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Upregulation of vascular endothelial growth factor (VEGF) in the retinas of transgenic mice overexpressing interleukin-1ß (IL-1ß) in the lens and mice undergoing retinal degeneration

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Summary. IL-1ß is a pro-inflammatory agent associated with angiogenesis and increased vascular permeability. To determine whether IL-1ß elicits these responses through an upregulation of VEGF, transgenic mice that overexpress IL-1ß in the lens were evaluated at various time points for the localization of VEGF, the location and extent of blood-retinal barrier (BRB) breakdown, and the origin and extent of neovascularization (NV). In homozygous and heterozygous transgenic mice, but not controls, intense VEGF immunoreactivity was scattered throughout the retina at postnatal days 5-7 (P5-7), just after the onset of inflammatory cell infiltration. VEGF staining in the retina remained widespread, but weak from P9-15. Beginning at P15, the intensity of VEGF immunoreactivity achieved a second peak, which it maintained through adulthood. This peak coincided with significant retinal destruction due to massive inflammation. The onset of BRB breakdown coincided with the upregulation of VEGF (P5-7) and widespread BRB breakdown was demonstrated from about P9. From P9-12, aggregates of cells positive for Griffonia simplicifolia isolectin-B4, a marker for vascular endothelial cells, formed on the retinal surface. These cells migrated into the retina at P12-15 with the more superficial cells forming a network of vessels and the deeper cells remaining in small clusters, thus demonstrating that NV occurs much later than BRB breakdown. Non-transgenic FVB/N mice, which undergo retinal degeneration beginning at about P9, also demonstrate the latter peak of VEGF upregulation and the accompanying BRB breakdown, but not the early upregulation. VEGF immunostaining of transgenic and non-transgenic mouse retinas was eliminated by pre-incubation of the VEGF antibodies with VEGF peptide. The data suggest that the early peak of VEGF upregulation (P5-7) and its accompanying BRB breakdown is due to IL-1ß expression and is likely to be dependent on inflammatory cell infiltration. The latter peak appears to be related to retinal destruction.

Key words: Interleukin-1ß, Vascular endothelial growth factor, Blood-retinal barrier, Retinal degeneration, Angiogenesis

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Introduction

Neovascularization (NV) is a complication leading to visual deficits that is associated with a number of ocular disorders, such as diabetic retinopathy, age related macular degeneration, corneal NV associated with Herpes keratitis, and neovascular glaucoma. A variety of factors are associated with pathological NV, but some of these are not primarily considered as angiogenic factors and may induce NV indirectly through another mediator. Interleukin-1ß (IL-1ß), the secreted isoform of interleukin-1, is an example of such a factor. IL-1ß is produced following infection, injury, or antigenic challenge. Its primary function is thought to be that of a proinflammatory factor and IL-1ß, together with tumor necrosis factor-α and IL-6, are the principal regulators of local inflammation in man (Hoekzema et al., 1990). IL-1ß is also a potent inducer of experimental intraocular inflammation. An intraocular injection of IL-1ß into rabbits or rats results in the rapid recruitment of monocytes, neutrophils, and polymorphonuclear leukocytes into the retina (Bhattacherjee and Henderson, 1987; Hoekzema et al., 1990; Martiney et al., 1990, 1992; Kulkarni et al., 1993; Cuff et al., 1996; Bamforth et al., 1997). Coincident with the peaks of leukocyte infiltration are peaks of blood-retinal barrier (BRB) disruption (Watson et al., 1989; Martiney et al., 1990, 1992; Cuff et al., 1996; Bamforth et al., 1997). IL-1ß can also initiate endothelial proliferation and angiogenesis

(Schöbitz et al., 1994) and it can induce NV in the brain (Giulian et al., 1988), but it is not certain whether this represents a direct action by IL-1ß.

In transgenic mice that overexpress IL-1ß in the lens, under control of the αA-crystallin promoter, NV occurs initially in the cornea and in front of the retina (preretina) and progresses throughout the entire retina, eventually including all tissues in the phthisical adult eye (Wawrousek et al., 1994). Corneal and preretinal NV begins at about postnatal day 9 (P9), but this is preceded by an inflammatory response, characterized by massive leukocyte infiltration and increased vascular permeability, which occurs as early as P4. It is possible that the angiogenic effect of IL-1ß in this model is dependent on leukocyte infiltration and the subsequent introduction of other factors. IL-1ß can stimulate the production of the adhesion molecules, E-selectin and ICAM-1 (Klein et al., 1995), prostaglandins E2 and F2α (Dayton and Major, 1996; Van Dam et al., 1996), IL-6 (Van Dam et al., 1996), and VEGF (Ben-Av et al., 1995; Li et al., 1995; Jackson et al., 1997; Ristimaki et al., 1998; Valter et al., 1999; El Awad et al., 2000; Levitas et al., 2000; Tanaka et al., 2000), which may play a role in indirectly mediating its actions. VEGF is capable of causing increased vascular permeability (Senger et al., 1983, 1986, 1990), of participating in an inflammatory response (Clauss et al, 1996; Melder et al., 1996; Lu et al., 1999), and in promoting NV (Aiello et al., 1995; Tolentino et al., 1996; Robinson et al., 1996; Ozaki et al., 1997, 2000; Okamoto et al., 1997; Tobe et al., 1998). Since IL-1ß can induce VEGF in other systems, it is likely to mediate its ocular angiogenic activity through an induction of VEGF. A recent study shows that IL-1ß, secreted by macrophages, may promote angiogenesis in choroidal neovascular membranes by stimulating VEGF production in RPE cells (Oh et al., 1999), supporting this hypothesis. The present study was conducted to show whether VEGF is upregulated in the retinas of transgenic mice that overexpress IL-1ß and to determine if its upregulation correlates with NV and BRB breakdown.

Materials and methods

Transgenic mice that overexpress IL-1ß were generated using the human IL-1ß vector with the murine αA-crystallin promoter as previously described (Wawrousek et al., 1994; Lai et al., 1996). Transgene DNA was injected into the pronucleus of single-celled mouse embryos of the FVB/N strain to generate transgenic mice, which were then bred to homozygosity. The transgenic line was initiated in FVB/N mice, which are one of the most commonly used inbred strains for the generation of transgenic animals due to the large pronuclei of its fertilized oocytes, which are ideal for microinjection, and for their vigorous reproduction and consistently large litter size (Gimenez and Montoliu, 2001). A drawback for using this strain is the fact that it carries the gene, *Pde6b(rd1)* (formerly called rd), which causes retinal degeneration. This deficit can be overcome by crossing these mice with strains that carry the wild-type allele at the *Pde6b* locus (Gimenez and Montoliu, 2001). To insure that the effects seen in the transgenic mice were not a reflection of the rd phenotype, homozygous IL-1ß transgenic mice were crossed with C57BL/6 mice to yield heterozygous offspring with a normal retinal phenotype. In addition to homozygous and heterozygous IL-1ß transgenic mice, non-transgenic FVB/N mice, C57BL/6 mice, and the offspring of a cross between these two strains, which also have a normal retinal phenotype, were examined in this study. Transgenic mice were sacrificed by cervical dislocation and the eyes were promptly fixed in buffered formalin after carefully removing the cornea. Homozygous IL-1ß transgenic mice were sacrificed at postnatal day 2 (P2; n=2), P4 (n=5), P6 (n=2), and P12 (n=4). Heterozygous IL-1ß transgenic mice were obtained by mating homozygous mice with C57BL/6 mice. Heterozygous IL-1ß transgenic mice were sacrificed at P3 (n=4), P5 (n=5), P7 (n=8), P9 (n=8), P15 (n=3), P19 (n=8), P24 (n=10), P32 (n=2), and P35 (n=2). In addition, eyes from 2 transgenic mice each at 6.5 and 12 months old and eyes from intercellular adhesion molecule-1 (ICAM-1) knockout mice (derived from Jackson Laboratory stock, Bar Harbor, ME) carrying the transgene at 35 days (n=3), 12 months (n=2), and 15 months of age (n=4) were also fixed. For controls, eyes from normal FVB/N mice were fixed and examined at P3 (n=2), P4 (n=2), P5 (n=2), P6 (n=2), P7 (n=2), P9 (n=2), P12 (n=2), P13 (n=1), P15 (n=2), P19 (n=2), P24 (n=2), P28 (n=3), and adult (n=1). Eyes from normal C57BL/6 mice were fixed and examined at P3 (n=2), P5 (n=2), P7 (n=3), P9 (n=3), P12 (n=3), P15 (n=3), P17 (n=1), P19 (n=2), P24 (n=3), and P32 (n=3). Eyes from the F1 offspring of a cross between normal FVB/N and C57BL/6 mice were fixed and examined at P2 (n=2), P5 (n=3), P7 (n=3), P9 (n=3), P12 (n=4), P15 (n=2), P19 (n=2), and P24 (n=3). The eyes were embedded in paraffin and sectioned. One section from each eye was stained with hematoxylin and eosin (H&E) and the remaining slides were used for immunohistochemistry or for histochemical staining.

Sections were stained for VEGF using polyclonal rabbit antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) and generating a brown reaction product with diaminobenzidine (Research Genetics, Huntsville, AL) or a red reaction product with HistoMark Red (Kirkegaard & Perry, Gaithersburg, MD), as previously described (Vinores et al., 1997, 2001). Incubation times with each chromogen were standardized so that the relative staining intensities of different specimens can be compared. Selected serial sections were stained using goat anti-mouse VEGF antibodies (R&D Systems, Minneapolis, MN) to verify that both antibodies labeled the same cells. VEGF antibodies were pre-incubated with a 10 molar excess of VEGF peptide (R&D Systems) prior to applying them to the sections to confirm that the staining was specific (Vinores et al., 1997). Staining for albumin was performed using

polyclonal rabbit anti-rat albumin antibodies (Nordic, Tilburg, The Netherlands) as previously described (Vinores et al., 1990, 2001). The vessels were visualized using histochemical staining for Griffonia simplicifolia isolectin-B4 (GSA; Vector, Burlingame, CA) as previously described (Vinores et al., 1992; Seo et al., 2000).

Results

Morphology

For the first 7 postnatal days, there was no morphological difference between IL-1ß transgenic mice and non-transgenic FVB/N, C57BL/6, or FVB/Nx C57BL/6 mice and there was no phenotypic difference between homozygous and heterozygous IL-1ß transgenic mice at any of the time points examined. The outer nuclear layer, inner nuclear layer, and ganglion cell layer began to segregate on P3 in all mice. The nerve fiber layer, inner plexiform layer, and inner nuclear layer thickened at P5. The outer plexiform layer had formed and the outer segments of the photoreceptors were forming at P7. In normal C57BL/6 mice, the outer segments of the photoreceptors were very short at P9, but at P12-15, marked lengthening of the outer segments was evident. By P19, C57BL/6 mice had a mature retina. In FVB/N mice, the outer segments never lengthened and there was thinning of the outer nuclear layer, which was pronounced at P15 and reduced to a single cell thickness at P19. By P24, there was a total absence of photoreceptors and retinal detachment ensued. The phenotype of FVB/NxC57BL/6 mice was indistinguishable from that of C57BL/6 mice.

In IL-1ß transgenic mice, the first sign of inflammatory cell infiltration occurred at P4 and significant inflammation was evident within the retina at P9. This marked inflammatory response was accompanied by disruption of the photoreceptors, particularly in the posterior retina (Fig. 1A). By P12, transgenic mice exhibited inflammation with some perivascular foci in the inner retina, disruption of the inner nuclear layer (Fig. 1B), and occasional retinal detachment with subretinal exudate (Fig. 1C). At P15, there was very pronounced inflammation in transgenic mice with disruptive changes affecting all layers of the retina (Fig. 1D). The disruptive changes that resulted from the pronounced inflammation masked the retinal degeneration characteristic of FVB/N mice; therefore, similar phenotypes were observed in homozygous IL-1ß transgenics in the FVB/N background and heterozygous IL-1ß transgenics in the FVB/NxC57BL/6 background. The disease continued to progress, but there were fewer inflammatory cells in the vitreous at P19 (Fig. 1E). By P24, the retina was adherent to the lens (Fig. 1F). The adult eye became completely phthisical with no normal retinal structure (Fig. 1G,H). A comparison of retinal phenotypes is presented in Table 1.

VEGF

C57BL/6, FVB/N, and C57BL/6xFVB/N mice showed weak, diffuse staining for VEGF in the inner third of the retina (from the inner plexiform layer to the inner limiting membrane), with the ganglion cells staining somewhat stronger from P2-P7. Beginning at P9, the inbred FVB/N mouse retinas begin to stain more intensely, particularly in the ganglion cells and in the outer segments of the photoreceptors (Fig. 2A,B). As the photoreceptors degenerate beyond P12, VEGF staining spreads throughout the retina and intensifies from P19 through adult (Fig. 2C,D). VEGF immunoreactivity also spreads throughout the retina in C57BL/6 and C57BL/6xFVB/N mice, but the staining is weaker and more diffuse than in FVB/N mice.

Weak diffuse staining for VEGF was also evident in the inner retinas of IL-1ß transgenic mice at P3 (Fig. 3A), but by P5-P7, the VEGF signal had intensified and was scattered throughout the retina (Fig. 3B,C). Only mice expressing the IL-1ß transgene demonstrated this peak of VEGF upregulation. From P9-P15, VEGF staining in the retina remained widespread, but appeared weaker (Fig. 3D). From P15, the intensity of VEGF staining achieved a second peak and remained intensely positive throughout the retina to adulthood (Fig. 3E,F). Heterozygous IL-1ß mice in the C57BL/6xFVB/N

Table 1. Summary of retinal phenotype.

	C57BL/6 AND C57BL/6xFVB/N	FVB/N	IL-1ß TRANSGENICS
Morphology	Normal	Retinal degeneration pronounced at P15, total by P24 with retinal detachment	Inflammation beginning at P4, significant with disruption of photoreceptors by P9. Disruptive changes affecting all layers by P15.
VEGF	Weak, diffuse staining in inner 1/3 of retina eventually spreading throughout retina	More intense staining beginning at P9, intensifies as photoreceptors degenerate	Staining intensifies & spreads throughout retina at P5-7, becomes weaker from P9-15, achieves second peak beginning at P15
BRB	Albumin confined to vessels	Focal leakage at P9, extensive leakage at P12-15	Albumin along inner retinal surface & in vitreous at P2-4, focal vascular leakage at P5-7, widespread leakage from P9
NV	No NV	No NV	Clusters of GSA+ cells aggregated along inner retinal surface at P9-12, migrated into retina at P12-15. More superficial cells formed vascular network, deeper cells dispersed

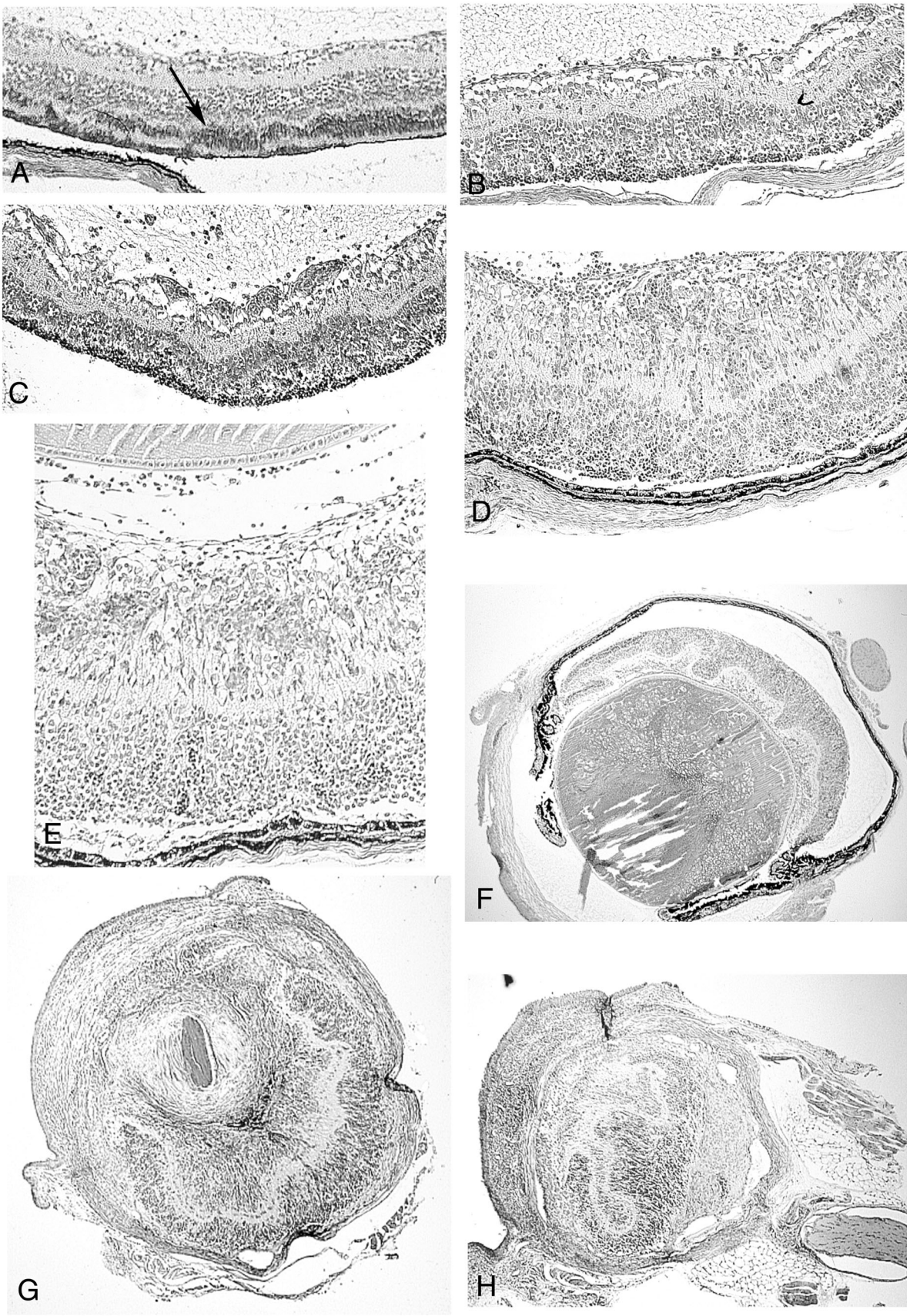

Fig. 1. Sections from IL-1ß transgenic mice stained with hematoxylin and eosin. **A.** Disruption of the photoreceptor layer (arrow) is evident at P9. **B.** Inflammation and disruption of the outer retina progressed by P12 with some disruption of the inner nuclear layer. **C.** Perivascular foci of inflammation are visible at P12. Retinal detachment has occurred and a subretinal exudate is present (bottom left). Note disruption of photoreceptors. **D.** At P15, there was pronounced inflammation throughout the retina with disruption of all layers. **E.** At P19, inflammation and disruption of the retinal architecture was evident. Note persistent inflammatory cells in the vitreous (top). **F.** A detached retina, devoid of photoreceptors, adhering to the lens at P24. **G, H.** Two examples of adult eyes from IL-1ß transgenic mice show a complete loss of normal retinal structure and the formation of a phthisical eye. A-C, x 100; D, x 200; E, x 400; F-H, x 50

background were indistinguishable from the homozygous IL-1ß mice in the pure FVB/N background with respect to VEGF immunoreactivity. Adult transgenic mice with normal ICAM-1 demonstrated widespread VEGF staining throughout the retina, whereas the retinas of ICAM-1 knockout mice expressing the IL-1ß transgene exhibited only scattered foci of VEGF positivity or were VEGF-negative. VEGF immunostaining on transgenic and non-transgenic retinas using rabbit antibodies was confirmed using distinct goat anti-mouse VEGF antibodies and was eliminated by pre-incubation of the VEGF antibodies with VEGF peptide (Fig. 4).

Blood-Retinal Barrier (BRB)

Immunohistochemical staining for albumin was used to visualize areas of BRB breakdown. In C57BL/6 and C57BL/6xFVB/N mice, there was weak to moderate staining of the formative vessels on the surface of the retina with weak staining of ganglion cells and occasionally some of the cells in the inner portion of the inner nuclear layer (Fig. 5A). Staining within the retinal vessels intensified as the vessels matured (Fig. 5B). FVB/N mice showed similar staining of vessels in the inner retina. Ganglion cells and cells in the inner nuclear layer stained somewhat stronger (Fig. 5C). Some focal retinal vascular leakage was first demonstrated at P9 (Fig. 5D) and became more extensive by P12-15 (Fig. 5E), eventually spreading to the outer retina (Fig. 5F).

In IL-1ß transgenic mice, from P2-4, the presence of albumin could be observed in some cells along the inner surface of the retina, in the vitreous, and within vessels in the vitreous (Fig. 6A,B). By P5-7, the same time as VEGF upregulation was commencing, focal areas of vascular leakage could be demonstrated in the inner retina to the level of the inner plexiform and inner nuclear layers and occasionally deeper within the retina (Fig. 6C). From P9 through adult, BRB breakdown became widespread with extravascular albumin being localized throughout the retina (Fig. 6D-J). In some cases, retinal vascular leakage could be traced to a specific location and in other occasions, albumin was diffusely spread through all layers of the retina.

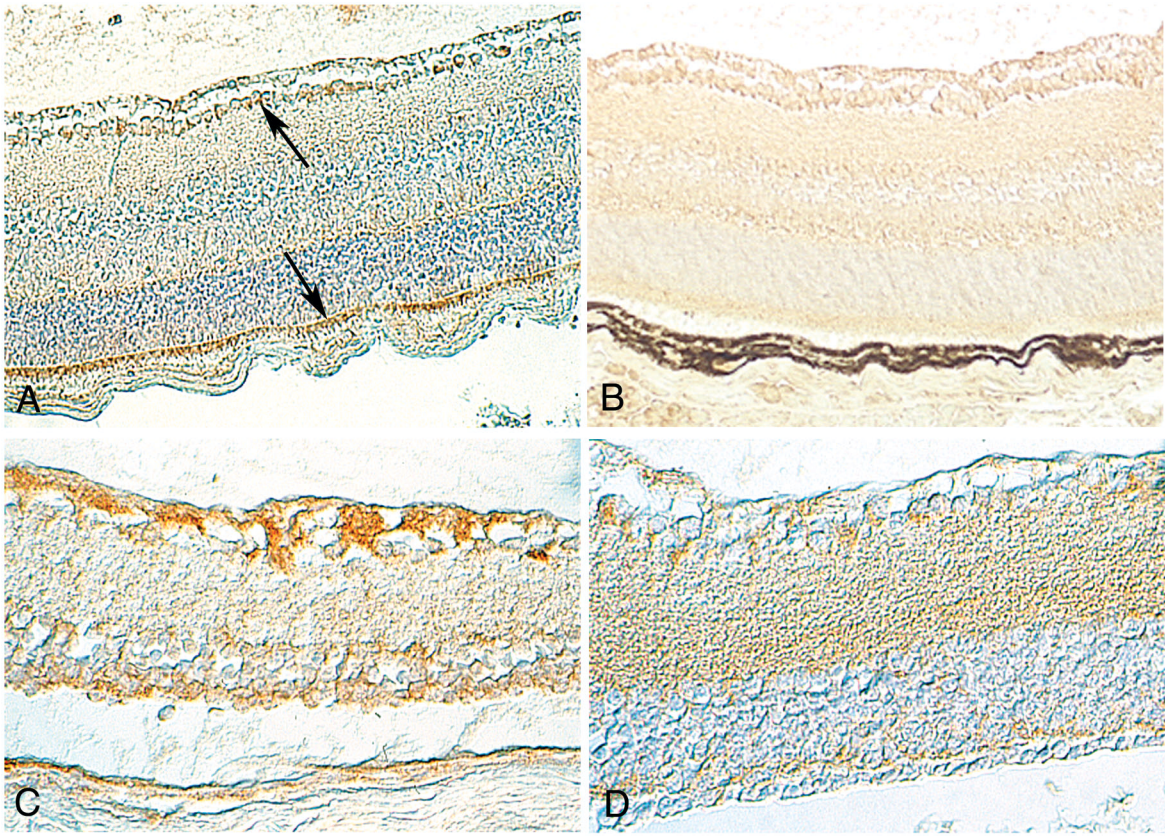

Fig. 2. VEGF immunolocalization in FVB/N mouse retinas using rabbit antibodies. **A.** At P12, retinal staining for VEGF is most conspicuous in the ganglion cells (top arrow) and remnants of the outer segments of the photoreceptors (bottom arrow). **B.** By contrast, normal C57BL/6 mice of the same age show only very weak staining for VEGF. **C.** VEGF immunostaining has spread throughout the retina and intensified in FVB/N by P24. **D.** Prominent VEGF staining is evident, particularly in cells along the inner retinal surface (top) in the adult FVB/N mouse. Brown staining indicates a positive immunohistochemical signal. x 200

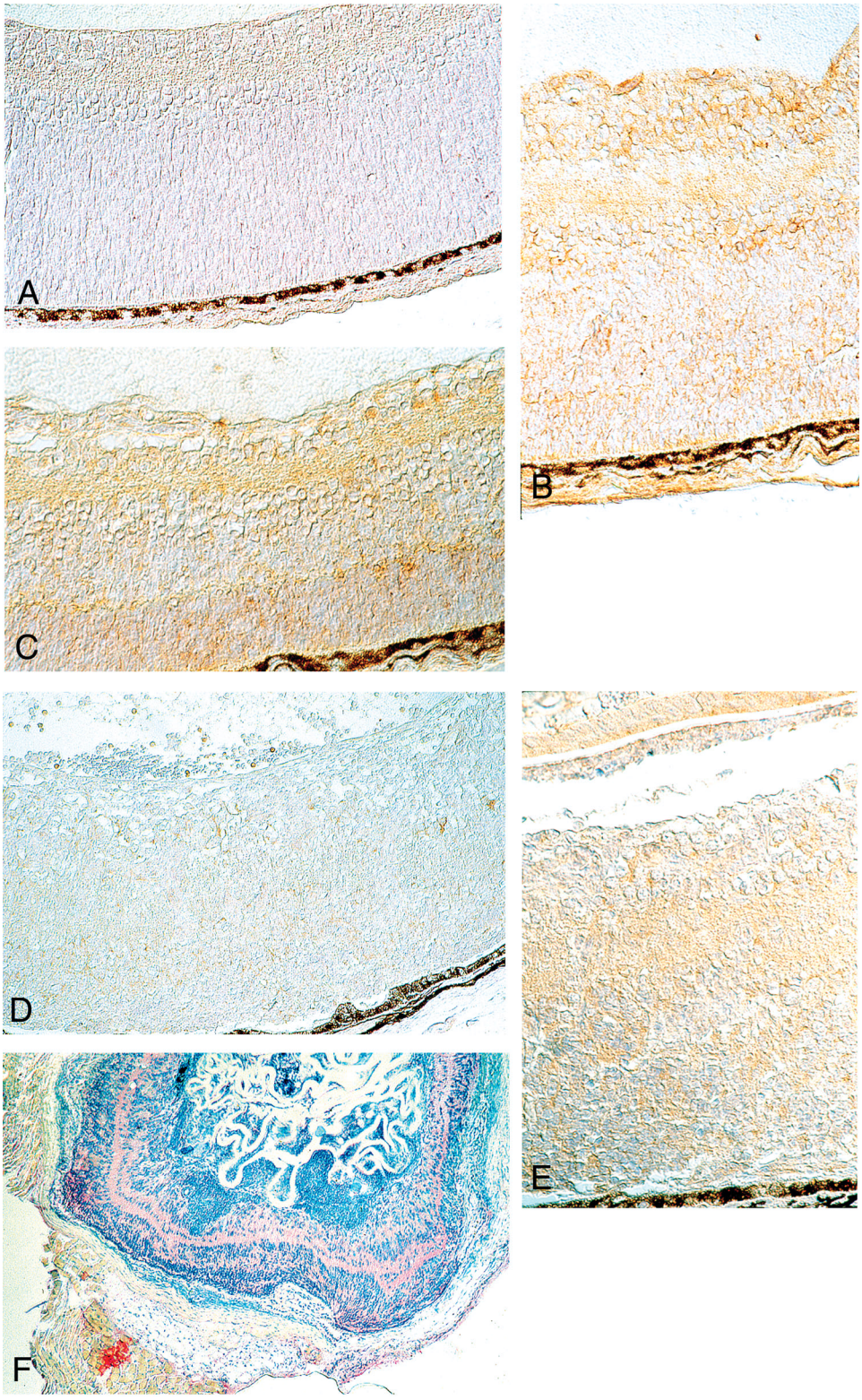

Fig. 3. VEGF immunolocalization in IL-1ß transgenic mice using rabbit antibodies. **A.** Weak, diffuse staining for VEGF in the inner retina (top) is evident at P3. **B.** Prominent staining for VEGF from the inner plexiform layer to the inner limiting membrane (top) with scattered positive foci throughout the retina at P5. **C.** At P7, the VEGF staining is similar to that seen at P5. **D.** Weak, but scattered positivity for VEGF in the retina at P15. **E.** More intense, widespread immunoreactivity for VEGF is demonstrated at P24. **F.** Positive staining (red reaction product) throughout the remnants of the retina in the adult, phthisical eye. A, C, D, x 200; B, E, x 400; F, x 50

Neovascularization

A histochemical stain for GSA is useful for visualizing vascular endothelial cells. This technique clearly labeled normal vessels in the retina, choroid, and vitreous, but no pathological endothelial proliferation or NV was seen in C57BL/6, FVB/N, or C57BL/6xFVB/N mice. In both normal and IL-1ß transgenic mice, GSA decorated vessels in the vitreous, surrounding the lens, and on the retinal surface from P2-7 (Fig. 7A,B). The vessels within the vitreous began to regress and, beginning at about P7, the vessels on the surface of the retina began to penetrate the retina. From about P9, the GSA staining of transgenic mouse retinas began to deviate from that of non-transgenic mouse retinas. Clusters of GSA-positive cells aggregated along the inner surface of the retina at P9-12. These clusters migrated into the retina at P12-15 (Fig. 7C-E). By P19, the clusters had dispersed into smaller aggregates (Fig. 7F). The more superficial cells formed a network of vessels, while the cells that had migrated deeper into the retina remained as small cell aggregates (Fig. 7G-I).

Discussion

Overexpression of IL-1ß induces NV in the brain (Giulian et al., 1988; Schöbitz et al., 1994) and causes retinal NV in transgenic mice (Wawrousek et al., 1994), but it is not clear whether IL-1ß directly stimulates vascular endothelial cells to proliferate or if its mitogenic activity is mediated indirectly through an induction of another factor. The upregulation of VEGF seen in the retinas of IL-1ß transgenic mice suggests that the angiogenic activity of IL-1ß may actually be mediated by VEGF. VEGF upregulation in IL-1ß transgenic mice demonstrates two peaks. The first of these occurs at about P5-7 and the second begins at about P15 and continues through adult.

Only the mice carrying the IL-1ß transgene developed the first peak of VEGF overexpression at about P5-7, showing that this upregulation is directly related to the expression of IL-1ß. The second peak of VEGF overexpression, which occurs from about P15 through adult, was also demonstrated in non-transgenic FVB/N mice. The FVB/N mice had undergone pronounced retinal degeneration at this time point and retinal destruction due to inflammation was occurring in transgenic mice, suggesting that this latter peak of VEGF upregulation may be related to retinal degeneration and the accompanying release of factors that may lead to VEGF induction. VEGF upregulation is likely to be related to inflammation. IL-1ß is a powerful inducer of intraocular inflammation (Bhattacherjee and Henderson, 1987; Hoekzema et al., 1990; Martiney et

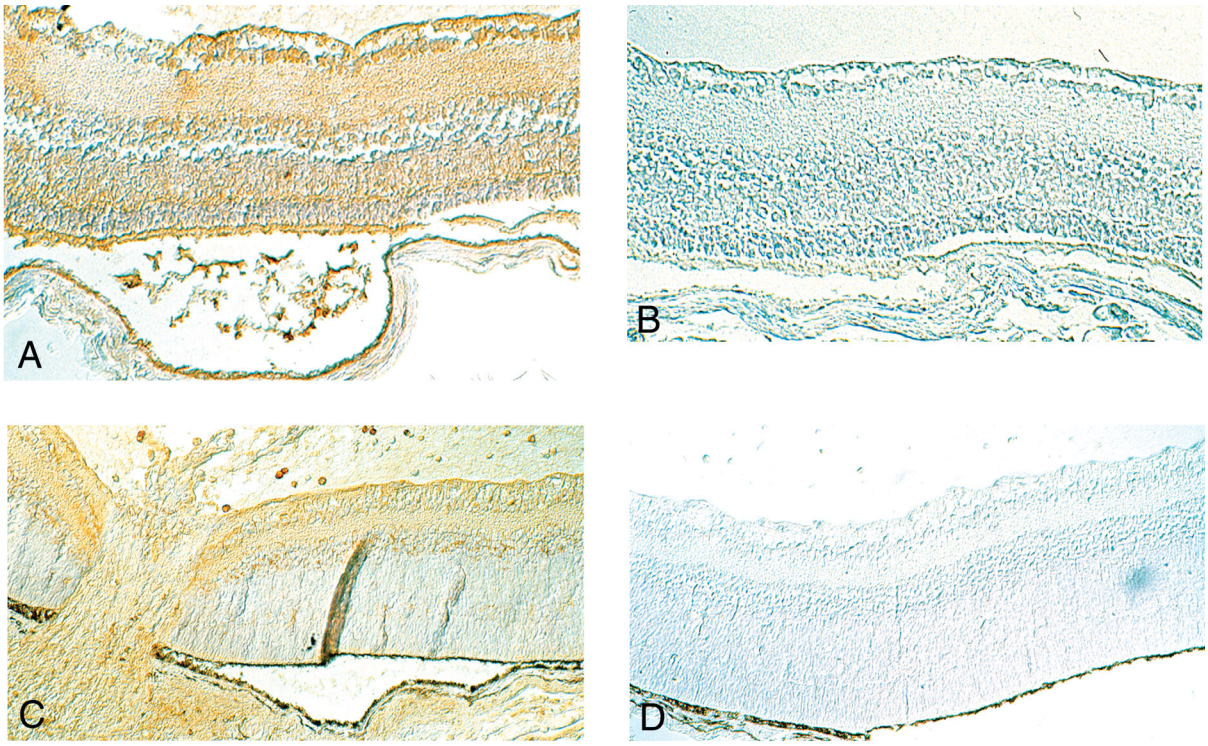

Fig. 4. Corresponding sections of retina from non-transgenic FVB/N mice **(A, B)** and heterozygous IL-1ß transgenic mice **(C, D)** stained for VEGF with (B, D) or without (A, C) pre-incubation of goat anti-mouse VEGF antibodies with VEGF peptide. In all cases, pre-incubatin of the antibodies with the control peptides eliminates immunohistochemical staining, demonstratin the specificity of the staining. **(A, B)** P15. **(C, D)** P5. x 100

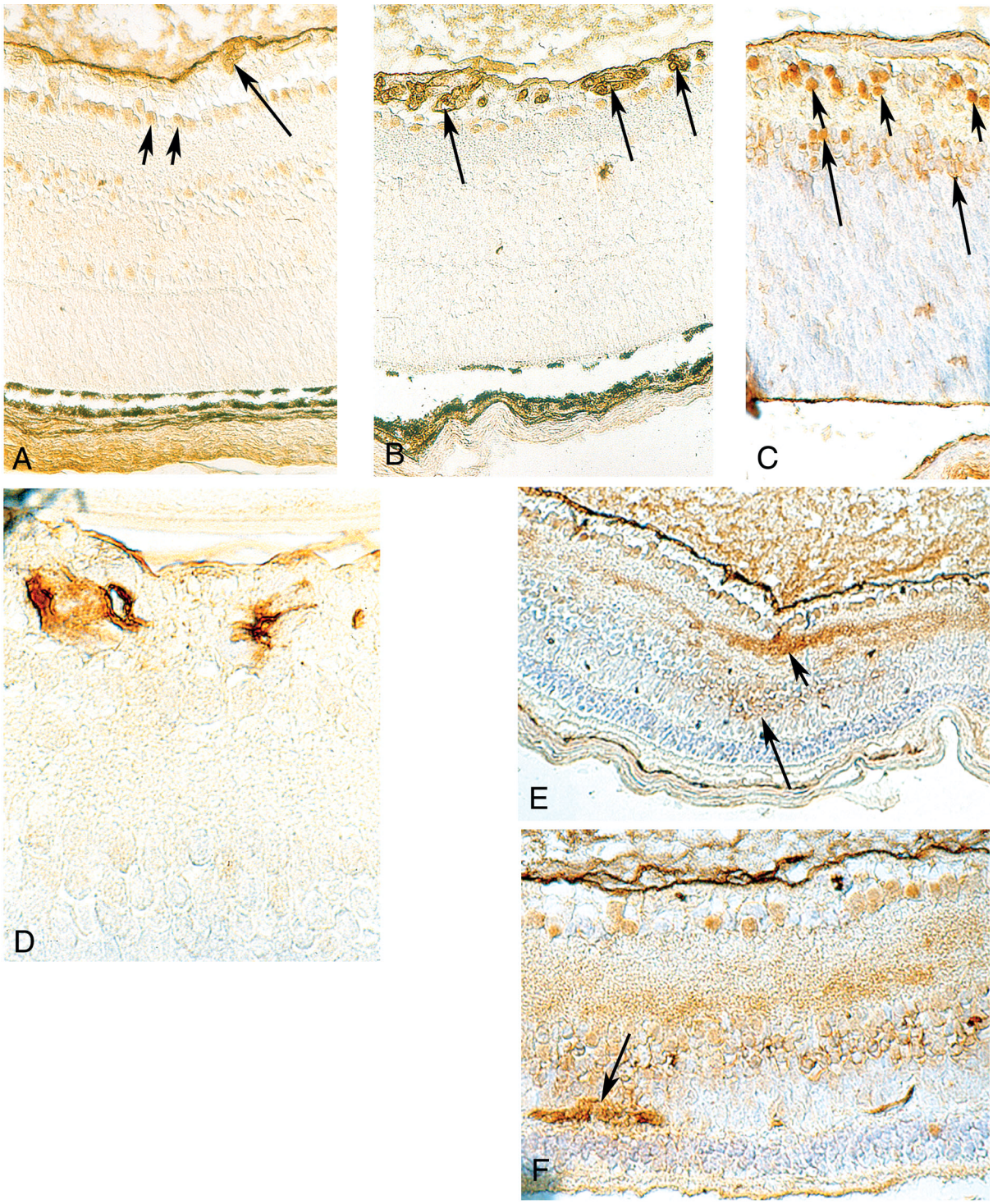

Fig. 5. Immunohistochemical staining for albumin to localize sites and determine extent of BRB breakdown in non-transgenic C57BL/6 **(A, B)** and FVB/N **(C-F)** mice. **A.** Positive staining along the retinal surface (top) and within vessels (long arrow); ganglion cells are weakly positive (short arrows) at P7. **B.** More intense staining is evident in more mature vessels in the inner retina (arrows) at P9. Ganglion cells continue to stain weakly. **C.** Similar staining along the retinal surface in FVB/N mouse (top) at P3 with positivity of ganglion cells (short arrows) and some cells in the inner nuclear layer (long arrows). **D.** Focal leakage of albumin through inner retinal vessels at P9. **E.** Focal vascular leakage in the inner retina (short arrow) with penetration of albumin into the deeper layers of the retina, as far as the outer plexiform layer (long arrow) at P15. **F.** Widespread extravasation of albumin through the inner retina (top) with some vascular leakage appearing to originate from the deep capillary bed in the outer plexiform layer (arrow) at P15. A-C, F, x 400; D, x 1000; E, x 200

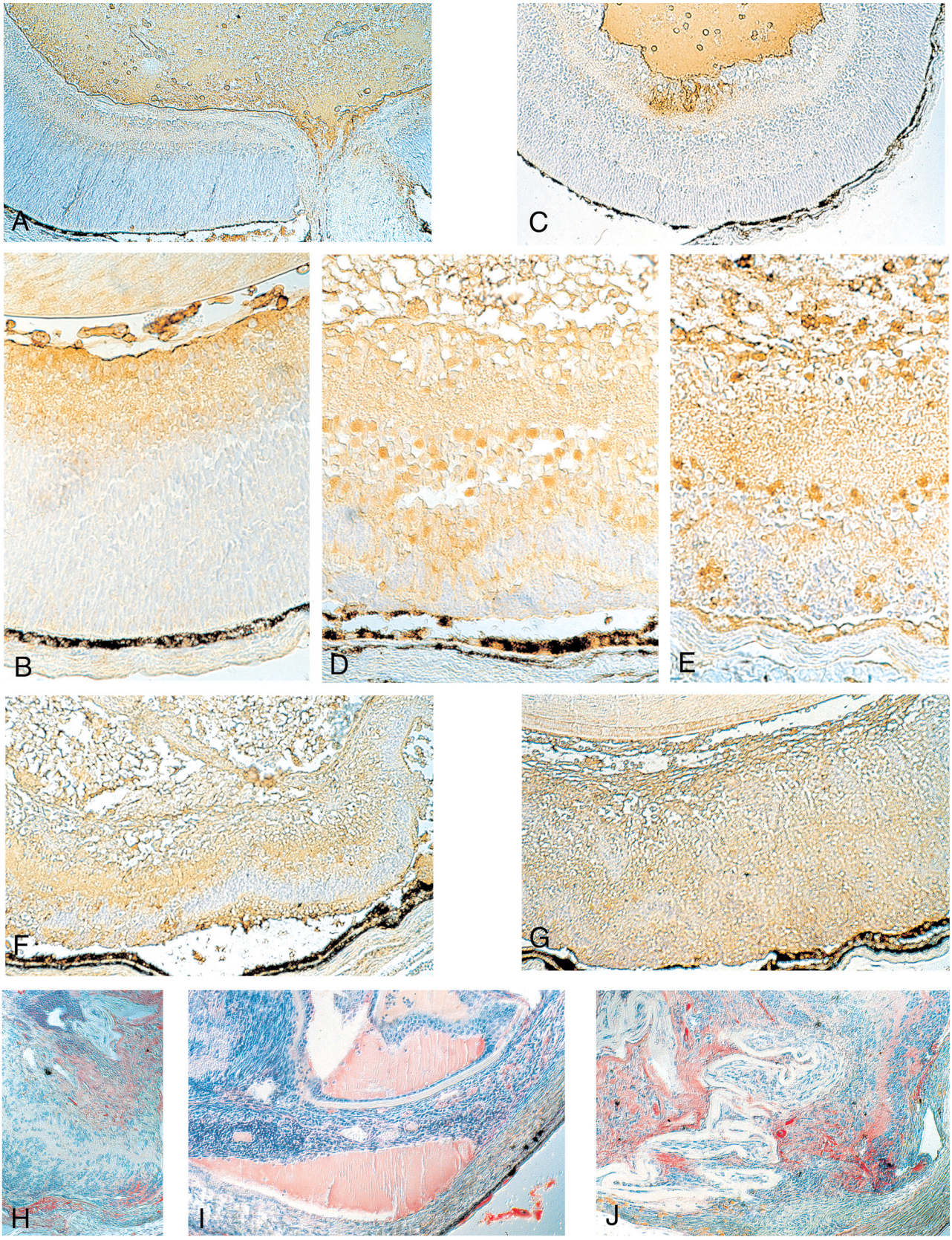

Fig. 6. Immunohistochemical localization of albumin to localize sites and determine extent of BRB breakdown in IL-1ß transgenic mice. **A.** Albumin positivity is found throughout the vitreous (top) and weakly in the innermost layers of the retina at P3. **B.** Positive staining is evident in the perilenticular vessels (top) and in the innermost layers of the retina at P3. **C.** A focal breech of the BRB in the inner retina at P7. **D.** Widespread BRB breakdown at P9. **E.** More intense staining for extravasated albumin throughout all layers of the retina at P12. Widespread BRB breakdown at P15 **(F)** and P19 **(G)**. **H.** Extravascular albumin (red reaction product) in the remnants of the inner (top) and outer (bottom) retina in the adult. **I.** Positive staining (red) in retinal exudate and throughout the retinal remnants in the adult. **J.** Widespread positivity (red) in the residual retina of the adult. A, C, F, I, x 100; B, D, E, x 400; G, H, J, x 200

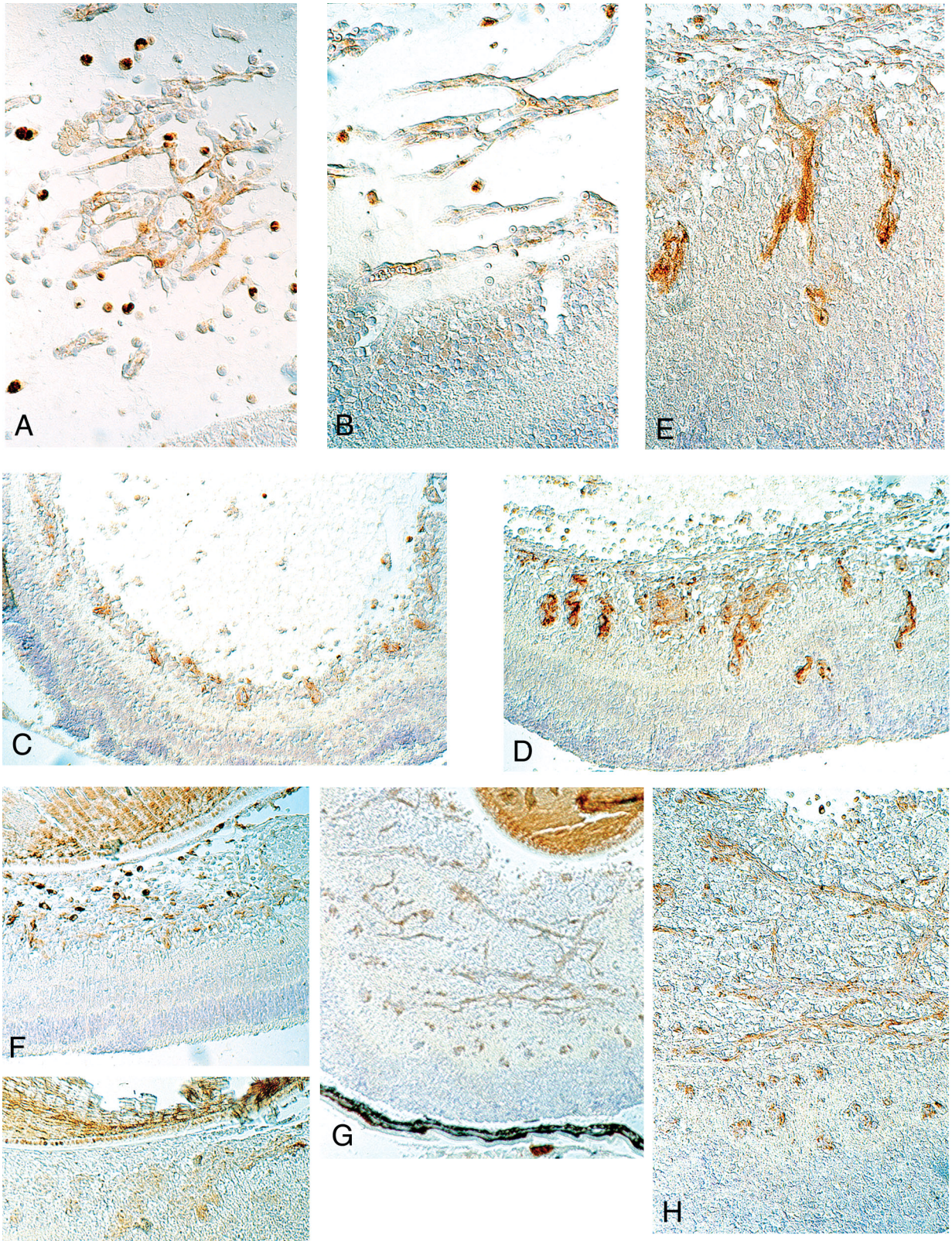

Fig. 7. Histochemical staining with GSA to label vascular endothelial cells in IL-1b transgenic mice. **A.** Labeling of vessels within the vitreous at P4. **B.** Positive staining of vessels within the vitreous and on the surface of the retina at P5. **C.** GSA-positive cell aggregates on the surface of the retina at P12. **D.** Clusters of GSA-positive cells migrated into the retina at P15. **E.** Invasion of the retina by cords of GSA-positive cells at P15. **F.** By P19, the GSA-positive cell clusters had dispersed into smaller aggregates. **G.** The more superficial GSA-positive cells formed a vascular network (top) while the cells that had migrated deeper into the retina remained as small cell clusters at P19. **H.** Higher magnification of the superficial vascular network and deeper cell clusters at P19. **I.** Further dispersion of the GSA-positive cell clusters at P24. A, B, E, H, x 400; C, x 100; D, F, G, I, x 200

al., 1990, 1992; Kulkarni et al., 1993; Cuff et al., 1996; Bamforth et al., 1997) and VEGF production and secretion is associated with inflammatory cells (Webb et al., 1998; Edelman et al., 1999; Scapini et al., 1999; Thickett et al., 1999; Coste et al., 2000; Kasama et al., 2000; Philipp et al., 2000; van der Flier et al., 2001; Vinores et al., 2001b). The initial upregulation of VEGF occurs just after the infiltration of inflammatory cells, supporting this hypothesis. Thus, the upregulation of VEGF in the retinas of IL-1ß transgenic mice could be dependent on the influx of inflammatory cells. The reduced staining for VEGF in the retinas of adult ICAM-1 knockouts expressing the IL-1ß transgene further supports this hypothesis, since ICAM-1 facilitates the influx of inflammatory cells and in its absence, inflammatory and immune responses are impaired (Sligh et al., 1993).

VEGF is known to promote NV and cause a breakdown of the BRB (Aiello et al., 1995; Tolentino et al., 1996; Robinson et al., 1996; Okamoto et al., 1997; Ozaki et al., 1997, 2000; Tobe et al., 1998). Both of these events occur in IL-1ß transgenic mice and could be attributable to VEGF, but the BRB breakdown occurs shortly after the initial VEGF upregulation, whereas endothelial proliferation and NV did not occur until much later. Previous experiments have shown that the maximum increase in retinal vascular permeability induced by VEGF in rabbits or mice occurs within 6 hours after exposure to VEGF (Luna et al., 1997; Derevjanik et al., 2002), whereas, the induction of NV in rabbits or mice requires a sustained release of VEGF for a week or more (Okamoto et al., 1997; Ozaki et al., 1997), indicating that the vascular permeability-promoting activity requires much less time than the angiogenic activity of VEGF. Although the timing of VEGF upregulation and BRB breakdown coincided, there is no evidence that VEGF, rather than other pro-inflammatory factors, is responsible for the BRB breakdown in IL-1ß transgenic mice. In fact, selective, but not specific inhibitors of VEGF receptor kinase, which decreased VEGF-induced breakdown of the BRB, did not block retinal vascular leakage induced by IL-1ß (Saishin et al., 2003), suggesting that IL-1ß does not cause BRB breakdown indirectly through VEGF.

Elevated VEGF levels in the retinas of FVB/N mice is a surprising result, since several previous studies have shown that vascular density is decreased in these mice (Matthes and Bok, 1984; Blanks and Johnson, 1986; Wang et al., 2000). This could be explained by the simultaneous upregulation of an angiogenesis inhibitor. TGFß has been shown to suppress vascular endothelial cell proliferation (Jennings et al., 1988; McAvoy and Chamberlain, 1990; Chakravarthy and Archer, 1992; Pertovaara et al., 1994; Behzadian et al., 1995; Kulkarni et al., 1995; Yoshimura et al., 1995; Zhao and Overbeek, 2001) and its simultaneous upregulation with VEGF in experimental autoimmune uveoretinitis appears likely to suppress the angiogenic activity of VEGF (Vinores et al., 1998). A preliminary evaluation showed an upregulation of TGFß in FVB/N mice, but not until P19, which is well after the onset of VEGF induction, suggesting that TGFß is not the anti-angiogenic agent that operates in this model. Another possible candidate for inhibiting VEGF-mediated NV is SPARC (secreted protein, acidic and rich in cysteine), which modulates the mitogenic activity of VEGF through a direct binding interaction and reduces the association of VEGF with its cell surface receptors in addition to reducing VEGF-mediated tyrosine phosphorylation of mitogen-activated protein kinases (Kupprion et al., 1998); however, this remains to be investigated.

In summary, there is an upregulation of VEGF in the retinas of IL-1ß transgenic mice that occurs after the initial onset of inflammatory cell infiltration (P5-7), suggesting that the inflammatory cells recruited by IL-1ß may indirectly mediate this induction. In inbred FVB/N mice, with or without the IL-1ß transgene, severe retinal degeneration had occurred by P15 and was accompanied by a second peak of VEGF upregulation. Heterozygous IL-1ß transgenic mice, in the FVB/NxC57BL/6 background, underwent retinal destruction due to massive inflammation at the same time point and also demonstrated concurrent VEGF upregulation. These results suggest the second peak of VEGF upregulation may be associated with the destruction/degeneration of photoreceptors. NV and breakdown of the BRB are major clinical problems and these data contribute to a better understanding of their pathogenesis and provide a model to test therapeutic intervention.

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