Int. J. Dev. Biol. 47: 59-64 (2003)

Original Article

Disruption of eyelid and cornea development by targeted overexpression of the glucocorticoid receptor

JOSÉ LUIS CASCALLANA[1], ANA BRAVO[1], ANGUSTIAS PAGE[2], IRINA BUDUNOVA[3], THOMAS J. SLAGA[3], JOSÉ L. JORCANO[2] and PALOMA PÉREZ*[,4]

[1]*Department of Animal Pathology. Faculty of Veterinary Science, University of Santiago de Compostela, Lugo, Spain,* [2]*Project on Cell and Molecular Biology and Gene Therapy, CIEMAT. Madrid Spain,* [3]*AMC Cancer Research Center, Denver CO, USA and* [4]*Instituto de Biomedicina de Valencia (CSIC) Valencia, Spain*

ABSTRACT Glucocorticoid hormones act through the glucocorticoid receptor (GR) and they affect almost all physiological systems in the organism. We have previously reported that transgenic mice overexpressing GR under the control of the keratin *k5* promoter (K5-GR mice) display severe phenotypic alterations in the epidermis and other ectoderm derivatives (Pérez *et al.,* 2001). In this work, we aimed to characterize the pathological consequences of GR targeted overexpression in the eyelid and cornea at late developmental stages. Despite glucocorticoids being widely prescribed as a topical treatment in ophthalmology, their potential role during ocular development in the embryo is not well understood. As shown by scanning electron microscopy analysis as well as by our histopathological and immunohistochemical data, long-term and newborn transgenic embryos showed unfused eyelids, along with proptosis of the globe and exposure of the anterior surface. In addition, epithelial defects were evident at the cornea. Our results indicate that GR overexpression affected the proliferation rate of targeted epithelia of the cornea and eyelid, thus demonstrating that GR was responsible for the arrest of epithelial proliferation of the developing eyelid edges, as well as for their destruction. We conclude that constitutive targeted overexpression of GR in the eyelid and corneal epithelium dramatically impairs ocular function in these transgenic mice.

KEY WORDS: *glucocorticoid receptor, transgenic mouse, epithelial cell, eye development*

Introduction

The biological effects of glucocorticoids (GCs) are mediated via the glucocorticoid receptor (GR), which acts as a ligand-dependent transcription factor by regulating gene expression (Weatherman *et al.,* 1999). We have recently generated a transgenic mouse model in which the overexpression of GR is driven by 5.2 kb of the keratin *k5* promoter (K5-GR mice) (Pérez *et al.,* 2001). K5-GR transgenic embryos exhibited multiple defects in skin and other stratified epithelia, collectively resembling the clinical findings in the human syndrome Ectodermal Dysplasia (ED). Among these phenotypic alterations, K5-GR transgenic mice exhibited open eyelids at birth unlike the control littermates (Pérez *et al.,* 2001). In this work, we have studied in detail the phenotypic defects found in the eye of K5-GR mice, since ocular anomalies are often associated with many EDs (reviewed in Freire-Maria and Pinheiro, 1984). Eyelid development, closure and subsequent reopening are a feature common to all mammals. The process of eyelid closure in the mouse embryo is a consequence of the active proliferation of the epithelium of each eyelid streaming across the corneal surface of the eye towards each other (Findlater *et al.,* 1993). Once epithelial fusion has taken place, the dermis continues to develop causing it to extend in towards the junctional zone. This process takes place with a degree of variation within a single litter, but eyelids are invariably fused at 16.5 d.p.c. to enclose the conjunctiva sac, start to separate at 3-5 days after birth and, finally, reopen at about 12 days after birth (Findlater *et al.,* 1993; Kaufman and Bard, 1999). In newborn K5-GR mice, we found a disruption of the eye development characterized by the failure of eyelid fusion during embryogenesis and anterior segment dysgenesis. The reported ocular defects were consistently found in transgenic lines 72 and 285. Transgenic mice of line 72 died perinatally, but mice of line 285

Abbreviations used in this paper: d.p.c., days post-conception; GC, glucocorticoid hormone; GR, glucocorticoid receptor; K5, keratin 5; NF-κB, nuclear factor kappa B; SEM, scanning electron microscopy.

***Address correspondence to:** Dr. Paloma Pérez. Instituto de Biomedicina de Valencia (CSIC). Jaime Roig 11, E-46010 Valencia, Spain. Fax: +34-96-369-0800. e-mail: paloma.perez@ibv.csic.es

0214-6282/2003/$25.00

Printed in Spain
www.ijdb.ehu.es

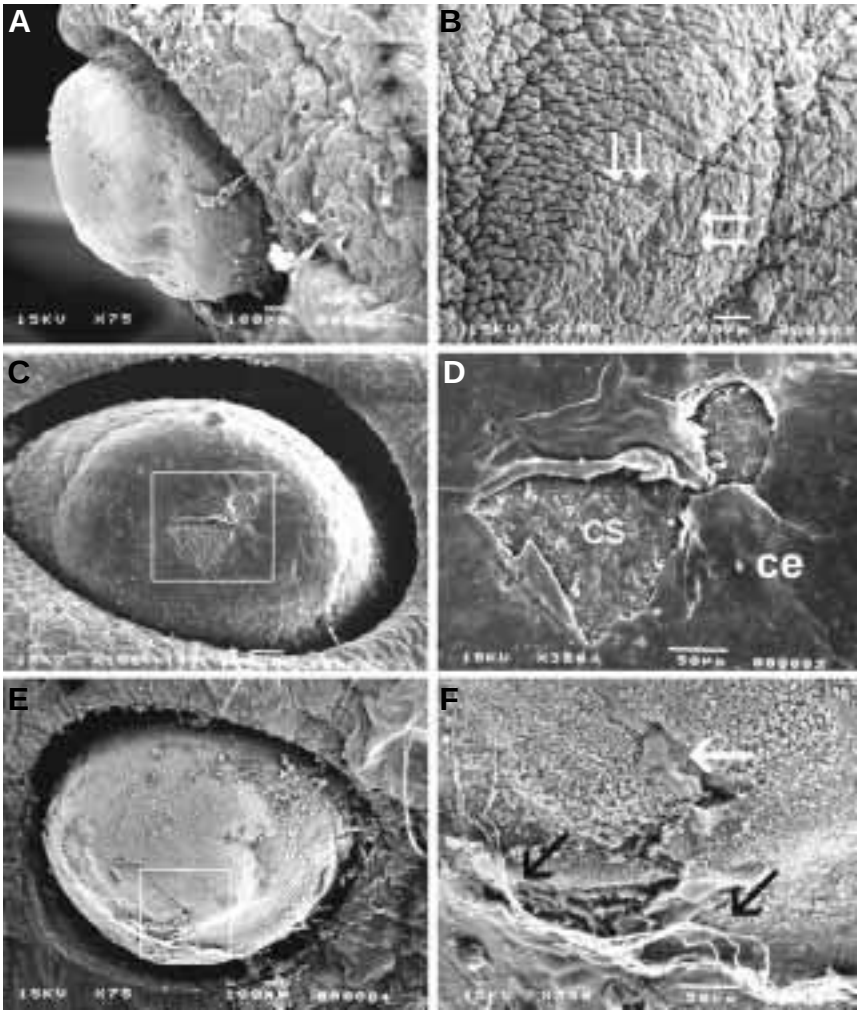

Fig. 1. Scanning Electron Microscopical (SEM) analysis of the K5-GR transgenic eye in long-term embryos. (A) *17.5 d.p.c. transgenic embryo eye at 75X magnification showing no closure of both eyelids and proptosis of the globe.* **(B)** *17.5 d.p.c. control eye at 100X magnification showing complete closure of both eyelids (double arrows). (C-F) Eyes from 18.5 d.p.c. transgenic embryos.* **(C)** *Central ulcer in the cornea at 100X magnification.* **(D)** *Higher magnification (350X) of the epithelial defect shown in C; note that the corneal stroma (cs) is exposed through the disrupted corneal epithelium (ce).* **(E)** *Epithelial desquamation affects almost the complete surface of the cornea (75X).* **(F)** *Higher magnification (350X) of the region boxed in E to show the lower edge of the extensive ulcer; note that conjunctive stromal fibers are exposed (black arrows) and a remaining epithelial shred could be seen (white arrow).*

survived and exhibited variable degrees of microphthalmia associated with corneal opacity and extensive calcium deposits at the corneal stroma (data not shown). The study of K5-GR mice strongly supports a crucial role for GR in the development and differentiation of the eyelids and cornea.

Results

K5-GR transgenic mice showed a lack of eyelid closure that was unequivocally detected at 16.5 d.p.c. embryos, that exhibited defects in one or both eyelids (Pérez *et al.,* 2001). Since the epithelial cells of the developing eyelids as well as those in the cornea normally express keratin 5 and therefore are targeted for transgene expression (Ramírez *et al.,* 1994), we aimed to study in detail the ocular defects found in K5-GR mice throughout development. By using scanning electron microscopy (SEM), we showed that eyelid fusion has already taken place in 17.5 d.p.c. wild type embryos (Fig. 1B, arrows point at the fused eyelids). In sharp contrast, 17.5 d.p.c. transgenic embryos showed transgenic embryos showed unfused eyelids along with proptosis of the globe and exposure of the anterior surface (Fig. 1A). At 18.5 d.p.c., SEM studies evidenced the presence of epithelial defects either in the central cornea (Fig. 1C, see D for higher magnification) or in practically the whole surface of the cornea (Fig. 1E, see F for higher magnification).

We next performed histological examination in embryo head sections from transgenic and control mice at different stages during ontogenesis (Figs. 2,3). We detected the earliest signs of histological differences between transgenic and non-transgenic eyes at 15.5 d.p.c embryos (Fig. 2). In transgenic mice, the epithelia in the central cornea and eyelids edges appeared thinner and vacuolated as compared with control mice. From 16.5 d.p.c. embryos to newborn mice, eyelids maintained fused to enclose the conjunctiva sac in wild type mice (Fig. 3A.1, 17.5 d.p.c. control mouse is shown as a representative, intermediate state in wild type eye development). However, in transgenic littermates we noticed a failure in eyelid fusion at 16.5 d.p.c. and throughout development (Fig. 3 B1-E1 and F). In 16.5 d.p.c. transgenic mice, we first noticed vacuolization associated with atrophic changes in the internal part of the eyelid epithelia (Fig. 3, compare B2 and A2). We also observed severe atrophic changes in the central region of the cornea, characterized by vacuolar appearance and flattening of the epithelium (Fig. 3, compare B4 and A4). In 17.5 d.p.c. transgenic mice, besides the arrested eyelid development, the atrophic changes in the cornea extended to its whole surface and its contact with the normally arranged lens caused a noticeable obliteration of the anterior chamber (Fig 3, notice that the atrophic cornea in C1 is half the width of that in the wild type littermate in A1). At this embryo stage, we detected the earliest necrotic changes in the epithelium of the central cornea and eyelid edges of transgenic mice (Fig.3C4 and 3C2, respectively). Eyelid edges showed the histological appearance of coagulation necrosis at the internal part of the epithelium, which appeared thinner, eosinophilic and with loss of cellular details (Fig 3C2, arrows). The central cornea displayed pathological development of blood vessels (Fig. 3C4, arrow) and severe stromal atrophy associated with coagulation necrosis of the overlying epithelium (Fig. 3C4, arrowhead; compare with A4 in the wild type). Remarkably, necrotic changes affecting eyelid edges and central cornea in the transgenic mice became more dramatic as development proceeded. In long-term embryos and newborn transgenic mice, necrotic changes affected not only the epithelium but also the conjunctive layer in the eyelid edges (Fig. 3D2, 18.5 d.p.c. embryo) or the whole tarsal plate (Fig. 3E2, newborn mouse). The surrounding necrotic areas were

usually infiltrated by macrophages (Fig. 3E2, arrows). In 18.5 d.p.c. embryos, the central cornea lacked epithelial covering by a shedding process of the necrotic cells (Fig. 3D4 and data not shown). In newborn mice, the corneal stroma displayed severe hyalinization (Fig. 3E4, arrow). In long-term embryos (Fig 3D1, arrows) and newborn K5-GR mice (Fig. 3E1, arrows, and F arrowheads), the necrotic process affected not only the cornea but also the anterior part of the lens. Since coagulation necrosis attracts macrophages that release enzymes into the tissue to digest the necrotic cellular debris (Slauson and Cooper, 2002), the observed anterior necrosis of the lens displayed by a subset of K5-GR transgenic mice might be a consequence of macrophage lytic effect. In such cases, the necrotic process progressed to corneal perforation with destruction of the lens (Fig. 3F, arrowheads) and detachment of a major portion of the sensory retina (Fig. 3F, arrows). All the ocular epithelia derived from surface ectoderm were destroyed in these transgenic mice, except the conjunctiva forniceal epithelium (Fig. 3F, asterisks).

Penetrance of the reported ocular phenotype in long-term embryos and newborn transgenic mice (n=22) was as follows: 27.27% presented upper eyelid defects and necrotic changes in the central cornea, 50% exhibited defects in both eyelids and necrotic changes affecting the cornea and the anterior part of the lens and 22.73% of transgenic mice displayed the most severe phenotype (both eyelids defects, necrosis of the cornea and lens with corneal perforation and detachment of the sensory retina).

We have previously demonstrated that the 5.2 kb sequences of the *keratin* 5 promoter used for driving transgene expression are efficiently targeting the gene of interest to the same epithelial compartments as endogenous *keratin* 5 (Ramírez *et al.,* 1994). Keratin 5 is a marker for the basal proliferating layer of the epithelium in the eyelids and the cornea. Therefore, in K5-GR transgenic mice, the expression pattern of K5 and the GR transgene should overlap. To prove this point, we did immunostaining of transgenic and non-transgenic mouse eye sections by using anti-keratin K5 and anti-GR antibodies at different developmental time points. At 15.5 d.p.c., K5 was detected in the corneal (Fig 2C) and eyelid (Fig 2G) epithelia of non-transgenic mouse eye sections. In 15.5 d.p.c. transgenic littermates, the eyelid developing edges still maintained continuous keratin 5 expression (Fig 2P), however, a slight discontinuous pattern was already noticeable in the epithelium of the central cornea corresponding to the cytoplasm vacuolated areas (Fig 2L, arrowheads). At this embryo stage, GR localization in the epithelium of the cornea and eyelids was both cytoplasmic and nuclear in the controls (Fig 2D and 2H respectively) whereas it was predominantly nuclear in transgenic littermates (Fig 2M, cornea and 2Q, eyelid). We have previously shown that overexpressed GR localized to the nuclei of basal cells of the epidermis, another stratified epithelium (Pérez *et al.,* 2001). Since GR exerts an anti-proliferative role in some stratified epithelia, such as epidermis, we evaluated whether GR overexpression affected the proliferation rate of targeted epithelia of the cornea and eyelid. To this, we performed immunostaining in eye sections of 15.5 d.p.c. embryos using an antibody against Ki67 nuclear antigen, which is expressed in all proliferating cells (Fig. 2). Determination of the proliferating rate in non-transgenic mice (n=3) showed 42.06% at the eyelid edge epithelium and 42.17% in the cornea (Fig. 2I and 2E, respectively). Importantly, these proliferating rates were clearly decreased in the transgenic littermates (n=3) down

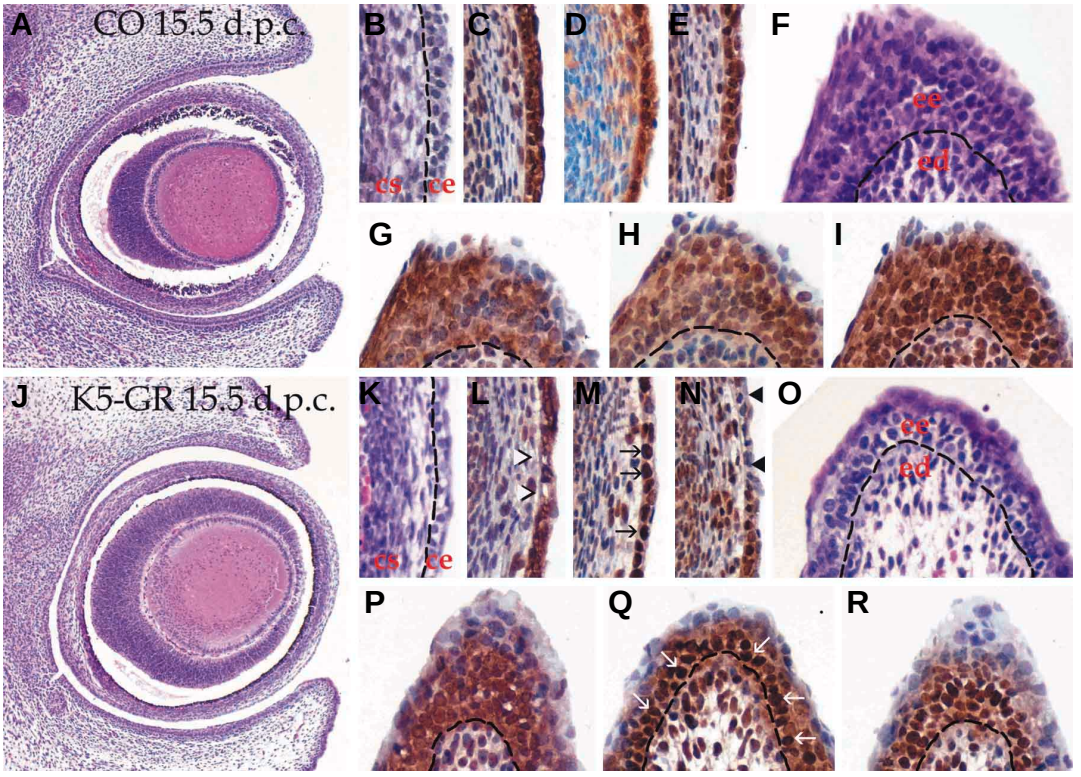

Fig. 2. Earlier stage of abnormal development of the eyelids and the cornea in K5-GR transgenic embryos. **(A-I)** *15.5 d.p.c. control eye sections.* **(J-R)** *15.5 d.p.c. transgenic littermate eye sections. Hematoxylin/eosin staining of the whole eye (A,J), cornea (B,K) or eyelids (F,O). (B,F,K,O) Dotted lines denote basement membrane. Immunostaining of the cornea (C-E, L-N) or the eyelid (G-I, P-R) using an anti-keratin K5 antibody (C,G,L,P), an anti-GR antibody (D,H,M,Q) or an anti-Ki67 antibody (E,I,N,R). Note the slight discontinuous K5 pattern in the epithelium of the central cornea corresponding to vacuolated cells (L, arrowheads, compare with C). GR localization in the epithelium of the cornea and eyelids is both cytoplasmic and nuclear in controls (D,H) but predominantly nuclear in transgenic mice (M,Q, arrows). Ki-67 expression is noticeably decreased in the central cornea (N, arrowheads) and eyelid edge (R) of transgenic compared to control mice (E,I). Abbreviations: ce, corneal epithelium; cs, corneal stroma; ee, eyelid edge epithelium; ed, eyelid edge dermis. Magnification: (A,J), 40X; (B-I, K-R), 200X.*

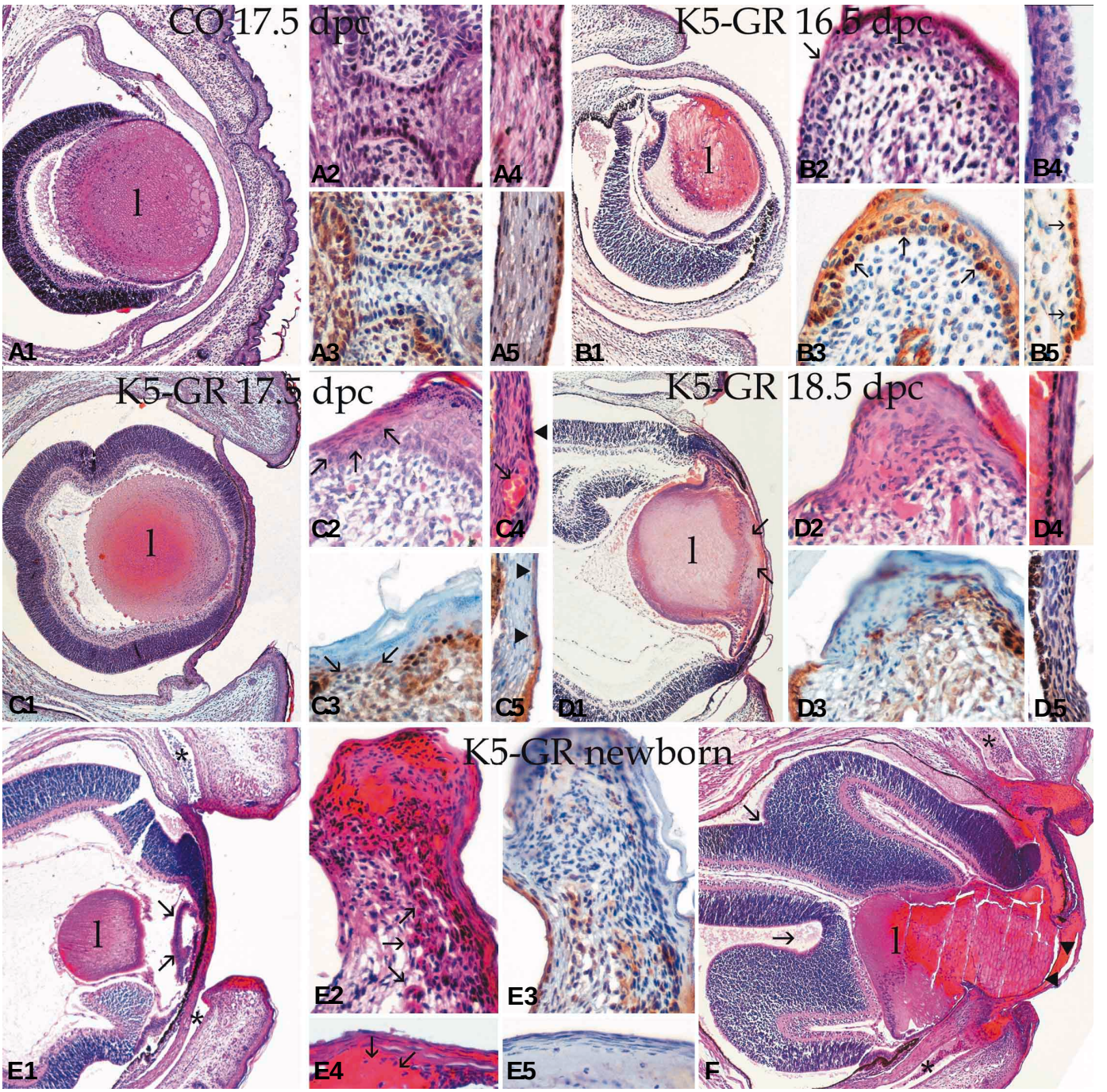

Fig. 3. Severe ocular anomalies in long-term embryos and newborn K5-GR transgenic mice. **(A1-A5)** *17.5 d.p.c control eye sections. Transgenic eye sections in 16.5 d.p.c.* **(B1-B5)** *17.5 d.p.c.* **(C1-C5)**, *18.5 d.p.c. embryos* **(D1-D5)** *and newborn mice* **(E1-E5, F)**. *Hematoxylin/eosin staining of the whole eye (A1, B1, C1, D1, E1, F), the eyelids edges (A2, B2, C2, D2, E2), or the cornea (A4, B4, C4, D4, E4); (l) lens. Eyelids failed to close in transgenic mice throughout development (B1, C1, D1, E1, F), in contrast to long-term control embryos (A1). Abnormalities in eye development progressed from atrophy associated to vacuolar appearance in the epithelium of the internal eyelids edges (B2, arrow) and the central cornea (B4) to coagulation necrosis of these epithelia (C2, arrows; C4, arrowhead) with pathological vascularization of the corneal stroma (C4, arrow). Necrotic areas comprised the conjunctive layer of eyelid edges in 18.5 d.p.c. transgenic embryos (D2) or the whole tarsal plate in newborn mice (E2, arrows point at macrophage infiltrates). Notice atrophy of the corneal stroma in 18.5 d.p.c. embryos (D4) and clear hyalinization in newborn transgenic mice (E4, arrows). Necrosis also affected the anterior part of the lens at different developmental points (D1, E1, arrows; F, arrowheads). Asterisks in E1 and F indicate the only unaffected epithelia of the conjunctival fornices. Arrows in F denote sensory retinal detachment. Immunostaining of the eyelids (A3-E3) and the cornea (A5-E5) using an anti-GR antibody. Note continuous nuclear localization of GR in the basal layer of the eyelids (B3, arrows) and corneal epithelium (B5, arrows) in 16.5 d.p.c. transgenic embryos. In long-term embryos and newborn transgenic mice, the necrotic areas in the eyelids (C3 arrows; D3, E3) and the cornea (C5, arrowheads; D5, E5) lack GR expression. In D5 and E5 the cornea has lost the epithelial layer; in D5, the pigmented epithelium at the left belongs to iridal tissue adhered to corneal stroma. Magnification: (A1, B1, C1, D1, E1, F), 40X; (A2-A5, B2-B5, C2-C5, D2-D5, E2-E5), 200X.*

to 26.60% in the eyelid and 26.31% in the corneal epithelium (Fig. 2R and 2N, respectively). In 16.5 d.p.c. transgenic embryos, the epithelia were atrophic, but not necrotic yet; in fact, immunostaining with an anti-GR antibody demonstrated GR transgene expression in basal cell nuclei of the eyelids (Fig. 3.3, arrows) and corneal epithelium (Fig. 3B5, arrows). In 17.5 d.p.c. transgenic embryos, the necrotic areas at the internal portion of eyelid edges and the central cornea lacked K5 and GR expression (Fig 3C3, arrows and C5, arrowheads and data not shown). This feature became progressively more pronounced in long-term embryos (Fig. 3D3 and 3D5) and newborn mice (Fig. 3E3 and 3E5). Overall, our data strongly suggest that targeted overexpression of GR in these epithelia exerts an anti-proliferative role responsible for the arrest of epithelial proliferation of the developing eyelid edges as well as for their destruction. In this context, eyelid fusion and the normal development of the cornea were precluded. Consistently with the severe ocular anomalies found in long-term embryos and newborn transgenic mice, adult mice displayed a remarkable corneal opacity and, in many cases, a completely obliterated eye (data not shown).

Discussion

It is well known that exogenous GCs retard fetal growth and that prenatal GCs alter the rate of maturation of organs such as the lung, heart, kidney, gut and epidermis (Seckl *et al.,* 1999), which suggest a relevant role of glucocorticoid hormones in early life. We previously reported that K5-GR mice exhibited major phenotypic alterations including epidermal hypoplasia, dysplastic hair follicles and skin defects associated to incomplete closure of one or both eyelids (Pérez *et al.,* 2001). Despite GCs are widely prescribed as a topical treatment in ophthalmology, their potential role during ocular development in the embryo is not well understood. In this work, we aimed to study the pathological consequences of GR targeted overexpression in the eyelid and cornea by using K5-GR mice as a model (Pérez *et al.,* 2001).

Our data indicate that GR has a crucial role for the development of anterior ocular structures, as shown by the SEM analysis, as well as by our histopathological and immunohistochemical data (Figs. 1, 2 and 3). When only one eyelid was absent, it was always the upper one (data not shown). A major point in this study is to show that targeted overexpression of GR, the nuclear hormone receptor that mediates glucocorticoid action, exerts an anti-proliferative function in epithelial cells of developing eyelids and cornea in transgenic embryos. At 15.5 d.p.c. of embryonic development, transgenic mice showed decreased proliferation in the eyelid and cornea, resulting in atrophic changes at 16.5 d.p.c. and finally necrosis in long-term embryos and newborn mice. The reported phenotype in the eyelids and corneal epithelium can be easily explained since these epithelial cells are targeted for transgene expression by means of the *k5* promoter from 13.5 d.p.c. (Ramírez *et al.,* 1994). In 16.5 d.p.c. transgenic embryos, transgene expression was evident at the nuclei of basal cells in the eyelids and cornea and correlated with a remarkable delay in the eyelid development (Fig. 3B1 to 3B5). At 17.5 d.p.c., the epithelia of the eyelid edges and cornea of transgenic embryos showed the first necrotic changes (Fig. 3C1 to 3C5) that were extensively pronounced in long-term embryos (Fig. 3D1 to 3D5) and newborn mice (Fig. 3E1 to 3E5 and 3F). Eyelid defects affected either both or only the upper one, which could be explained as a consequence of the earlier development of the lower eyelid in the mouse (Rugh, 1990). Consequently, the anti-proliferative effect of the transgene as well as the necrotic changes observed in long-term embryos and newborn mice were more extensive in the upper eyelids as compared to the lower (Fig. 3E1 and F).

Impaired development of the cornea in the transgenic mice is a consequence of an abnormal destruction of the epithelium due to transgene expression (Fig. 2 and 3). Additionally, the lack of eyelid fusion in K5-GR transgenic mice fails to provide a protected environment *in utero* and in the early post-natal period, which may interfere with corneal differentiation, as suggested by some authors (Addison and How, 1999).

Cicatrization and opacity of the cornea with vascularization of the stroma, as well as dystrophic calcification, descemetocele and adherent leukoma are recognized as late sequelae of corneal inflammatory diseases in humans, both ulcerative and nonulcerative (Haustein, 1983). Thus, varying degrees of destruction of the stroma may follow an initial destruction of the corneal epithelium. Consistently, K5-GR transgenic embryos developed necrotic changes in the corneal epithelium that appeared as central ulcers (Fig. 1C and D) or extensive desquamation of the corneal surface (Fig. 1E and F). In the surviving adult transgenic mice, we always found corneal opacity with extensive calcium deposits, leukoma adherens and descemetocele that could be considered as sequelae of the corneal ulceration that they suffered during development (data not shown).

Overall, the phenotype of K5-GR mice recapitulate the defects reported in patients with ectodermal dysplasia (ED), which often display recurrent corneal epithelial defects from birth and blindness due to severe ocular anomalies (Apple and Rabb, 1998;, Donahue *et al.,* 1999). Recently, some other transgenic mouse models of Ectodermal Dysplasia with ocular defects have been reported, having in common a defective NF-κB function (Yoshida *et al.,* 2000; Schmidt-Ulrich *et al.,* 2001; Naito *et al.,* 2002). We have previously reported that skin of K5-GR mice exhibited reduced NF-κB binding activity (Pérez *et al.,* 2001), which is in agreement with the known biological antagonism between GR and NF-κB (Mckay and Cidlowski, 1999). Our present work thus suggests that glucocorticoid hormones interference with NF-κB activity elicits a disruption in formation of the outer epithelial layers that provide protection to the mammalian eye, besides affecting other ectoderm derivatives. Since the observed phenotypic eye defects found in K5-GR mice were much pronounced than in other mouse models with defective NF-κB function (Yoshida *et al.,* 2000; Schmidt-Ulrich *et al.,* 2001; Naito *et al.,* 2002), additional mechanisms of GR, most probably acting through DNA-binding-dependent function, may be responsible for the reported ocular defects. K5-GR mice may thus be a powerful tool to understand the molecular mechanisms underlying the ocular defects associated with human syndrome ED.

Materials and Methods

Animals

Transgenic mice were generated as previously described (Pérez *et al.,* 2001). All mice were handled in accordance with the ARVO Statement for the Use of Animals in Ophtalmic and Vision Research.

Antibodies

The primary antibodies used included rabbit polyclonal antibodies to GR (sc-1004, Santa Cruz, CA) and anti-keratin K5 (Babco, Berkeley, CA), and mouse monoclonal antibody anti-Ki67 (Novocastra Lab. Ltd, UK). The

secondary biotin-conjugated anti-rabbit or anti-mouse antibodies were from Jackson ImmunoResearch Laboratories, Inc. West Grove, PA.

Histological and Immunohistochemical Analysis

For histological studies, whole embryos and heads from newborn mice were formalin-fixed. For immunohistochemistry, samples were fixed in 70% ethanol. Embryos and newborn heads were embedded in paraffin as a whole. Consecutive 3 to 4 μm-thick sections were obtained. For histopathology, sections were routinely stained with hematoxylin/eosin. Embryos were obtained by cesarean derivation at 15.5, 16.5, 17.5 and 18.5 days post-conception (d.p.c.; the morning of the day that the vaginal plug was seen was considered as day 0.5 p.c.). For immunohistochemistry, paraffin sections were blocked with 1% bovine serum albumin (BSA), then incubated with the primary antibody for at least one hour. Slides were washed three times with PBS, then incubated with conjugated secondary antibodies for one hour (biotin-conjugated anti-rabbit or anti-mouse from Jackson ImmunoResearch Laboratories, Inc. West Grove, PA). Finally, the reaction was visualized with the Avidin-Biotin-Complex (ABC) kit from DAKO (Vectastain Elite, Vector Laboratories, Inc, Burlingame, CA) using diaminobenzidine as chromogenic substrate for peroxidase. Slides were mounted and analyzed by light microscopy, and microphotographs were taken at the indicated magnification.

Scanning Electron Microscopy

Heads from control and transgenic 17,5 and 18.5 d.p.c. embryos were fixed in 2.5% glutaraldehyde in phosphate buffer. After dehydration, samples were dried by critical point drying (Balzers CPD 020), coated with gold-palladium by a Jeol JFC-1100 Ion Sputter and examined with a Jeol JSM-T220A Scanning Electron Microscope.

Acknowledgements

This work was supported by grants from the Spanish CICYT (PM98-0039), SAF2002-04368-CO2-01 and the USA National Cancer Institute (RO1-CA-79065-01).

References

ADDISON, W.H.F. and HOW, H.W. (1999). The development of the eyelids of the albino rat, until the completion of disjunction. *Am. J. Anat.* 29: 1-31.

APPLE, D.J. and RABB, M.F. (1998). Ocular pathology. 5th edition. Mosby Inc, St. Louis. Missouri, USA.

DONAHUE, J.P., SHEA, C.J. and TARAVELLA, M.J. (1999). Hidrotic ectodermal dysplasia with corneal involvement. *Journal of AAPOS* 3 (6): 372-375.

FINDLATER, G.S., McDOUGALL, R.D. and KAUFMAN, M.H. (1993). Eyelid development, fusion and subsequent reopening in the mouse. *J. Anat.* 183: 121-129.

FREIRE-MARIA, N. and PINHEIRO, M. (1984). Ectodermal dysplasias: a clinical and genetic study. New York, Alan R. Liss, pp. 5-31.

HAUSTEIN, J. (1983). On the ultrastructure of the developing and adult mouse corneal stroma. *Anat. Embryol.* 168: 291-305.

KAUFMAN, M.H. and BARD, J.B.L. (1999). The anatomical basis of mouse development. San Diego, Academic Press, Inc.

McKAY, L.I. and CIDLOWSKI, J.A. (1999). Molecular control of immune/inflammatory responses: interactions between nuclear factor-κB and steroid receptor signaling pathways. *Endocrine Reviews* 20(4): 435-459.

NAITO, A., YOSHIDA, H., NISHIOKA, E., SATOH, M., AZUMA, S., YAMAMOTO, T., NISHIKAWA, S. and JUN-ICHIRO, I. (2002). *TRAF6*-deficient mice display hypohidrotic ectodermal dysplasia. *Proc.Nat. Acad. Sci.* 99(13): 8766-8771.

PEREZ, P., PAGE, A., BRAVO, A., DEL RÍO, M., GIMÉNEZ-CONTI, I., BUDUNOVA, I., SLAGA, T.J. and JORCANO, J.L. (2001). Altered skin development and impaired proliferative and inflammatory responses in transgenic mice overexpressing the glucocorticoid receptor. *FASEB J.* (July 24) 10.1096/fj.00-0772fje.

RAMIREZ, A., BRAVO, A., JORCANO, J.L. and VIDAL, M.A. (1994). Sequences 5´ of the bovine keratin 5 gene direct tissue- and cell-type specific expression of a *lacZ* gene in the adult and during development. *Differentiation* 58: 53-64.

RUGH, R. (1990). The mouse: its reproduction and development. Oxford Science Publications.

SCHMIDT-ULLRICH, R., AEBISCHER, T., HULSKEN, J., BIRCHMEIER, W., KLEMM, U. and SCHEIDEREIT, C. (2001). Requirement of NF-κB/Rel for the development of hair follicles and other epidermal appendices. *Development* 128: 3843-3853.

SECKL, J.R., NVIRENDA, M.J., WALKER, B.R. and CHAPMAN, K.E. (1999). Glucocorticoids and fetal programming. *Biochemical Society Transactions* pp. 27-32.

SLAUSON, D.O. and COOPER, B.J. (2002). Mechanisms of Disease. A textbook of Comparative General Pathology. 3th Edition. Mosby Inc, St. Louis. Missouri, USA.

WEATHERMAN, R.V., FLETTERICK, R.J. and SCANLAN, T.S. (1999). Nuclear receptor ligands and ligand-binding domains. *Ann. Rev. Biochem.* 68: 559-582.

YOSHIDA, K., HU, Y. and KARIN, M. (2000). IκB Kinase α is essential for development of the mammalian cornea and conjunctiva. *Invest. Ophtalmol. Vis. Sci.* 41(12): 3665-3669.

Received: October 2002
Reviewed by Referees: November 2002
Modified by Authors and Accepted for Publication: December 2002