31^{+} and VEGF-R2^{low}CD31^{+} endothelial cells, respectively *(G).* Data are shown as means ± SDs. Statistical analysis between age- or condition-matched FADS/*Postn*$^{+/+}$ and FADS/*Postn*$^{-/-}$ mice was performed by 1-sided Mann-Whitney *U* test; **P* < .05, ***P* < .01.

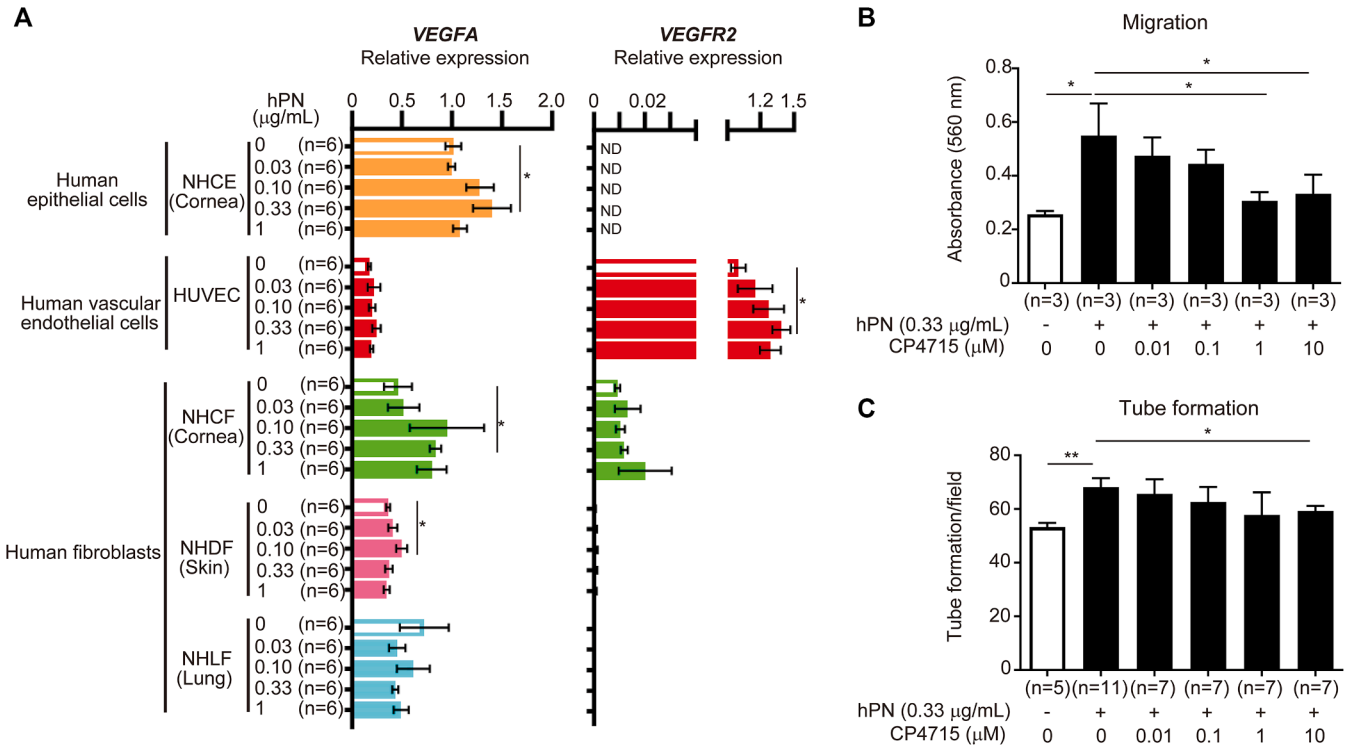

FIG 4. CP4715 inhibits periostin-induced migration and tube formation of vascular endothelial cells. **(A)** Quantitative real-time PCR analysis of effects of periostin stimulation on *VEGFA* and *VEGFR2* expression in NHCE *(orange),* NHCF *(light green),* NHDF *(pink),* NHLF *(light blue),* and HUVEC *(red).* Cells were stimulated with *(solid bars)* or without *(open bars)* recombinant human periostin at indicated concentrations for 8 hours. Relative gene expression levels were normalized to *GAPDH.* Mean expression levels of *VEGFA* in untreated NHCE and *VEGFR2* in untreated HUVEC are displayed as 1, respectively. *ND,* Not detected. (**B** and **C**) Effects of CP4715 on periostin-induced migration *(B)* and tube formation *(C)* of HUVEC. *Solid* and *open bars* indicate periostin-treated or untreated groups, respectively. Data are shown as means ± SEMs. Statistical analysis was performed by 1-sided Mann-Whitney *U* test; **P* < .05. *NHCE,* Normal human corneal epithelial cells.

ECs (GSE121922; see Fig E3, *A,* in the Online Repository available at www.jacionline.org). Accordingly, we found that IL-13 increased periostin expression in NHCF at both mRNA and protein levels (Fig E3, *B*). Taken together, these findings suggest that fibroblasts are major periostin-producing cells in the corneal stroma.

Because periostin is thought to play an important role in neovascularization by enhancing the VEGF/VEGF-Rs axis,[25-27] we examined whether genetic disruption of periostin affects VEGF-A and VEGF-R2 expression in the cornea of FADS mice. VEGF-A expression in the corneal epithelium and stroma was considerably higher in FADS/*Postn*$^{+/+}$ than in FADS/*Postn*$^{-/-}$ mice (Fig 3, *F*). Periostin was mainly localized around the VEGF-A–positive regions in the stroma and was partially colocalized with those regions. VEGF-R2 was highly expressed in the corneal epithelium and blood vessels of the corneal stroma of FADS/*Postn*$^{+/+}$ mice (Fig 3, *G,* and see Fig E4 in the Online Repository available at www.jacionline.org). The ratio of VEGF-R2^{hi} to VEGF-R2^{low} cells in CD31^{+} ECs was higher in FADS/*Postn*$^{+/+}$ than in FADS/*Postn*$^{-/-}$ mice (Fig 3, *H*). These results indicate that in FADS mice, periostin contributes to corneal neovascularization by upregulating expression of VEGF-A and VEGF-R2.

Periostin/$\alpha_V\beta_3$ integrin axis causes migration and tube formation of VEC

Given that periostin deficiency downregulated the expression of VEGF-A and VEGF-R2 in FADS mice, we explored whether periostin was directly involved in expressing these neovascularization-related molecules. We examined the effects of periostin on *VEGFA* and *KDR*/VEGF-R2 expression in various normal human primary cells—corneal fibroblasts (NHCF), corneal epithelial cells, umbilical vein ECs (HUVEC), dermal fibroblasts (NHDF), and lung fibroblasts (NHLF). Periostin increased *VEGFA* mRNA expression in normal human corneal epithelial cell and NHCF, less so in NHDF, but not at all in HUVEC or NHLF (Fig 4, *A*). Alternatively, periostin increased *KDR*/VEGF-R2 mRNA expression in HUVEC, but not in NHCF, NHDF, or NHLF (Fig 4, *A*). These results suggest that periostin acts directly on NHCF, NHDF, and ECs, inducing VEGF-A in NHCF and NHDF, and VEGF-R2 on ECs, followed by promoting neovascularization by enhanced VEGF-A and VEGF-R2.

It has been previously reported that periostin enhances migration and tube formation of VEC, both of which are crucial for neovascularization.[35-37] We confirmed that periostin upregulated both migration and tube formation of VEC, as previously reported (Fig 4, *B* and *C*). Moreover, CP4715 significantly inhibited

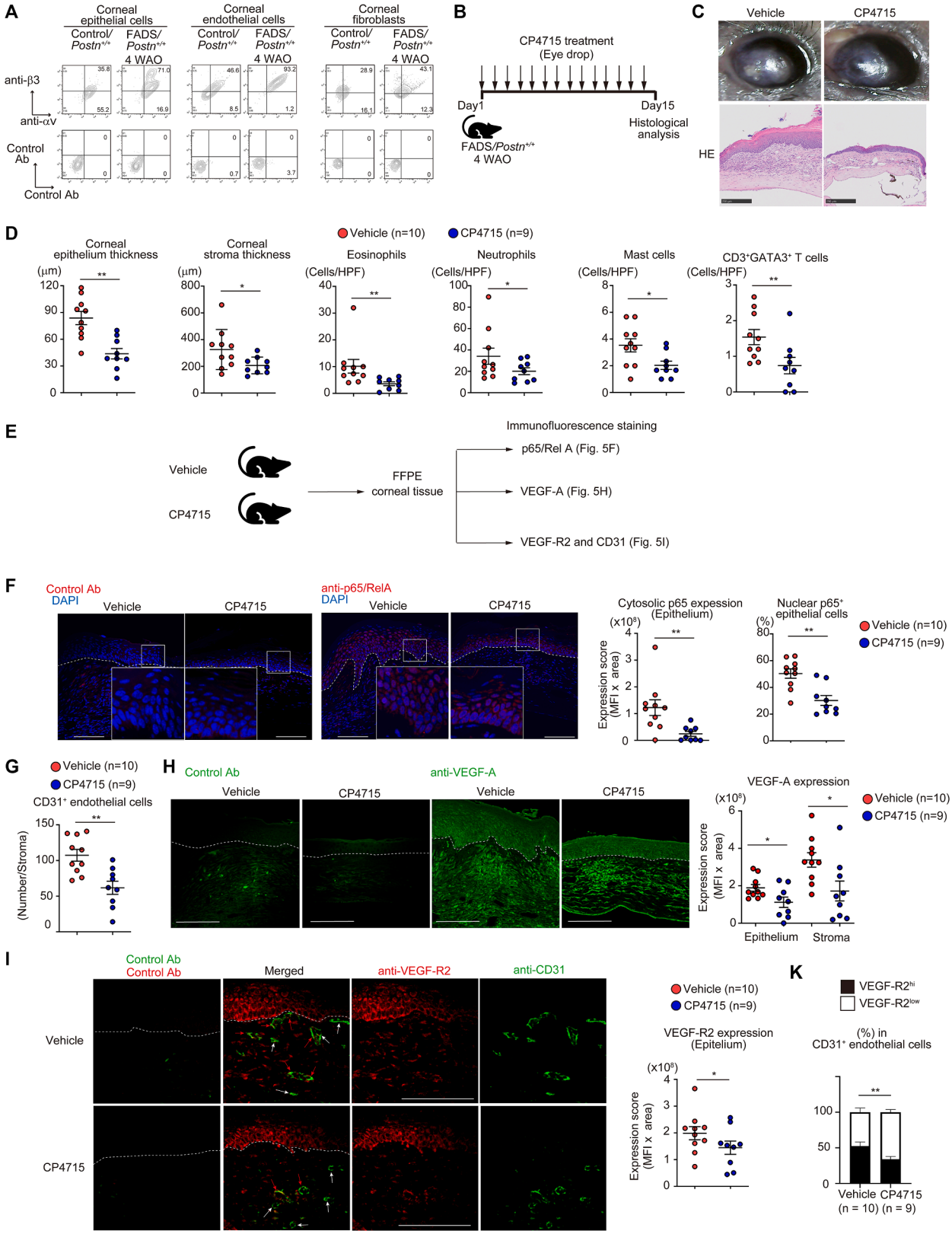

FIG 5. CP4715 improves corneal lesions of FADS mice. **(A)** Cell surface expression of α_V and β_3 integrins on corneal epithelial cells, endothelial cells, and fibroblasts in FADS mice at 4 weeks after onset (WAO) of corneal lesion and age-matched control mice. **(B)** Experimental protocol. **(C)** Macroscopic features and hematoxylin and eosin staining of corneal lesions of FADS mice after CP4715 administration. Scale bar, 250 μm. (**D** and **G**) Thickness of corneal epithelium and stroma and number of eosinophils, neutrophils, MCs,

periostin-mediated migration and tube formation of VEC in a dose-dependent manner. We found that periostin did not physically interact with VEGF-R2, and VEGF-R2 tyrosine kinase inhibitor (Ki8751) had no effects on the periostin-mediated tube formation (data not shown) (see Fig E5 in the Online Repository available at www.jacionline.org). These results suggest that the periostin/$\alpha_V\beta_3$ integrin pathway is directly involved in the process of neovascularization in the ocular lesions of FADS mice.

Pharmacologic inhibition of periostin improves corneal neovascularization and ocular inflammation in FADS mice

On the basis of the importance of periostin for inflammation and neovascularization in the ocular lesions of FADS mice, we examined the effects of pharmacologic inhibition of the periostin/$\alpha_V\beta_3$ integrin pathway on the lesions. The $\alpha_V\beta_3$ integrin was expressed on corneal epithelial cells, fibroblasts, and ECs with both the α_V and β_3 subunits more on fibroblasts and only the β_3 subunit more on epithelial cells (Fig 5, *A*). We administered CP4715 to the eyes of FADS mice with corneal lesions at 4 weeks after their onset, then daily for 14 days (Fig 5, *B*). CP4715 significantly reduced all corneal epithelial hyperplasia, corneal stromal hyperplasia, and infiltration of inflammatory cells such as eosinophils, neutrophils, MCs, and $CD3^+Gata3^+$ T cells (T_H2/Tc2) in the corneal stroma (Fig 5, *D*). However, CP4715 did not alter the size of visible corneal plaque (Fig 5, *C,* and see Fig E6, *A,* in the Online Repository available at www.jacionline.org). Similarly, CP4715 administration reduced infiltration of eosinophils, neutrophils, and MCs in the PC and dermis of eyelid skin (Fig E6, *B,* and see Fig E7 in the Online Repository). The schema of the experiments is depicted in Fig 5, *E.* CP4715 decreased cytosolic expression and nuclear localization of p65/RelA in the corneal epithelium of FADS mice as well (Fig 5, *F*). Moreover, CP4715 significantly reduced $CD31^+$ ECs in the stroma (Fig 5, *G*) and decreased VEGF-A expression in both the corneal epithelium and stroma; VEGF-R2 expression in the corneal epithelium also decreased (Fig 5, *G-I*). The ratio of VEGF-$R2^{hi}$ to VEGF-$R2^{low}$ cells in $CD31^+$ ECs in the corneal stroma was decreased by administering CP4715 (Fig 5, *K*). Taken together, these results demonstrate that in FADS mice, pharmacologically inhibiting the periostin/$\alpha_V\beta_3$ integrin pathway by CP4715 effectively improves inflammation and neovascularization.

DISCUSSION

We recently established a FADS mouse as a model of AKC by deleting *Ikk2* in facial dermal fibroblasts.[32] FADS mice spontaneously exhibit AKC-like phenotypes—severe blepharitis, infiltration of MCs, eosinophils, and T_H2/Tc2 in the conjunctival tissues and corneal stroma, corneal epithelium defects, and neovascularization.[32] Moreover, we found that in FADS mice, topical administration of betamethasone, and also to a lesser extent tacrolimus, improved AKC-like phenotypes.[32] Thus far, it remains unclear how deletion of *Ikk2* in fibroblasts leads to the appearance of AKC-like lesions. However, to our knowledge, our FADS mouse is the only mouse model of AKC, and it is a useful tool to elucidate the pathogenesis of AKC and to develop therapeutic agents against it. In this study, we investigated the role of periostin in the pathogenesis of AKC, applying both genetic and pharmacologic approaches to FADS mice. We observed the importance of the periostin/$\alpha_V\beta_3$ integrin pathway in type 2 and non–type 2 inflammation, the proliferation of epithelium and stroma of cornea, and neovascularization, although expression of non–type 2 cytokines was not fully analyzed. We have previously shown that AKC patients with coexistence of type 2 and non–type 2 inflammation are resistant to tacrolimus.[18] It is possible that evaluation of periostin levels in tears may enable stratification of patients with refractory AKC whose disease will have good response to treatment targeting the periostin/$\alpha_V\beta_3$ integrin pathway. These findings suggest that periostin is a key mediator for the onset of AKC and that the periostin/$\alpha_V\beta_3$ integrin pathway is a promising target as we seek to develop therapeutic agents.

We have shown that in FADS mice, periostin plays a critical role in both type 2 and non–type 2 inflammation in the pathogenesis of AKC-like lesions. We base this on the finding that both periostin deficiency and administration of CP4715 downregulated the infiltration of neutrophils, eosinophils, MCs, and T_H2/Tc2 in the cornea, PC, and eyelid dermis (Figs 2 and 5; and see Figs E1, E2, E6, and E7). We have previously demonstrated that in facial skin of FADS mice, periostin is required for activating NF-κB in keratinocytes, leading to the expression of cytokines and chemokines recruiting neutrophils and MCs such as *Ccl3, Ccl4, Cxcl1,* and *Cxcl2*.[24] In this study, we have shown that periostin was required for the activation of NF-κB/RelA in the corneal epithelium as well (Fig 2, *F*), which would explain why periostin is necessary for infiltration of neutrophils and MCs. However, in the present study, periostin was critical for type 2 inflammation in ocular lesions—in contrast with previous finding that periostin deficiency does not downregulate eosinophil infiltration in the skin.[24] Because we were not able to examine the expression of type 2 cytokines and chemokines as a result of small samples from the ocular lesions, which are hard to separate for both histologic and gene expression analyses, we do not know the exact effects of periostin on them. Similarly, it remains unclear how periostin regulates expression of non–type 2 cytokines and chemokines. In the corneal epithelium, activating NF-κB/RelA may induce the expression of cytokines and chemokines for recruiting eosinophils and T_H2/Tc2, in addition to neutrophils and MCs. Alternatively, periostin may induce the

$CD3^+Gata3^+$ T cells *(D),* and $CD31^+$ endothelial cells *(G)* in corneal stroma after CP4715 *(blue circles)* or vehicle *(red circles)* administration. **(E)** Schematic summary of immunofluorescence staining. (**F, H,** and **I**) Immunofluorescence staining for NF-κB p65/RelA *(F)* and VEGF-A *(H),* and double staining of CD31 and VEGF-R2 *(I)* in corneas of FADS mice after CP4715 *(blue circles)* or vehicle *(red circles)* administration. Cytosolic p65/RelA expression levels and percentages of nuclear p65/$RelA^+$ cells in corneal epithelium *(F),* expression score of VEGF-A in corneal epithelium and stroma *(H),* and ratio of VEGF-$R2^{hi}$ *(black)* and VEGF-$R2^{low}$ *(white)* cells in $CD31^+$ endothelial cells in corneal stroma *(K). Red* and *white arrows* indicate VEGF-$R2^{hi}CD31^+$ and VEGF-$R2^{low}CD31^+$ endothelial cells, respectively. Scale bars, 200 μm (*F* and *H*) and 100 μm *(I). MFI,* Mean fluorescence intensity. *Dashed lines* depict borders of epithelium and stroma (*F, H,* and *I*). Data are shown as means ± SEMs. Statistical analysis was performed by 1-sided Mann-Whitney *U* test; $*P < .05$, $**P < .01$.

expression of cytokines and chemokines for recruiting eosinophils and T_H2/Tc2 in an NF-κB/RelA–independent manner. This would be a characteristic of the inflammation mechanism in ocular lesions that is different from that in skin lesions.

In this study, we showed the importance of periostin in neovascularization in ocular lesions of FADS mice, at least partially by directly enhancing the VEGF-A/VEGF-R2 pathway (Figs 3 and 4). It has been understood that the VEGF/VEGF-R pathway is central to the neovascularization of AKC.[38,39] The actions of periostin on the VEGF/VEGF-R pathway are efficient and coordinated; periostin induces expression of VEGF-A in corneal epithelial cells and fibroblasts, whereas in ECs, it induces the expression of VEGF-R2 (Fig 4). Moreover, we have shown that the actions of periostin on the VEGF/VEGF-R pathway are enough to enhance the tube formation of VEC (Fig 4, *C*), although this effect was independent of tyrosine kinase activity of VEGF-R2 (Fig E5). Because it has been reported that FAK, a downstream molecule of $\alpha_V\beta_3$ integrin, promotes tube formation by activating actin cytoskeleton organization,[40] the periostin/$\alpha_V\beta_3$ integrin/FAK pathway may also contribute to tube formation. Alternatively, it is known that various inflammatory cells—MCs, eosinophils, and neutrophils—secrete VEGF,[41-43] so periostin would enhance the VEGF/VEGF-R pathway indirectly as well by inducing infiltration of inflammatory cells. Taken together with the previous studies,[24,32] the present study highlights neovascularization as a novel function of periostin in the pathogenesis of AKC.

Our study showed that CP4715, an inhibitor of $\alpha_V\beta_3$ integrin, periostin's receptor, downregulated all inhibition, proliferation, and neovascularization of ocular lesions, including by inhibiting the tube formation of ECs (Fig 4, *B* and *C,* and Fig 5), suggesting that CP4715 would be a promising therapeutic agent against AKC. Although immunosuppressants or glucocorticoid eye drops are now usually used to treat AKC, we have to be careful about adverse effects of these agents, such as suppression of broad immunoresponses.[3] In contrast to other allergic diseases such as asthma, eosinophilic chronic rhinosinusitis, and AD,[44,45] however, to date, no molecularly targeted drug is available for AKC. Taking into account that, as the present study indicates, periostin may be critical in AKC's pathogenesis, and given that eye drops are both feasible and efficient, we can expect that CP4715 will be developed as a molecularly targeted drug against AKC—one, we hope, with fewer adverse effects. Several humanized anti-VEGF antibodies have already been developed and are used for treating retinal angiogenic diseases as well as being efficacious for neovascularization after cornea graft transplantation.[46,47] However, there has been no report showing that an anti-VEGF antibody is effective for treating AKC. The present study indicates that inhibiting the periostin/$\alpha_V\beta_3$ integrin pathway inhibits, at least to some extent, the VEGF/VEGF-R pathway.

Moreover, we think that targeting $\alpha_V\beta_3$ integrin, the receptor of periostin, has more advantages than targeting periostin itself. It has been reported that VEGF-R2 cooperates with $\alpha_V\beta_3$ integrin to induce c-Src kinase activation and subsequent β_3 integrin tyrosine phosphorylation, which is critical for VEGF-induced formation of the VEGF-R2/$\alpha_V\beta_3$ integrin complex. In addition, MK-0429, another inhibitor against $\alpha_V\beta_3$ integrin, reduces tube formation of VEC induced by VEGF-A.[48,49] It has been previously shown that TNC, an alternative ligand of $\alpha_V\beta_3$ integrin, facilitates corneal neovascularization by upregulating the expression of VEGF.[50] Actually, TNC expression was elevated in the ocular tissues of FADS mice (Fig 1). Furthermore, Szeto et al in 2023 demonstrated that the interaction between Thy1 and $\alpha_V\beta_3$ integrin on naïve T cells is critical for polarization into T_H2 cells.[51] Thus, targeting $\alpha_V\beta_3$ integrin may inhibit the actions of ligands other than periostin that are important in the pathogenesis of AKC.

A limitation of this study is the small sample size of AKC patients. In the future, a multicenter study should be performed on a larger sample size. Regardless, we hope that going forward, novel therapeutic agents against AKC will be developed that are based on the importance of the periostin/$\alpha_V\beta_3$ integrin pathway.

DISCLOSURE STATEMENT

Supported in part by Japan Society for the Promotion of Science Grants-in-Aid for Scientific Research grant JP21K08476 (to K.I.) and grant JP22K08566 (to S.N.).

Disclosure of potential conflict of interest: K. Izuhara has received research grants from Maruho, Torii Pharmaceutical, and Shino-Test. The rest of the authors declare that they have no relevant conflicts of interest.

We thank Dovie R. Wylie for the critical review of this report. We also thank Maki Watanabe and Tomoyo Yoshida for their technical assistance. We thank Manolis Pasparakis and Gail R. Martin for their kindness in providing *Ikk2*$^{f/f}$ and *Nestin*^{Cre} mice.

Key messages

- **Both genetic deficiency and pharmacologic inhibition of the periostin/$\alpha_V\beta_3$ integrin pathway ameliorate AKC-like ocular lesions in FADS mice by reducing corneal neovascularization as well as type 2 and non–type 2 inflammation in corneal stroma and conjunctiva.**
- **The periostin/$\alpha_V\beta_3$ integrin pathway constitutes a promising therapeutic target for treating AKC.**

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