

Complex regulation of TGF β expression by retinoic acid in the vitamin A-deficient rat

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Summary

We report the results of a histochemical study, using polyclonal antipeptide antibodies to the different TGF β isoforms, which demonstrates that retinoic acid regulates the expression of TGF β in the vitamin A-deficient rat. Basal expression of TGF β 2 diminished under conditions of vitamin A deficiency. Treatment with retinoic acid caused a rapid and transient induction of TGF β 2 and TGF β 3 in the epidermis, tracheobronchial and alveolar epithelium, and intestinal mucosa. Induction of TGF β 1 expression was also observed in the

epidermis. In contrast to these epithelia, expression of the three TGF β isoforms increased in vaginal epithelium during vitamin A deficiency, and decreased following systemic administration of retinoic acid. Our results show for the first time the widespread regulation of TGF β expression by retinoic acid *in vivo*, and suggest a possible mechanism by which retinoids regulate the functions of both normal and pre-neoplastic epithelia.

Key words: TGF β , retinoic acid, vitamin A deficiency.

Introduction

Polypeptide growth factors and ligands for nuclear hormone receptors are elements of a complex cellular signalling network. The transforming growth factor β s (TGF β) and the retinoids are global regulators of the growth and differentiation of many cell types (Roberts and Sporn, 1984, 1990). Epithelial cells in particular are extremely sensitive to both of these agents. Pioneering work of Wolbach and Howe (1925, 1932) and Fell and Mellanby (1953) demonstrated that the keratinizing or secretory phenotype of an epithelium is regulated by tissue retinoid levels. Subsequent biochemical analysis has shown that the expression of specific differentiation markers of individual epithelial cell types is also regulated by retinoic acid (Fuchs and Green, 1981; Jetten *et al.* 1989). In addition, the extensive proliferation of epithelial cells in vitamin A deficiency (Wolbach and Howe, 1925), as well as the ability of exogenous retinoids to suppress neoplastic progression of premalignant epithelial cells (Sporn, 1976; Moon and Itri, 1984) indicates the importance of retinoids in the maintenance of epithelial homeostasis.

Three distinct isoforms of transforming growth factor β , TGF β 1, TGF β 2 and TGF β 3 have been identified in mammals (Roberts and Sporn, 1990). Each

is a potent growth inhibitor for most cultured epithelial cell types (Tucker *et al.* 1984; Roberts and Sporn, 1990), and can induce specific differentiation markers in several cases (Masui *et al.* 1986; Jetten *et al.* 1986; Kurokawa *et al.* 1987). Although little is known about the mechanisms that regulate their expression *in vivo*, the different isoforms are expressed in specific temporal and spatial patterns during embryogenesis (Heine *et al.* 1987; Lehnert and Akhurst, 1988; Pelton *et al.* 1989, 1990; Fitzpatrick *et al.* 1990).

We have recently shown that retinoic acid can induce the expression of TGF β 2 both in mouse keratinocytes in culture and in the intact epidermis *in vivo* (Glick *et al.* 1989). These data indicated that TGF β 2 could mediate some of the effects of retinoic acid on the epidermis, and suggested that this mechanism could reflect a more global interaction between retinoids and the TGF β family in the physiology of other cell types. In order to extend this observation *in vivo*, we have utilized the vitamin-A-deficient rat as a model system. Using antisera specific for TGF β 1, TGF β 2 and TGF β 3, we have analyzed the expression of each isoform under conditions of vitamin A deficiency and subsequent systemic administration of retinoic acid. Our data show a complex regulation of each TGF β in different epithelia and suggest that this interaction is important

not only for the regulation of the TGF β s *in vivo* but also for understanding the specific actions of retinoids on distinct epithelial tissues.

Materials and methods

Generation of vitamin A-deficient rats

Vitamin A-deficient diet, obtained from TEKLAD (TD 69523), has been described (Clamon *et al.* 1975). Pregnant Fisher rats were obtained from the Frederick Cancer Research Facility and maintained on a mixture of normal rat chow and vitamin A-deficient diet. After birth, the pups were placed solely on the deficient diet, weaned, randomized and separated by sex at 4 weeks. The control group of animals was maintained on the deficient diet and given weekly oral doses of 200 μ g retinyl acetate (Hoffmann-La Roche). Each animal in the deficient group was weighed 3 times weekly until the growth plateau was reached, after which the animals were weighed daily. Under these conditions, the animals became vitamin-A-deficient approximately 60 days after birth. Experiments were initiated after the animals had maintained a weight plateau for 5 days, at which time the average weight was 135 grams. Deficient littermates were separated into 2 groups, each receiving either an oral dose of 100 μ g of all-*trans*-retinoic acid (Hoffmann-La Roche) in 100 μ l DMSO or 100 μ l DMSO alone. Animals were killed by CO₂ narcosis at 1, 2, 4 and 8 days post-treatment. Tissues were removed and fixed in neutral buffered formalin, embedded in paraffin and sections (5 μ m) made on gelatin-coated slides.

Immunohistochemistry

Polyclonal TGF β antibodies were raised in rabbits using synthetic peptides as immunogens. Two antibodies to TGF β 1 were used with similar results: anti-pre(266–278–1) was raised to a peptide corresponding to amino acids of 266–278 of human pro-TGF β 1 as described (Wakefield *et al.* 1987), and anti-LC(1–30–1) was raised to amino acids 1–30 of the mature TGF β 1 protein (Heine *et al.* 1987). Results shown are with antibody pre(266–278–1). Anti-LC(50–75–2) was raised to amino acids 50–75 of mature human TGF β 2 as described (Flanders *et al.* 1990). The anti-TGF β 3 antibody was raised to amino acids 50–60 of mature human TGF β 3 (K. C. Flanders personal communication). All antibodies were affinity-purified through columns of the immunizing peptide coupled to Affi-gel 10 (anti-TGF β 2 and anti-TGF β 3) or Sepharose (anti-TGF β 1). The anti-TGF β 1 and TGF β 3 antibodies do not cross-react with heterologous isoforms. The anti-TGF β 2 antibody does not cross-react with either TGF β 1 or TGF β 3 in an ELISA, but cross-reacts slightly with TGF β 3 in a western blot. However, in several tissues where only TGF β 3 is detected, there is no staining with the anti-TGF β 2 antibody, strongly suggesting that there is no significant cross-reactivity under the conditions used for immunohistochemistry (A. Glick, B. McCune, unpublished observations). TGF β s 1, 2 and 3 were localized in sections by incubation with antibodies at IgG concentrations of 8–10 μ g ml⁻¹. Specificity of immunohistochemical staining was shown both by replacing the primary antibody with normal rabbit serum, and by preincubation of each of the primary antibodies with a 50-fold molar excess of the respective immunizing peptide prior to incubation with the sections. Bound primary antibodies were localized using biotinylated goat anti-rabbit IgG and ABC reagents from Vector Labs. The peroxidase stain was developed with 3,3'-diaminobenzidine, and sections were counterstained with hematoxylin. For each tissue, sections

from the deficient animals, normal control and retinoic acid treated animals were processed simultaneously.

Results

Retinoid-deficient female rats were generated by maintaining newborn animals on a vitamin A-deficient diet as described in detail in Materials and methods. As 'normal controls', littermates of the deficient animals were fed the deficient diet and supplemented with weekly intragastric doses of retinyl acetate. A subset of the deficient animals was given an oral dose of either 100 μ g all-*trans*-retinoic acid in 100 μ l DMSO, or DMSO alone. At specific times post-treatment, animals were killed and tissues fixed and processed for immunohistochemistry. A total of 20 rats was used in two independent sets of experiments.

Epidermis

To confirm previous results (Glick *et al.* 1989) and ascertain that systemic rather than topical application of retinoic acid could modulate TGF β expression, we first examined the epidermis. Fig. 1A–C show that there was little staining of the epidermis from the adult normal control animals with any of the anti-TGF β antibodies. Similar results were obtained in the vitamin-A-deficient animals. However, within 24 h after oral administration of retinoic acid to the deficient animals, there was a marked increase in staining of the epidermis by the anti-TGF β 1, TGF β 2 and TGF β 3 antibodies (Fig. 1D–F). Preincubation of each antibody with its respective immunogenic peptide blocked this staining (Fig. 1G–H), as did replacement of the primary antibody with normal rabbit serum (not shown). The increased staining after treatment was largely confined to the epidermis with little or no change in the dermis. Interestingly in these experiments, in contrast to results obtained with topical application of retinoic acid (Glick *et al.* 1989), there was little hyperproliferation of the epidermis, suggesting that this is not obligatory for the induction of TGF β expression. The increase in TGF β 1 staining in these experiments compared to topical administration of retinoic acid in mice (Glick *et al.* 1989) may reflect a species-specific response, or differences in route of delivery.

Intestinal mucosa

In the small intestine of the normal control animals, there was significant reactivity with the anti-TGF β 2 antibody, localized to cells of the lamina propria and epithelial cells lining the villi (Fig. 2A) and to the nuclei of the crypt cells (Fig. 2B). TGF β 1 and TGF β 3 were not detected in the normal small intestine (not shown). Interestingly, the intensity of TGF β 2 staining increased from the base of the villi towards the tip. In the colonic mucosa, there was slight staining of the surface epithelial cells only by the anti-TGF β 2 antibody; no staining of the crypts or lamina propria was seen (Fig. 3A).

There was little change in the morphology of the gut epithelium in vitamin A-deficient animals. However,

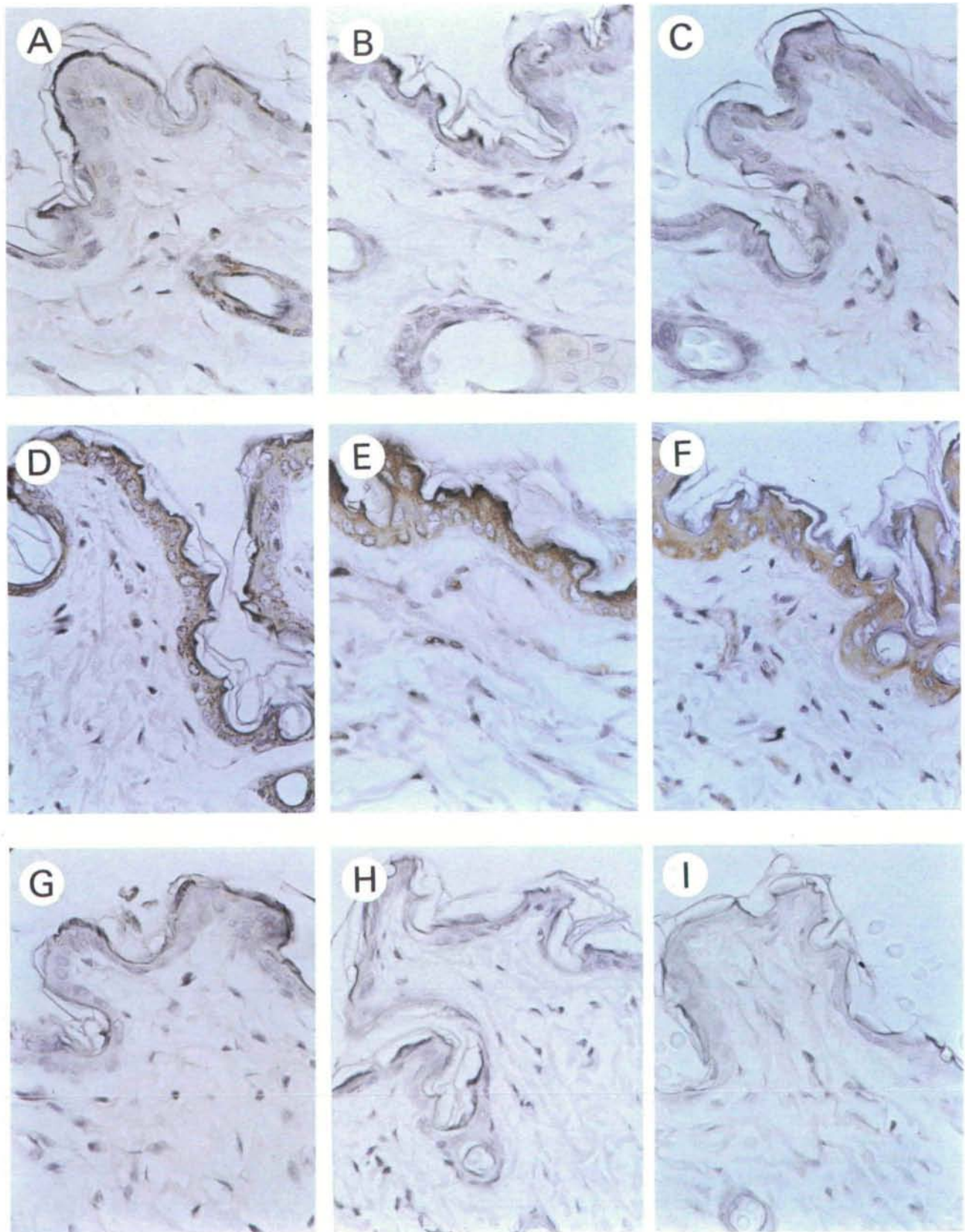


Fig. 1. Regulation of TGF β expression by retinoic acid in the epidermis. Sections were stained with anti-TGF β antibodies as described in Materials and methods. (A–C) Normal control animal; (A) anti-TGF β 1; (B) anti-TGF β 2; (C) anti-TGF β 3. (D–F) 24 h after treatment of vitamin A-deficient animal with 100 μ g all-*trans* retinoic acid; (D) anti-TGF β 1; (E) anti-TGF β 2; (F) anti-TGF β 3. (G–I) 24 h after treatment with retinoic acid, antibodies preincubated with respective immunogenic peptides; (G) anti-TGF β 1 with peptide; (H) anti-TGF β 2 with peptide 50–75–2; (I) anti-TGF β 3 with peptide 50–60–3. Magnification of all sections \times 300.

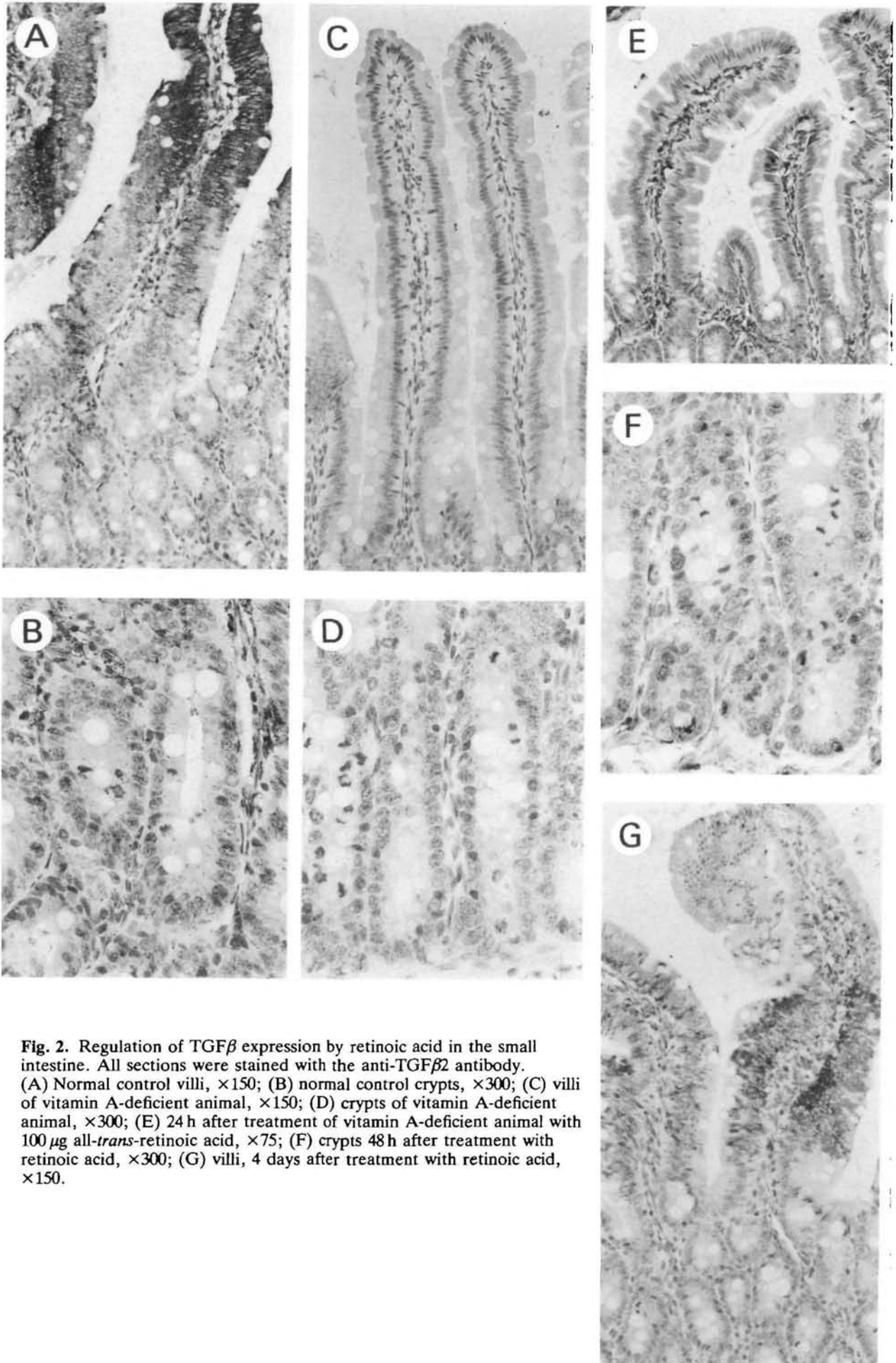


Fig. 2. Regulation of TGF β expression by retinoic acid in the small intestine. All sections were stained with the anti-TGF β 2 antibody. (A) Normal control villi, $\times 150$; (B) normal control crypts, $\times 300$; (C) villi of vitamin A-deficient animal, $\times 150$; (D) crypts of vitamin A-deficient animal, $\times 300$; (E) 24 h after treatment of vitamin A-deficient animal with $100 \mu\text{g}$ all-*trans*-retinoic acid, $\times 75$; (F) crypts 48 h after treatment with retinoic acid, $\times 300$; (G) villi, 4 days after treatment with retinoic acid, $\times 150$.

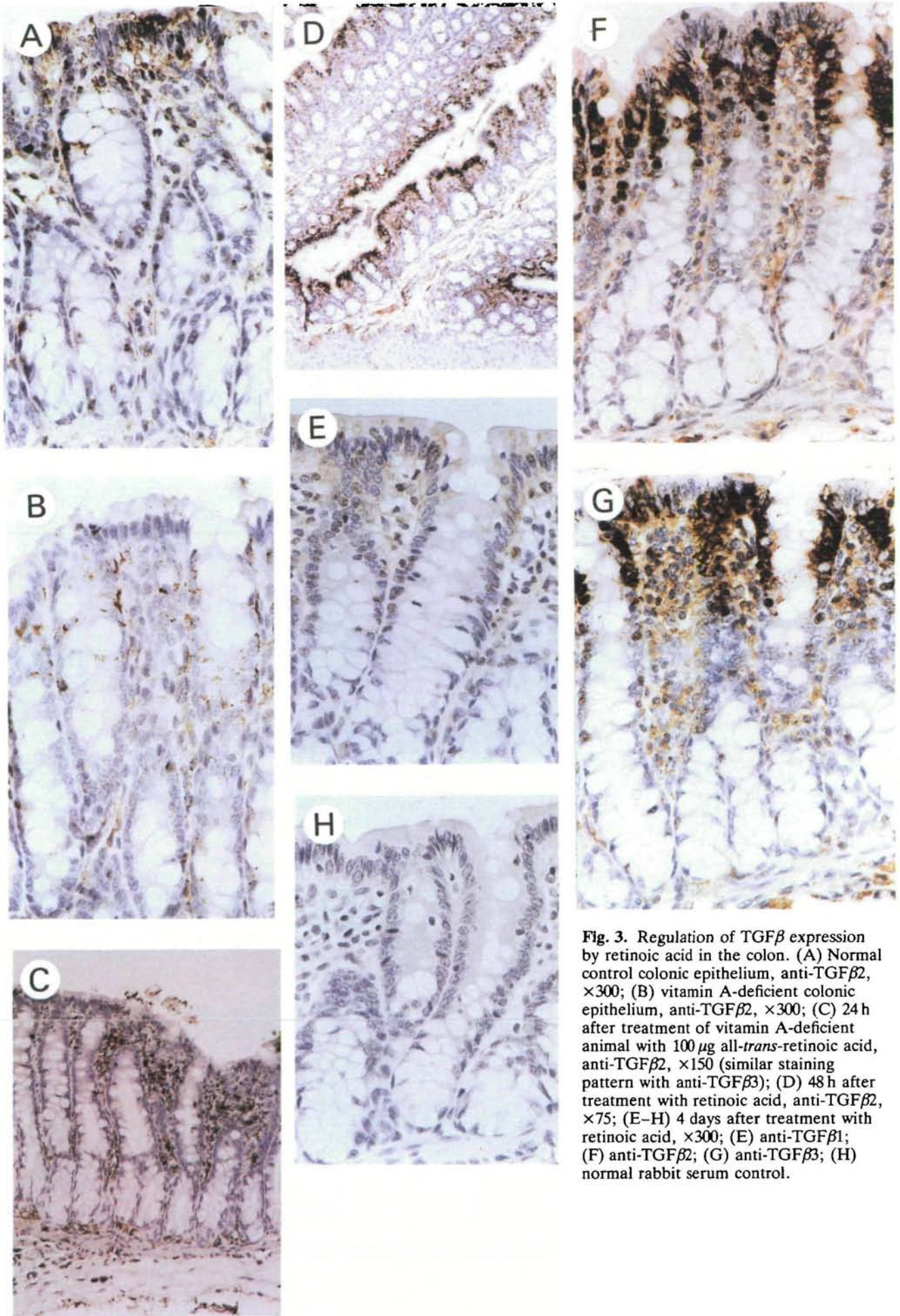


Fig. 3. Regulation of TGF β expression by retinoic acid in the colon. (A) Normal control colonic epithelium, anti-TGF β 2, $\times 300$; (B) vitamin A-deficient colonic epithelium, anti-TGF β 2, $\times 300$; (C) 24 h after treatment of vitamin A-deficient animal with 100 μ g all-*trans*-retinoic acid, anti-TGF β 2, $\times 150$ (similar staining pattern with anti-TGF β 3); (D) 48 h after treatment with retinoic acid, anti-TGF β 2, $\times 75$; (E-H) 4 days after treatment with retinoic acid, $\times 300$; (E) anti-TGF β 1; (F) anti-TGF β 2; (G) anti-TGF β 3; (H) normal rabbit serum control.

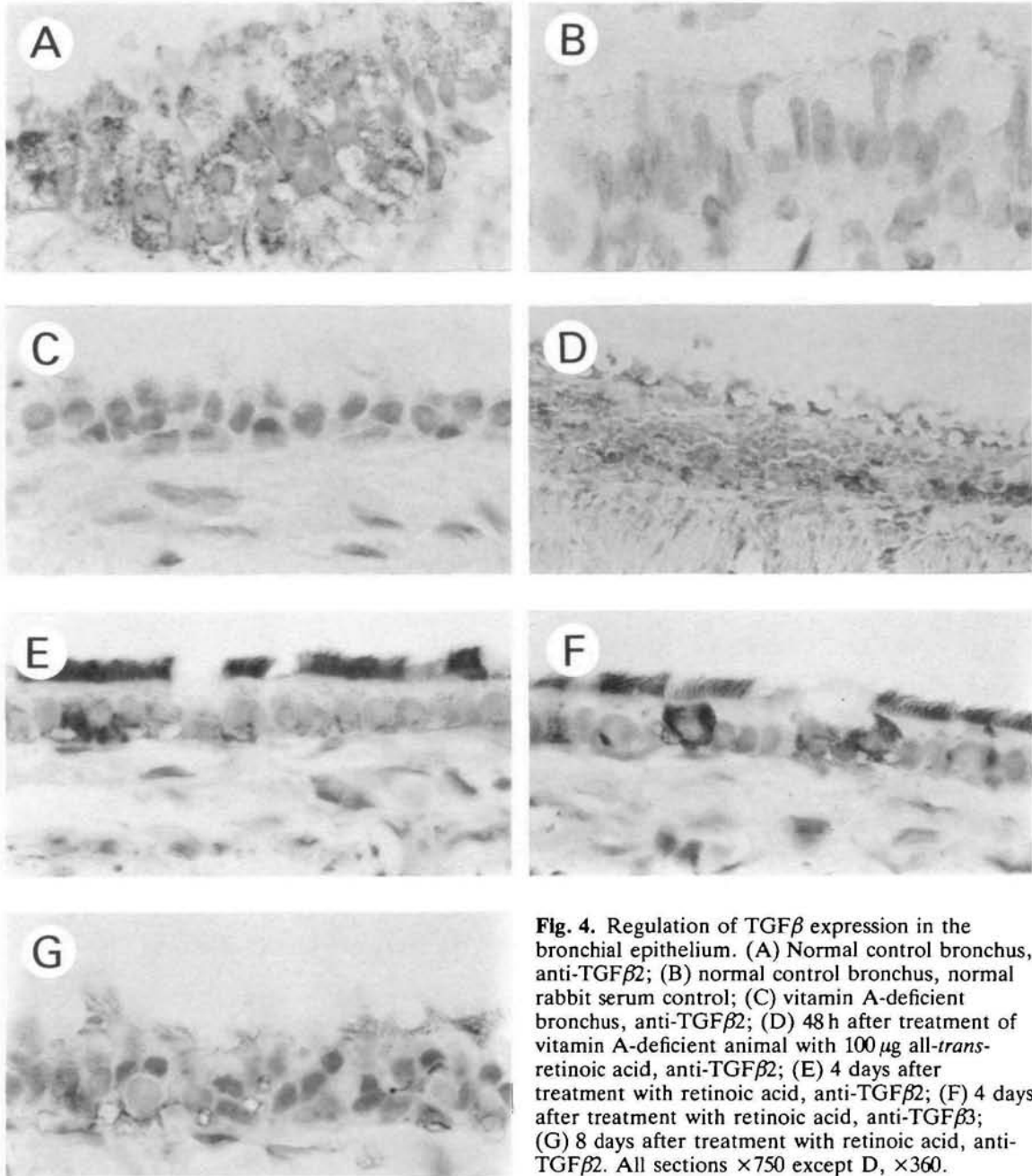


Fig. 4. Regulation of TGF β expression in the bronchial epithelium. (A) Normal control bronchus, anti-TGF β 2; (B) normal control bronchus, normal rabbit serum control; (C) vitamin A-deficient bronchus, anti-TGF β 2; (D) 48 h after treatment of vitamin A-deficient animal with 100 μ g all-*trans*-retinoic acid, anti-TGF β 2; (E) 4 days after treatment with retinoic acid, anti-TGF β 2; (F) 4 days after treatment with retinoic acid, anti-TGF β 3; (G) 8 days after treatment with retinoic acid, anti-TGF β 2. All sections \times 750 except D, \times 360.

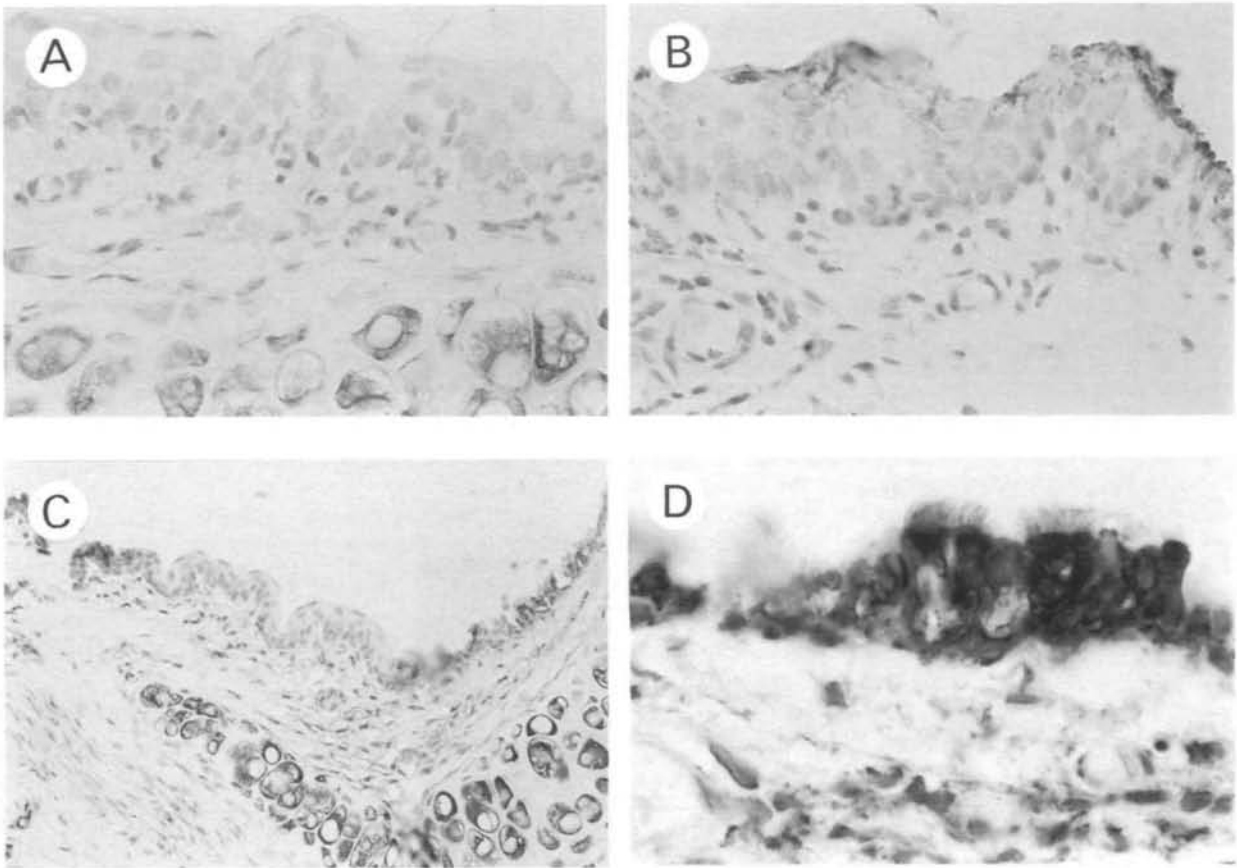


Fig. 5. Regulation of TGF β expression by retinoic acid in the tracheal epithelium. All sections stained with anti-TGF β 2 antibody. (A) Metaplastic lesion from vitamin A-deficient trachea $\times 375$; (B) metaplastic lesion 24 h after treatment with $100 \mu\text{g}$ all-*trans*-retinoic acid, $\times 375$; (C) metaplastic lesion and surrounding uninvolvement epithelium, $\times 200$; (D) 4 days after treatment with retinoic acid, $\times 600$.

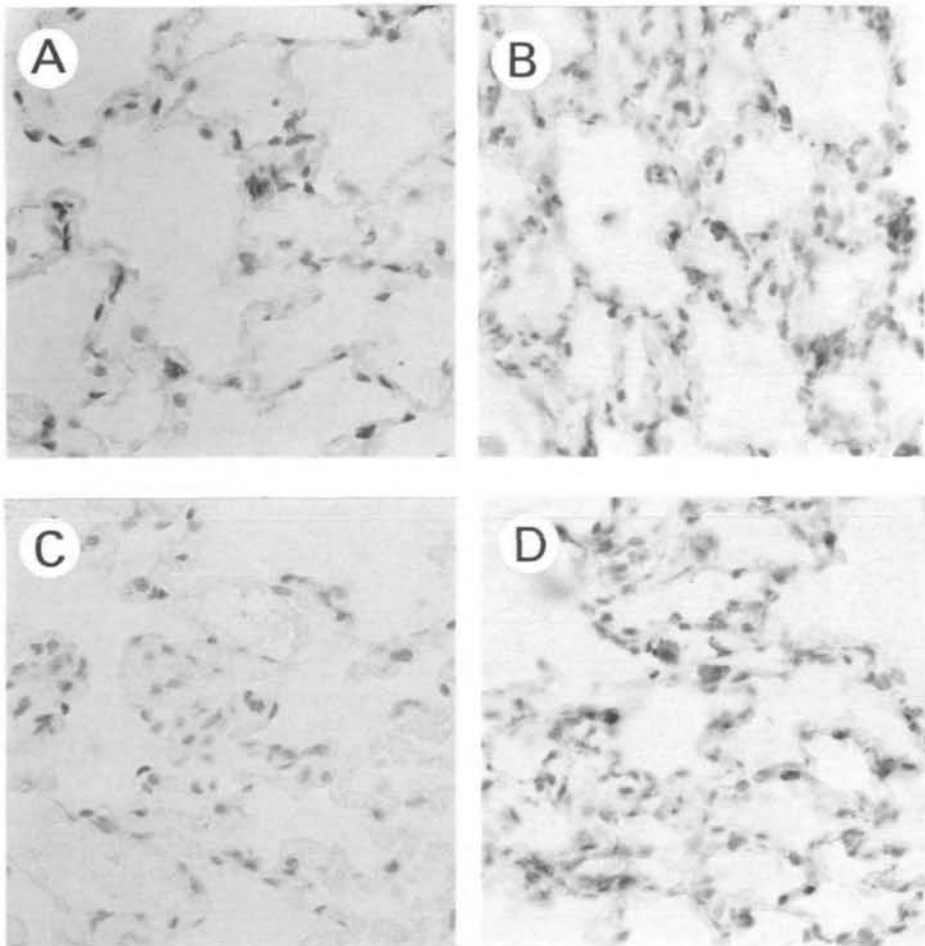


Fig. 6. Regulation of TGF β expression in the distal airway. (A,B) Stained with anti-TGF β 2; (C,D) stained with anti-TGF β 3. (A) Vitamin A-deficient animal; (B) 2 days after treatment with $100 \mu\text{g}$ all-*trans*-retinoic acid; (C) vitamin A-deficient animal; (D) 4 days after treatment with retinoic acid, $\times 600$.

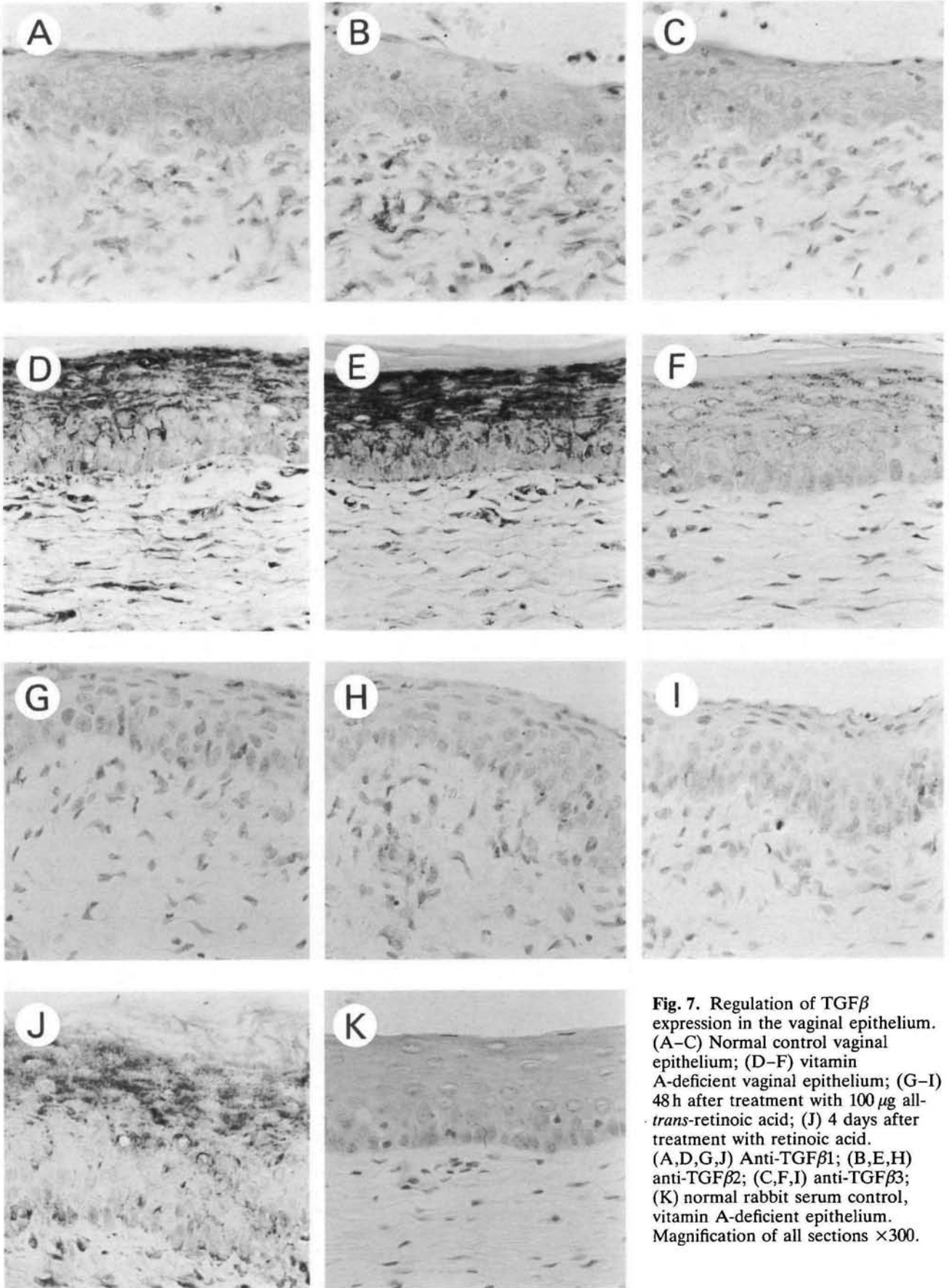


Fig. 7. Regulation of TGF β expression in the vaginal epithelium. (A-C) Normal control vaginal epithelium; (D-F) vitamin A-deficient vaginal epithelium; (G-I) 48 h after treatment with 100 μ g all-*trans*-retinoic acid; (J) 4 days after treatment with retinoic acid. (A,D,G,J) Anti-TGF β 1; (B,E,H) anti-TGF β 2; (C,F,I) anti-TGF β 3; (K) normal rabbit serum control, vitamin A-deficient epithelium. Magnification of all sections \times 300.

there was a striking loss of staining by the anti-TGF β 2 antibody in the lamina propria, surface epithelium and crypts of the small intestine (Fig. 2C,D) and in the surface epithelium of the colon (Fig. 3B).

Within 24 h of treatment of deficient animals with retinoic acid, there was an increase in staining with the anti-TGF β 2 antibody of the lamina propria in both the small intestine (Fig. 2E) and colon (Fig. 3C). At this time point, there was also a slight increase in TGF β 2 in the surface epithelium of the colon, and a lesser but distinct increase of TGF β 1 and TGF β 3 in the colonic lamina propria (not shown).

In the small intestine, 48 h after dosing the deficient animals with retinoic acid, there was a significant increase in staining by the anti-TGF β 2 antibody in the cells of the villus crypt (Fig. 2F). However, by 4 days, TGF β 2 was no longer seen in the crypts but rather was expressed primarily in cells midway along the villi (Fig. 2G), suggesting that the induction of TGF β 2 expression by retinoic acid initiates within the crypt and increases as cells differentiate and move up the axis of the villus. At this time point, there was very faint staining by the anti-TGF β 1 and TGF β 3 antibodies in the intestinal villi, which colocalized with that of TGF β 2.

Fig. 3D shows that 48 h after treatment with retinoic acid, the surface epithelium of the colon was stained intensely by the anti-TGF β 2 antibody. An increase was also seen with TGF- β 3 while there was no staining by the anti-TGF β 1 antibody. However, unlike the small intestine, by 4 days post-treatment, the colonic mucosa and lamina propria stained strongly with both anti-TGF β 2 and TGF β 3 antibodies while again only a slight increase was seen for TGF β 1 (Fig. 3E-G). This staining was absent when normal rabbit serum was used as the primary antibody (Fig. 3H). Within the surface epithelium, the reactivity was localized primarily to the basal layer of cells. While there was little or no staining of the colonic crypts, this may simply reflect the transit time of cells from the crypt. By the 8th day post-treatment, staining of TGF β 2 had disappeared in the small intestine; in the colon, both TGF β 2 and TGF β 3 were considerably diminished.

Respiratory epithelium

The pseudostratified epithelium of the normal trachea and bronchus is composed of tall columnar ciliated cells and secretory goblet cells interspersed with a population of stem cells. In the bronchial epithelium of the normal control animals, there was specific staining of the ciliated cells of the bronchus and bronchioles by the anti-TGF β 2 antibody (Fig. 4A,B), while there was little detectable TGF β 1 or TGF β 3.

The bronchial epithelium of the deficient animals had acquired a low cuboidal morphology with few ciliated cells evident; focal regions of basal cell hyperplasia were also seen. In these animals, the basal level of TGF β 2 expression was lost (Fig. 4C), and there was no expression of any of the TGF β isoforms in the hyperplastic bronchial areas. Within 2 days following

systemic administration of retinoic acid, a significant increase in staining by the TGF β 2 antibody was seen, primarily in basal cells in regions where the upper layers of squamous cells were either degenerating or being sloughed (Fig. 4D). At day 4, the cilia of the newly differentiated ciliated cells stained intensely with both anti-TGF β 2 and TGF β 3 antibodies (Fig. 4E,F), but ultrastructural localization has not yet been done to confirm this observation. In contrast, there was no increase in TGF β 1 at this or other time points. Again by the 8th day following treatment with retinoic acid, expression of TGF β 2 and TGF β 3 had disappeared (Fig. 4G).

The tracheal epithelium is also extremely sensitive to retinoid deficiency, which causes loss of ciliated cells and appearance of focal regions of keratinizing squamous metaplasia. In the normal control animals, there was only faint staining by the anti-TGF β 2 antibody, and this was entirely lost in the deficient animals, including areas of squamous metaplasia (Fig. 5A). Fig. 5B shows a striking and rapid response of the squamous lesions to the retinoic acid. Within 1 day post-treatment, all of the identifiable metaplastic lesions reacted strongly with the TGF β 2 and TGF β 3 antibodies and to a lesser extent with the anti-TGF β 1 antibody, primarily in the differentiated keratinizing cells. Significantly, at this time point there was little staining with any of the antibodies in the adjacent tracheal epithelium. However, the staining of the squamous lesions was transient and was largely absent at 2 days. In contrast, the adjacent epithelium as well as the hyperproliferative epithelium surrounding the squamous lesions reacted strongly with the anti-TGF β 2 and TGF β 3 antibodies by 4 days post-treatment. Fig. 5C shows the border between a metaplastic lesion and adjacent epithelium in which the increased expression of TGF β 2 and TGF β 3 occurs only in the adjacent epithelium. As in the bronchial epithelium, staining of the tracheal epithelium localized to both the cell body and cilia of the ciliated cells (Fig. 5D). Again all staining disappeared by 8 days after the initial treatment with retinoic acid.

Unlike the tracheobronchial epithelium, little is known about the effects of vitamin A deficiency on the cells of the distal airway. In normal control animals, there was significant staining only by the anti-TGF β 2 antibody, and this decreased considerably in the deficient animals (Fig. 6A). However, following treatment with retinoic acid, there was a dramatic increase in immunoreactive TGF β 2, which was detectable within 2 days (Fig. 6B), and an increase in TGF β 3 detectable by 4 days (Fig. 6D). For both isoforms, there was a generalized increase in staining throughout the cells of the alveoli; although the 5 μ m sections did not allow precise identification, the most intense reactivity appeared to be in type II pneumocytes. As in other tissues, the increase in expression of TGF β 2 and TGF β 3 was transient and had returned to levels found in the deficient animals by 8 days post-treatment. No change in TGF β 1 expression was observed in the distal airway.

Vaginal epithelium

Vaginal epithelium, which is normally stratified and squamous in rats, keratinizes in vitamin A deficiency (Wolbach and Howe, 1925), and expresses markers normally found in the differentiating epidermis (Roop, 1987). There was no vaginal staining by antibodies to any of the TGF β isoforms in normal control animals (Fig. 7A–C). In striking contrast, Fig. 7D–F show intense vaginal staining by antibodies to TGF β 1, TGF β 2 and TGF β 3 in vitamin A-deficient animals; the TGF β 2 staining was most prominent. Staining was localized to the cell layers analogous to the spinous and granular layers of the epidermis. Remarkably, within 24 h after dosing with retinoic acid (which caused a partial restoration of the normal stratified squamous epithelium), the staining by the TGF β antibodies was lost (Fig. 7G–I). Fig. 7J shows that the effect of retinoic acid was again transient; by 4 days post-treatment, there was a marked increase in expression of all TGF β isoforms in the newly keratinized vaginal epithelium.

Discussion

Retinoic acid and the TGF β s are key regulators of the growth and differentiation of cells. Our data show retinoic acid controls the expression of the TGF β family in diverse tissues *in vivo*. This action of retinoic acid has important implications for embryonic development, control of epithelial function in the adult, as well as the chemoprevention of cancer.

Using specific immunohistochemical reagents, we have examined the effects of retinoic acid on the expression of three TGF β isoforms in the epithelia of the epidermis, intestine, respiratory system and vagina *in vivo*. Our data show that systemic retinoic acid induces expression of all TGF β isoforms in the epidermis, and of TGF β 2 and TGF β 3 in the respiratory epithelium as well as in the intestinal mucosa and lamina propria. In contrast, retinoid deficiency results in increased expression of all isoforms in the vaginal epithelium, and subsequent addition of retinoic acid decreases expression. However, our data do not exclude other metabolites of vitamin A as the primary retinoids involved in the regulation of TGF β expression under normal dietary conditions.

Of the three isoforms, TGF β 2 appears to be the most sensitive to tissue retinoid levels. In tissues such as the bronchial and intestinal epithelium where TGF β 2 was detected in the normal controls, depletion of tissue retinoids resulted in the loss of this basal expression. The kinetics of induction of the isoforms was rapid, although there were differences in the lag time between tissues, which could represent unequal distribution of retinoic acid among various tissues or inherent differences in response. TGF β 2 expression appeared first in several tissues; this could indicate that the isoforms have different thresholds for induction by retinoic acid, or that other mechanisms such as autoinduction are responsible for the increases in TGF β 1 and TGF β 3 (van Obberghen-Schilling *et al.* 1988; Bascom *et al.* 1989).

However, the effects of retinoic acid on the expression of all three isoforms was transient; regardless of the type of response, a return to the deficient state of expression generally occurred within 8 days of the initial dose. This is consistent with data showing that 82–95 % of physiological or pharmacological doses of retinoic acid given to deficient animals is excreted from the body within 5 days (Roberts and DeLuca, 1967).

Our data show that TGF β s are induced by retinoic acid in both epithelial and mesenchymal components of specific tissues such as the small intestine and colon. In contrast, little increase in immunoreactive TGF- β was detected in the dermis, indicating the specificity of this response. A comparison of histological and *in situ* hybridization patterns would clarify whether more complex interactions between epithelial and mesenchymal components can occur as suggested by Lehnert and Akhurst (1989). While in these *in vivo* experiments it is not clear that the induction of TGF- β expression is a direct effect of retinoic acid, we have previously shown that cultured epidermal keratinocytes respond to retinoic acid with an increased level of both TGF β 2 peptide and transcripts; this occurred through a post-transcriptional mechanism (Glick *et al.* 1989). Whether similar mechanisms operate for the induction of TGF β 2 in other epithelia, as well as for the induction of TGF β 1 and TGF β 3 remains to be determined. These data suggest that the response does not occur through a direct interaction between retinoic acid receptors (Petkovich *et al.* 1987; Giguere *et al.* 1987; Zelent *et al.* 1989) and the TGF β 2 promoter. However, these *in vitro* studies do not exclude an indirect induction of TGF β *in vivo*, through the regulation of other growth factor or endocrine effectors by retinoic acid.

Implications for control of growth in epithelia

We have demonstrated a positive relationship between tissue retinoid levels and expression of the TGF β gene family in the epidermal, intestinal and respiratory epithelium and a negative relationship in the vaginal epithelium. Both retinoic acid and TGF β have significant effects on the growth and differentiation of these epithelia. Thus, in the lung and vagina, retinoid deficiency causes epithelial hyperplasia and expression of the squamous phenotype *in vivo*, and a similar regulation of squamous differentiation *in vitro*, although the effects on cell proliferation are variable (Jetten *et al.* 1987, 1989; Glick *et al.* 1989).

The TGF β isoforms are potent growth inhibitors for cultured epithelial cells (Tucker *et al.* 1984; Roberts and Sporn, 1990) and when administered *in vivo* to the breast (Silberstein and Daniel, 1987) and liver (Russell *et al.* 1988). In contrast, the vaginal epithelium is one of a few cell types for which TGF β stimulates growth *in vivo* (Takahashi *et al.* 1989). Expression of a specific TGF β isoform in the small intestine and during terminal differentiation of primary mouse keratinocytes (Glick *et al.* 1990) supports the hypothesis that programmed secretion of TGF β by the differentiating cells could act to control the proliferation of cells in the basal compartment.

The superficial similarity between hyperplasia and squamous metaplasia induced either by carcinogens or vitamin A deficiency (Harris *et al.* 1972) strongly suggests that mutations induced by carcinogens might interfere with the normal regulation of epithelia by retinoids. Furthermore, in experimental carcinogenesis of lung (Nettesheim and Williams, 1976) or digestive tract (Newberne and Rogers, 1973), vitamin A deficiency enhances the neoplastic response to chemical carcinogens; conversely in the respiratory tract (Crocker and Sander, 1970; Cone and Nettesheim, 1976), prostate (Lasnitzki, 1955), bladder (Sporn *et al.* 1977) and breast (Moon *et al.* 1976), retinoids can suppress the progression of premalignant epithelial lesions *in vitro* and *in vivo*. However, the mechanism by which retinoids can suppress progression of preneoplastic epithelial lesions is not clear.

Our data suggest that systemic levels of retinoids obtained through a normal diet maintain epithelial homeostasis in part through the local regulation of TGF β expression in responsive epithelia. Depletion of retinoid levels, or alterations in the response to retinoids induced by carcinogens, both could result in deregulation of TGF β expression and the loss of control of growth and differentiation. The rapid induction of TGF β expression in areas of tracheal squamous metaplasias suggests that suppression of preneoplastic lesions by exogenous retinoids could result from the autocrine induction of TGF β expression within a lesion, as well as paracrine suppression through enhanced secretion by the surrounding epithelium. We have previously shown that a significant fraction of the TGF β 2 induced by retinoic acid in primary newborn mouse keratinocytes is in the biologically active form (Glick *et al.* 1989), suggesting that the TGF β induced by retinoic acid *in vivo* is functional.

Implications for epithelial differentiation and development

Our results suggest that the induction of TGF β expression is an important element of the regulation of epithelial differentiation by retinoids *in vivo*. In certain epithelia, both *in vitro* and *in vivo*, the pathways of mucociliary or squamoid differentiation are determined by the presence or absence of retinoids (Sporn, 1976). *In vitro*, TGF β can also induce squamous differentiation in the respiratory epithelium (Masui *et al.* 1986; Jetten *et al.* 1989) and specific differentiation markers in an intestinal cell line (Kurokawa *et al.* 1987). However, there is no evidence to suggest that TGF β produced *in vivo* has similar effects on epithelial differentiation. Thus, the induction of squamous differentiation by TGF β in tracheal epithelial cells occurs only in the absence of retinoic acid (Jetten *et al.* 1989). This observation suggests that the control of epithelial differentiation *in vivo*, through the interaction of retinoids and induced TGF β , is likely to be very complex.

Although the experiments reported here involved adult tissues, the induction of a multifunctional cytokine by an agent that is likely to be an endogenous

morphogen (Tickle *et al.* 1982; Thaller and Eichele, 1987; Durston *et al.* 1989; Simeone *et al.* 1990), suggests the importance of this interaction in embryonic development as well. Thus it is possible that during embryogenesis, retinoic acid or other metabolites of vitamin A could act as a signal for the localized synthesis of the TGF β s, which in turn could propagate morphogenesis both through effects on the extracellular matrix, or the induction of specific pathways of cellular differentiation. In support of this, retinoic acid has been shown to induce expression of TGF β 2 in mouse embryonal carcinoma cells and embryonic stem cells (Mummery *et al.* 1990).

While further experiments will be required to explore the functional consequences of the regulation of TGF β expression by retinoic acid both in the adult and embryo, our present results firmly establish that the regulation of TGF β expression by retinoic acid is a significant aspect of the biology of retinoid action *in vivo*.

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