Vitamin A Deficiency Alters the Expression of Mucin Genes by the Rat Ocular Surface Epithelium

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Purpose. To study effects of depletion of retinoic acid on expression of the mucins ASGP (rMuc4), rMuc5AC, and rMuc1, by the corneal and conjunctival epithelia of the rat.

Methods. Nineteen-day-old Sprague–Dawley male rats were fed a casein-based vitamin A–deficient diet or casein-based diet with vitamin A as control. Rats from both groups were killed at 1, 3, 5, 13, 15, 18, and 20 weeks after initiation of feeding. Expression of the three mucin genes by the ocular surface epithelium was assayed by reverse transcription–polymerase chain reaction (RT-PCR) and in situ hybridization.

Results. In vitamin A–deficient rats, ASGP mRNA was not detected by RT-PCR after 15 weeks of feeding. rMuc5AC mRNA was detected by RT-PCR at 15 weeks, but by 18 and 20 weeks was no longer detectable. By in situ hybridization, ASGP mRNA was localized in the entire ocular surface epithelium after 1 week of feeding, was diminished but detectable above background by 13 weeks, and was not detectable at 20 weeks. rMuc5AC mRNA was detected in the goblet cells of vitamin A–deficient rats by in situ hybridization at 13 weeks, but was lost by 20 weeks, as were identifiable goblet cells. rMuc1 mRNA were detected by RT-PCR through all time points of 1 to 20 weeks in both vitamin A–deficient and control rats, indicating no significant change in rMuc1 mRNA expression with vitamin A deficiency.

Conclusions. Both the membrane-spanning mucin ASGP (rMuc4) and the secretory mucin rMuc5AC are directly or indirectly regulated by vitamin A in the ocular surface epithelium, whereas the membrane-spanning mucin rMuc1 is not. (*Invest Ophthalmol Vis Sci.* 2000;41:82–88)

Vitamin A and its derivatives are required for the normal growth and differentiation of epithelium both in vivo and in vitro.[1–5] Systemic vitamin A depletion results in keratinization and drying of the epithelium in the gastrointestinal tract, respiratory tract, and ocular surface.[6–8] In the eye, vitamin A deficiency is reported to be associated not only with keratinization and squamous metaplasia but also with a reduction in the goblet cell population and an increase in conjunctival epithelial cell mitosis.[9–11] Vitamin A has been shown to be involved in biosynthesis of glycoconjugates by the rat corneal epithelium, but it is not clear whether an increase in biosynthesis with vitamin A is due to synthesis of the glycoconjugate or to its glycosylation.[12] It is also not clear whether the reduction of goblet cells is due to altered glycosylation of the secretory mucins expressed by the goblet cells or to altered expression of the mucin gene.

The ocular surface, made up of stratified, nonkeratinizing cell layers, is covered by the tear film, which lubricates, hydrates, and protects the underlying epithelium. The innermost component of the tear film is mucus, composed mainly of secreted mucins, which are produced by conjunctival goblet cells, with potential additional contribution of membrane-spanning mucins expressed by corneal and conjunctival epithelia.[13] Mucins are highly glycosylated glycoproteins that have only recently been characterized at the molecular level.[14] Our laboratory has demonstrated that, of the nine cloned human mucins, MUC1, MUC4, and MUC5AC are expressed by the human ocular surface epithelium.[13,15,16] MUC1 is a membrane-spanning mucin found in most wet-surfaced epithelial tissues; mRNA to MUC1 is expressed by the entire ocular surface epithelium with MUC1 protein present primarily along the apical cell membrane of cells at the tear interface.[15] A rat homologue of human MUC1, designated rMuc1, has been cloned, and sequence is available in the GenBank (accession no. AF007554).

MUC4 appears to also have a wide tissue distribution and is expressed not only in simple epithelium of wet-surfaced mucosa but in stratified, squamous, nonkeratinizing epithelium of the ectocervix and trachea.[13,17] MUC4 is the second membrane-spanning mucin identified to date and is expressed by the conjunctival epithelium, but not by the corneal epithelium.[16] The human *MUC4* gene was recently reported to have over 80% homology in its 3′ membrane-spanning epidermal growth factor–like region[18] to a rat gene designated ascites sialoglycoprotein (ASGP).[19] ASGP may thus be the homologue to the human *MUC4* and can be referred to as *rMuc4*. The ASGP mucin, also known as *sialo mucin complex* (*SMC*), was recently demonstrated in both the conjunctival and corneal epithelia of the rat.[20]

The third mucin expressed at the ocular surface is MUC5AC, a large gel-forming mucin whose expression at the ocular surface is limited to the goblet cells of the conjunctiva.[16] This mucin, a member of the family of mucins that forms intermolecular associations through carboxyl and amino terminal cysteine-rich domains, may play an important role in form-

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TABLE 1. PCR Primers Used for Semiquantitative Analysis

PCR Primers	Size	Reference
rMuc1		
5′-TCGACAGGCAATGGCAGTAG-3′ (154–173)	286 bp	GenBank accession no.
5′-TCTGAGAGCCACCACTACCC-3′ (439–420)		AF007554
ASGP		
5′-CGTACTAGAGAACTTGGACATGC-3′ (4761–4783)	638 bp	Wu et al.[19]
5′-GGTAGGAGAACTTGTTCATGG-3′ (5398–5378)		
rMuc5AC		
5′-TATGAGGTGCGACTGCTTTG-3′ (726–745)	470 bp	Inatomi et al.[21]
5′-CACTGGCGTGGGCTCAAAGA-3′ (1195–1176)		
β-Actin		
5′-TTGTAACCAACTGGGACGATATGG-3′ (1552–1575)	764 bp	
5′-GATCTTGATCTTCATGGTGCTAGG-3′ (2991–2844)		
In situ hybridization probes		
ASGP	638 bp	Wu et al.[19]
rMuc5AC	228 bp	Inatomi et al.[21]

ing the gelled mucus layer of the tear film at the air–ocular surface epithelium interface. To establish animal models for the study of goblet cell differentiation and regulation and function of this gel-forming mucin, our laboratory has cloned a rat homologue of the human *MUC5AC* gene.[21] It is designated *rMuc5AC*, and a 3783-bp region of the 3′ end of the molecule, including a cysteine-rich domain, was sequenced. Previous in situ hybridization studies from our laboratory demonstrated that this rat homologue of *MUC5AC* is expressed in the goblet cells of the rat conjunctiva.[21]

Previous in vitro studies have indicated that vitamin A derivatives are required for maintaining mucosal cell differentiation, mucin production, and mucin gene expression.[22–26] Recent studies using cultured tracheobronchial epithelium showed that MUC5AC mRNA as assayed by either northern blot analysis[22] or by quantitative reverse transcription–polymerase chain reaction (RT-PCR)[27] is increased when cells were cultured in the presence of vitamin A. These in vitro results indicate the need for assessment of the role of retinoids in the regulation of mucin gene expression in vivo. It has long been known that keratinization occurs and that goblet cells disappear in the conjunctiva of vitamin A–deficient humans and in animal models using rats. Little is known, however, of how decreased vitamin A affects conjunctival or corneal epithelium at the mucin gene expression level. The purpose of our experiments was thus to use the vitamin A–deficient rat model to determine the effects of retinoic acid depletion on expression of the membrane-spanning mucins rMuc1 and ASGP and on the goblet cell–specific mucin rMuc5AC.

MATERIALS AND METHODS

Animals and Tissues

All procedures used in these studies adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Nineteen-day-old Sprague–Dawley male rats were fed a casein-based vitamin A–deficient diet (Teklad, Madison, WI).[28] Age-matched control male rats had vitamin A included in the casein-based diet. Body weight and ocular surface and skin characteristics were monitored for signs of vitamin A deficiency twice a week. At 1, 3, 5, 13, and 20 weeks after initiation of feeding, two rats from each group were killed. In addition, two rats from the vitamin A–deficient group were killed because of poor general condition, one at 15 and one at 18 weeks after initiation of feeding. Conjunctivas for RNA isolation were frozen in liquid nitrogen immediately after removal and stored at −80°C until RNA isolation. Anterior segments of the eyes with conjunctiva and lids included were fixed immediately after removal in 4% paraformaldehyde in 0.1 M phosphate buffer and processed through paraffin for in situ hybridization and histochemical staining.

RNA Isolation and RT-PCR of rMuc1, ASGP, and rMuc5AC

Total RNA was isolated from tissues of Sprague–Dawley rats using TRIzol reagent (Gibco, Grand Island, NY), according to the protocol provided by the manufacturer.

Recently, our laboratory successfully used semiquantitative RT-PCR to correlate the expression of endocervical mucin mRNA to blood hormone levels.[29] We used the same approach to study rMuc5AC, ASGP, and rMuc1 mRNA expression by the rat ocular surface during progression from mild to severe vitamin A deficiency. In this study, rat *β-actin* was used as the internal control.[30] Total RNA from rat conjunctiva (1 μg/sample) was subjected to reverse transcription using random hexamer primers as previously described.[15] A 1-μl aliquot (one twentieth of total volume) of the same RT products per sample was used in the PCR amplification reaction for each of the genes investigated.

Oligonucleotide primers to the nontandem repeat region of rMuc1, ASGP, and rMuc5AC were designed from published or GenBank sequences (Table 1). PCR amplifications were performed as previously described.[15] The sequence of each PCR product was confirmed by sequencing using the dideoxy-mediated chain termination method.

PCR conditions were optimized for all genes studied using RT product from adult rat conjunctival total RNA. The linear range of the amplification reaction for β-actin, rMuc5AC, ASGP, and rMuc1 was determined by checking amplification after each cycle from cycles 21 to 35 for β-actin, rMuc5AC, and ASGP, and from cycles 29 to 41 for rMuc1. These determinations showed 27 cycles to be in the midlinear phase for β-actin, rMuc5AC, and ASGP and 35 cycles to be in the midlinear phase

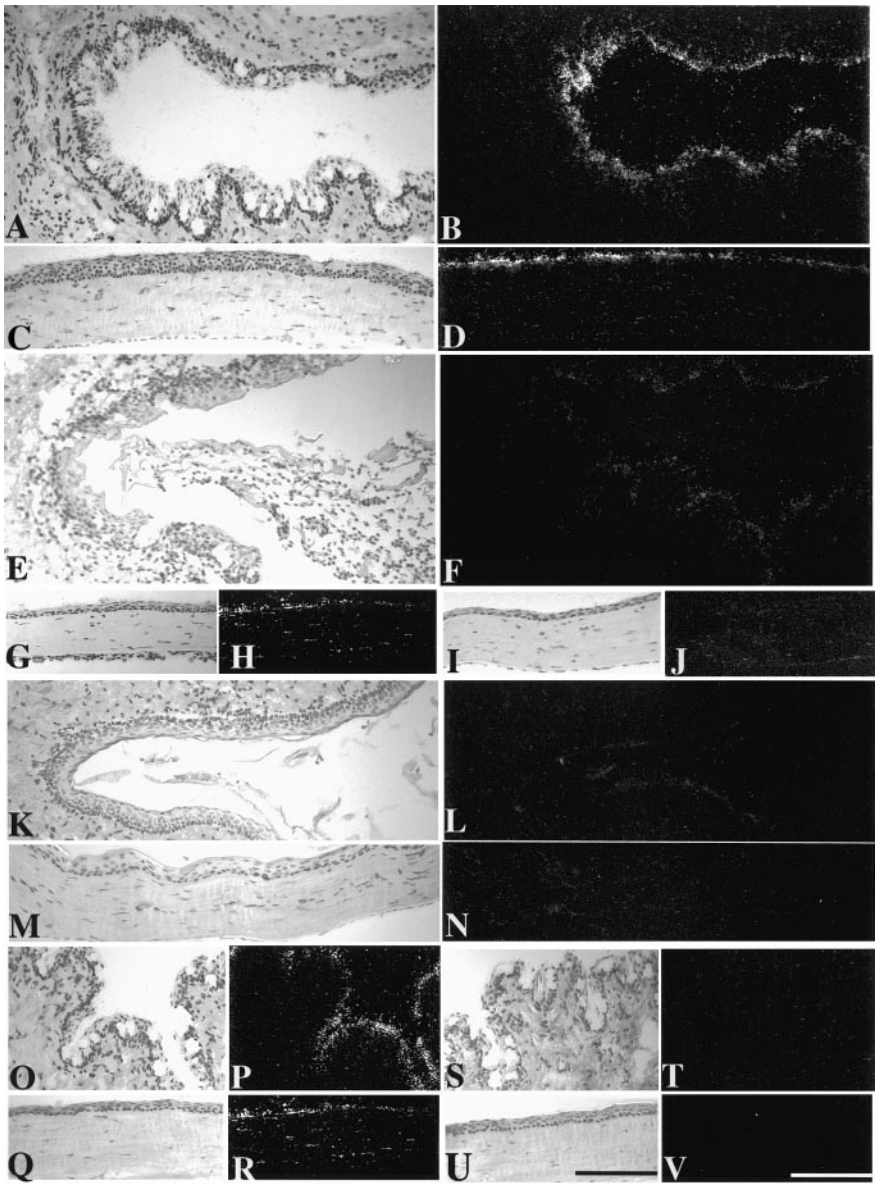

FIGURE 1. Histology and in situ hybridization of ASGP (rMuc4) mRNA in ocular surface tissues of vitamin A–deficient and control rats. Paired bright-field and dark-field micrographs are shown for all time points. Sections of rat ocular surface tissue hybridized with antisense probe (**A** through **R**) and sense probe as control (**S** through **V**). (**A** through **D**) show bright- and dark-field microscopy of conjunctival (**A**, **B**) and corneal (**C**, **D**) tissue from rats fed a vitamin A–deficient diet for 1 week. ASGP expression was detected in conjunctival (**C**) and corneal epithelium (**D**). (**E** through **J**) show tissues from rats fed a vitamin A–deficient diet for 13 weeks. Weak levels of ASGP expression were detected in conjunctival (**F**) and corneal epithelium (**H**) in mildly affected rats, whereas ASGP localization was not detected in the conjunctival (not shown) or corneal epithelium (**J**) of severely affected rats at 13 weeks. In rats fed a vitamin A–deficient diet for 20 weeks (**K** through **N**), the conjunctival (**K**, **L**) and corneal epithelia (**M**, **N**) were keratinized, and no ASGP mRNA was localized. Control rats fed a diet with vitamin A showed ASGP mRNA in conjunctiva (**O**, **P**) and cornea (**Q**, **R**). Sense controls are shown in (**S**) through (**V**). All micrographs are of the same magnification; bar, 50 μm.

for rMuc1. All PCR amplifications started with denaturation at 94°C for 5 minutes and ended with a final elongation at 72°C for 7 minutes. The parameters for PCR amplifications were as follows: rMuc5AC and β-actin: 27 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute; ASGP: 27 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 1 minute, and extension at 72°C for 1 minute; rMuc1: 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 1 minute, and extension at 72°C for 1 minute.

The PCR products were run on a 1.5% agarose gel stained with ethidium bromide and photographed with positive-negative film (Type 665; Polaroid, Cambridge, MA). The amount of amplified product was quantified for each sample from the negatives using a computing densitometer (model 300A; Molecular Dynamics, Sunnyvale, CA) and software (Image Quant, ver. 2.0; Molecular Dynamics). The final amount of PCR product was expressed as the ratio of mucin gene amplified to that of β-actin to account for any differences in starting amounts of RNA.

In Situ Hybridization and Histologic Analysis

To demonstrate when and where ASGP and rMuc5AC expression is altered, in situ hybridization was performed,[16] by using radiolabeled RNA probes to the 3′ end of ASGP and radiolabeled RNA probes to the tandem repeat of rMuc5AC. The cDNA used to generate the ASGP riboprobes was generated by RT-PCR using adult rat endocervix RNA and ASGP-specific primers (Table 1) and was subcloned into pGEM-T Easy vector (Promega, Madison, WI). The cDNA used for rMuc5AC has been described previously.[16] Hybridization was performed overnight at 52°C using 1×10^6 cpm/ml of ^{35}S-UTP-labeled sense or antisense riboprobe transcribed from plasmid Bluescript SK(−) using T3 or T7 RNA polymerase (Boehringer–Mannheim, Indianapolis, IN) for rMuc5AC and from pGEM-T Easy vector using T7 or SP6 RNA polymerase for ASGP. Sections were counterstained with hematoxylin and eosin. Six-micrometer sections were stained with periodic acid-Schiff (PAS) reagent by conventional techniques to determine goblet cell presence and distribution.

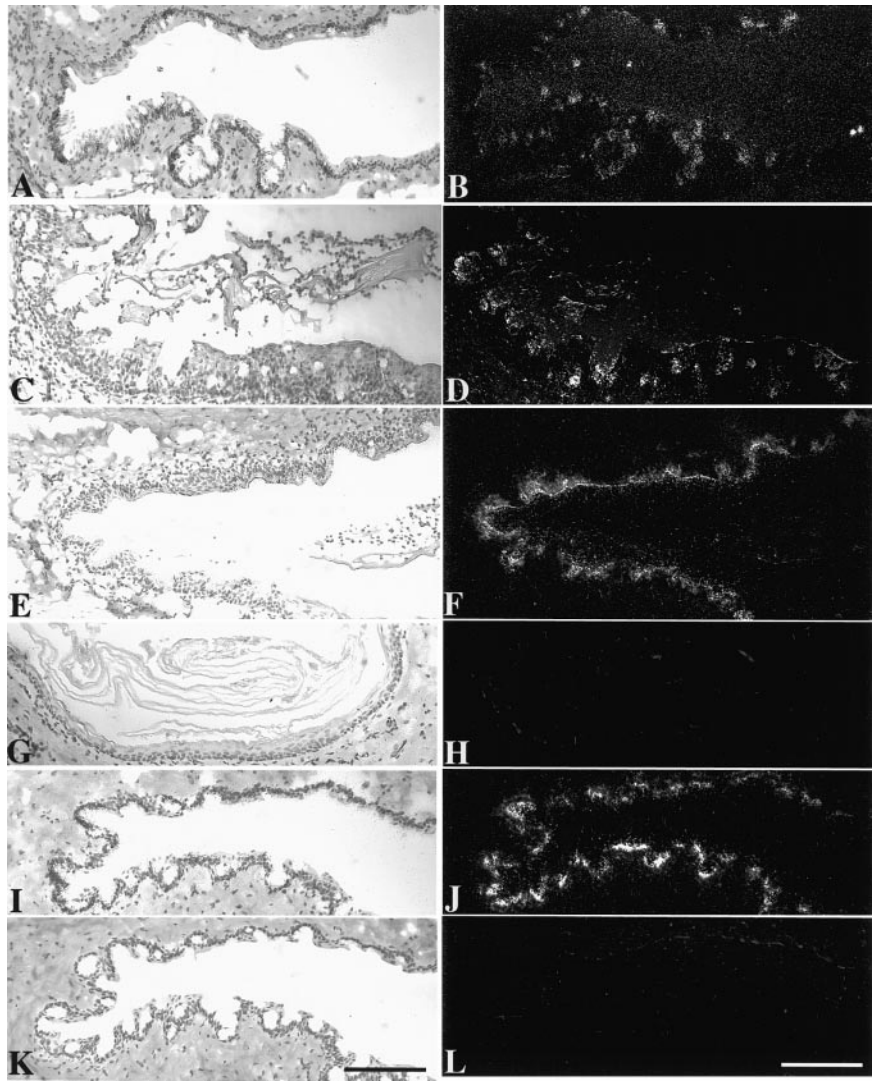

FIGURE 2. Localization of rMuc5AC in rat conjunctiva by in situ hybridization using ^{35}S-labeled riboprobes for tandem repeat region of rMuc5AC. Sections of rat conjunctiva from rats fed a vitamin A–deficient diet for 1 week (**A**, **B**), 13 weeks (**C** through **F**), and 20 weeks (**G**, **H**). At 13 weeks some rats were mildly affected (**C**, **D**) or more severely affected (**E**, **F**). Note that in severely affected eyes, rMuc5AC expression was more diffusely localized along the apical cells of the epithelium (**F**) and that few goblet cells were obvious by bright-field microscopy (**E**). In rats fed a vitamin A–deficient diet for 20 weeks, the epithelium was completely keratinized, goblet cells were absent (**G**), and rMuc5AC mRNA was not localized (**H**). Sections of conjunctiva of control rats fed a vitamin A–replete diet are shown in (**I**) and (**J**). Note strong localization of rMuc5AC in regions of goblet cell clusters. Sense probe did not localize to sections of control rat conjunctiva (**K**, **L**). All micrographs are of the same magnification; bar, 50 μm.

RESULTS

A change in the average weight of vitamin A–deficient male rats relative to that of control rats was first observed at 6 weeks after initiation of feeding of the vitamin A–deficient diet. The average weight of the vitamin A–deficient males remained constant for an additional 7 weeks, and additional weight loss began at 13 weeks after initiation of feeding. Severe general manifestations (hair loss, bleeding from nose) and severe ocular manifestations (accumulation of reddish secretions at the eyelid margin, loss of corneal clarity, corneal vascularization) appeared between 13 and 20 weeks after initiation of feeding.

Histologic Characteristics of Vitamin A–Deficient Rats

Of the time points examined (1, 3, 5, 13, 15, 18, and 20 weeks) the first difference between conjunctival epithelial or goblet cell morphology of vitamin A–deficient rats compared with control rats was observed at 13 weeks (Figs. 1, 2). At 13 weeks, one rat was more severely affected than others (compare Figs. 2C and 2E.) In the mildly affected eye, the conjunctival epithelium was partially keratinized with goblet cells present within regions of keratinization, and the conjunctival epithelium appeared to have more cell layers (Figs. 2C, 2E). Inflammatory cells were seen in the adherent mucus, and lymphocytes were observed in the conjunctival epithelium. In the severely affected eye, the degree of keratinization appeared to be greater, and the presence of morphologically distinct goblet cells was less, as was the thickness of the epithelium (Fig. 2E). At 20 weeks after initiation of feeding, the entire conjunctival epithelium of the vitamin A–deficient rat eyes was keratinized, and goblet cells were not evident (Figs. 1K, 2G).

PAS staining of goblet cells was detected from 1 to 13 weeks of feeding (Fig. 3). At 13 weeks, the partially keratinized conjunctival epithelium still had goblet cells intercalated within it (Figs. 3B, 3C). In severely affected eyes at 13 weeks (Fig. 3C), PAS staining was evident, but the shape of the goblet cells was not round. No PAS-positive cells were seen at 20 weeks in the completely keratinized conjunctival epithelium of vitamin A–deficient rat conjunctiva (Fig. 3D).

Semiquantitative RT-PCR Analysis of Message Levels of ASGP and rMuc5AC

To compare the mRNA expression of ASGP and rMuc5AC within experimental groups, the linear phase of amplification of each cDNA was demonstrated so that gene expression could be semiquantitatively compared. For ASGP, rMuc5AC, and the control housekeeping gene β-actin the linear phase of amplifi-

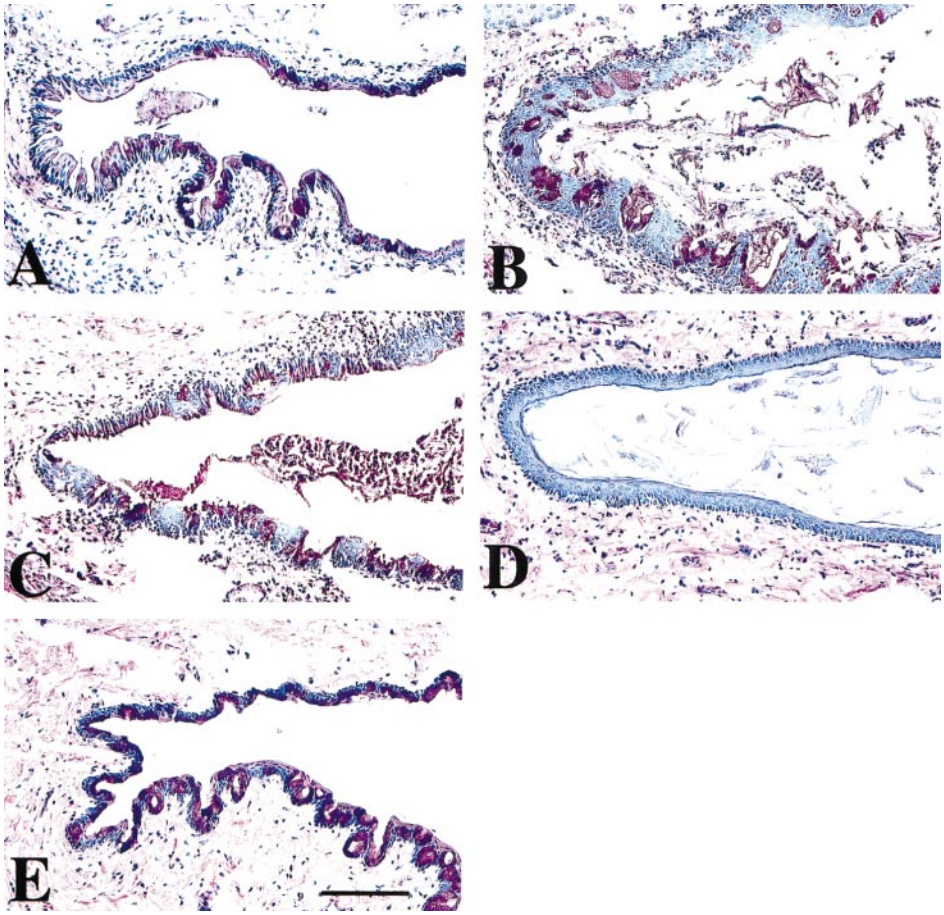

FIGURE 3. PAS staining of sections of rat conjunctival goblet cells in vitamin A–deficient (**A** through **D**) or control (**E**) rats. In rats fed a vitamin A–deficient diet for 1 week (**A**), goblet cells are still prominent. After consuming the vitamin A–deficient diet for 13 weeks, some rats were mildly (**B**) or severely (**C**) affected. Clusters of goblet cells were present in the thickened, partially keratinized epithelium of mildly affected rats (**B**); whereas, in severely affected rats, PAS staining was still evident, but goblet cell morphology was aberrant. In rats fed the deficient diet for 20 weeks, PAS staining and goblet cells were not evident in the conjunctival epithelium (**D**). In 20-week control animals, goblet cells with intense PAS staining were evident (**E**). All micrographs are of the same magnification; bar, 50 μm.

cation was established at 27 cycles. In control rats, ASGP, rMuc5AC, and β-actin mRNA was detected from 1 to 20 weeks after initiation of feeding. ASGP mRNA was not detected in ocular surface tissue at 15, 18, and 20 weeks after initiation of feeding (Fig. 4). In the deficient rats, rMuc5AC was present until 15 weeks, but the amount of mRNA was low. By 18 and 20 weeks, the vitamin A–deficient rats no longer expressed detectable amounts of rMuc5AC (Fig. 4).

Densitometric comparisons of both ASGP and rMuc5AC mRNA to that of β-actin were obtained in each of these groups (Fig. 5). The ratios of ASGP/β-actin mRNA and rMuc5AC/β-actin were higher than 1 until 3 weeks after initiation of feeding, when they both gradually decreased. There were no dramatic ratio changes between 3 and 13 weeks after initiation of feeding. After 13 and 15 weeks, ASGP mRNA and rMuc5AC, respectively, were not detected (Fig. 4), even with 40 cycles of PCR amplification (data not shown).

rMuc1 RT-PCR

rMuc1 mRNA did not amplify in either the experimental or control group at 27 cycles, but all groups showed amplification at 35 cycles. rMuc1 mRNA was detected from 1 to 20 weeks after initiation of feeding in both vitamin A–deficient and control rats (Fig. 4C). There was no apparent change in rMuc1 mRNA expression in either control or vitamin A–deficient rats through the 20 weeks of feeding.

In Situ Hybridization of ASGP

In situ hybridization using ^{35}S-labeled riboprobes for the 3′ domain of ASGP resulted in detection of expression of ASGP in the cells of both the corneal and conjunctival epithelia in control rats and rats taken at 1 week after initiation of feeding the vitamin A–deficient diet (Figs. 1B, 1D). The epithelium of the ocular surface labeled predominantly in the apical cell layers. Label was not obvious within goblet cells; however, at the level of the light microscope, using ^{35}S-labeled probes, label on the stratified cells cannot be distinguished from label at the base of the goblet cell where the nucleus and endoplasmic reticulum are packed tightly against the basal cell surface by the mucin packets. The labeling of ASGP mRNA in the conjunctival epithelium was weaker than the labeling detected in the corneal epithelium at 13 weeks after initiation of feeding in mildly affected deficient rats (Figs. 1F, 1H), whereas in severely affected rats, binding was weak to undetectable in both conjunctiva and cornea (Figs. 1I, 1J). At 20 weeks after initiation of feeding, the expression of ASGP was not detected in either cornea or conjunctiva (Figs. 1L, 1N). The expression of ASGP was detected in the ocular surface epithelial cells in the control rats at all time points (Figs. 1P, 1R). The sense sequence of the same region of ASGP, used as a negative control probe, did not show any binding in control or vitamin A–deficient groups (Figs. 1T, 1V).

In Situ Hybridization of rMuc5AC

In situ hybridization using ^{35}S-labeled riboprobes for the tandem repeat region of rMuc5AC resulted in detection of expression of rMuc5AC in the goblet cells of control-fed rats at all time points examined (Fig. 2J). The expression of rMuc5AC was also detected in the goblet cells at 1 week and 13 weeks after initiation of feeding of the vitamin A–deficient rats (Figs.

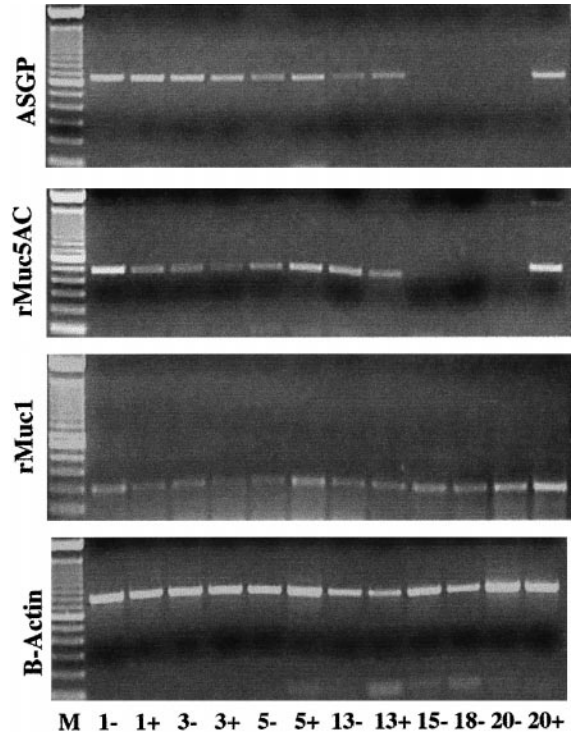

FIGURE 4. Assay of ASGP (rMuc4), rMuc5AC, and rMuc1 by RT-PCR in vitamin A–deficient and control rats. β-actin was used as control. Numbers at bottom indicate weeks consuming a vitamin A–deficient (−) or –replete (+) diet; M, 100-bp DNA marker. Note that ASGP mRNA did not amplify after 13 weeks of a deficient diet. A faint rMuc5AC PCR product was still present at 15 weeks, but was not present after 18 or 20 weeks of a deficient diet.

2B, 2D). In severely affected rats fed for 13 weeks, some conjunctival epithelial cells showed diffuse binding in areas along the apical region of the epithelium where the goblet cells were no longer morphologically identifiable (Figs. 2E, 2F). However, these regions did stain with PAS (Fig. 3C). At 20 weeks after initiation of feeding, expression of rMuc5AC was not detected in the keratinized conjunctival epithelium (Fig. 2H). The sense sequence of the same region of rMuc5AC, used as a negative control probe, did not show any binding in control or vitamin A–deficient groups (Fig. 2L).

DISCUSSION

A major conclusion of the present study is that both the membrane-spanning mucin ASGP and the gel-forming mucin rMuc5AC were downregulated by vitamin A deficiency. The second membrane-spanning mucin, Muc1, was not affected by the absence of vitamin A, even after severe keratinization. These data suggest that the membrane mucin ASGP (rMuc4) and the secretory mucin rMuc5AC are regulated by retinoic acid, whereas, the membrane-spanning mucin rMuc1 is not. This potential regulation of ASGP and rMuc5AC by retinoic acid may be at the transcriptional level with direct retinoic acid receptor interaction with the regulatory regions of the mucin genes. To date, the regulatory regions of neither ASGP nor rMuc5AC have been characterized, but data from cultured human tracheal cells show induction of MUC5AC mRNA by retinoids. These latter data suggest that there is potential direct transcriptional regulation of MUC5AC by retinoic acid.[27]

Retinoic acid receptors are members of the superfamily of nuclear receptors activated by lipophilic ligands. These receptors are of two categories: retinoic receptors (RARs) and retinoid X receptors (RXRs). Recently, Bossenbroek et al.[5] have demonstrated the presence of mRNA for all subtypes of RARs and RXRs in rabbit corneal epithelium and corneal and conjunctival fibroblasts. These data suggest that appropriate receptors are available within ocular surface tissues that would allow direct regulation of the mucin genes by vitamin A. Proof of retinoic acid regulation of the mucin genes within ocular surface epithelium awaits characterization of the regulatory region of each mucin gene and development of culture systems of ocular surface epithelia that would be useful for experimental analysis of mucin gene regulation.

A second major conclusion of this study is that mucin gene expression was downregulated as keratinization of the epithelium occurred, and on complete keratinization, expression of mucin genes ASGP and rMuc5AC was entirely lost. These data suggest a second potential method of regulation of the expression of the mucin genes. Their expression may be regulated indirectly or secondarily because of the alteration of the differentiation pathway of the epithelia. If the differentiation of a nonkeratinizing epithelium is switched to a keratinizing epithelial differentiation mode, as is the case in vitamin A deficiency, a whole set of genes is turned on or off. These genes may indirectly affect mucin gene expression. In support of this hypothesis is the demonstration that retinoids through RARs and RXRs inhibit mRNA expression of a group of squamous differentiation markers including transglutaminase type I, involucrin, keratin 5, and keratin 13 by cultured human bronchial epithelium.[31]

Our data regarding the effect of vitamin A deficiency on *rMuc5AC* gene expression are compatible with previous reports that retinol deficiency is associated with a reduction in the goblet cell population[11] and other studies that show ab-

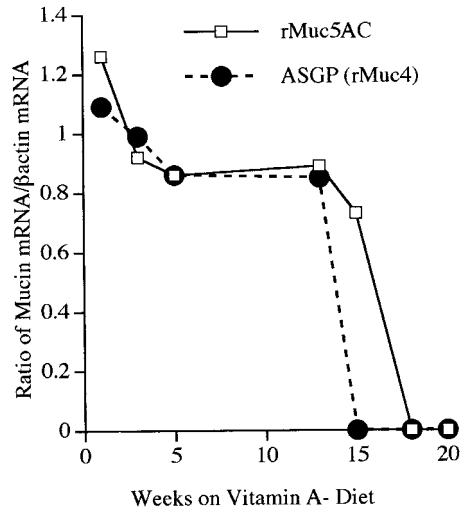

FIGURE 5. Semiquantitative RT-PCR analysis of ASGP and rMuc5AC mRNA in vitamin A–deficient rats compared with control rats. The ratio is the level of ASGP or rMuc5AC RT-PCR product divided by the level of β-actin PCR product, all from mRNA from vitamin A–deficient ocular surface tissue, which is, in turn, divided by the same ratio obtained for control-fed animals. The ratio was higher than 1 at and between 1 and 3 weeks after initiation of feeding. There was no apparent change of the ratio between 3 and 13 weeks. After 13 weeks, the ratio decreased rapidly.

sence of mucin expression in retinoid-deficient tracheal cultures that are characterized by squamous differentiation.[23] The data also demonstrate that the alteration in glycoconjugate expression—for example, mucin expression—is at the transcriptional level rather than at the level of glycosylation of the mucin, because mucin mRNA is lost.

Data from this study show that mucin gene expression is altered not only in goblet cells but in the stratified epithelium of cornea and conjunctiva as well. In the deficient rats of this study, the ASGP mucin mRNA of the stratified epithelium disappeared before the goblet cell mucin rMuc5AC mRNA. These findings may relate to the observation in humans that corneal xerophthalmia responds more rapidly to vitamin A deficiency than does goblet cell return and response.[10] Perhaps the stratified epithelial cells respond more rapidly to vitamin A than does the goblet cell population. Alternatively, the goblet cell stem cell population may require considerably more time to differentiate than does the stratified cell population.

In summary, we show that the expression of ocular surface mucins was downregulated in the vitamin A–deficient rat. We demonstrated that mRNA for rMuc5AC and ASGP were not detected after the appearance of severe keratinization, whereas rMuc1 mRNA expression was not affected by vitamin A deficiency. The expression of the membrane-spanning mucin ASGP and the gel-forming mucin rMuc5AC may be regulated by retinoic acid.

References

1. Fuches E, Green HJ. Regulation of terminal differentiation of cultured human keratinocytes by vitamin A. *Cell.* 1981;25:617–624.
2. Lotan R. Effects of vitamin A and its analogs (retinoids) on normal and neoplastic cells. *Biochim Biophys Acta.* 1980;605:33–91.
3. Peck GL, Elias PM, Wetzel B. Effect of retinoic acid on embryonic chick skin. *J Invest Dermatol.* 1977;69:463–476.
4. Tseng SCG, Hirst LW, Farazdaghi M, Green WR. Inhibition of conjunctival transdifferentiation by topical retinoids. *Invest Ophthalmol Vis Sci.* 1987;28:538–542.
5. Bossenbroek NM, Sulahian TH, Ubels JL. Expression of nuclear retinoic acid receptor and retinoid X receptor mRNA in the cornea and conjunctiva. *Curr Eye Res.* 1998;17:462–469.
6. Moore T, Holmes PD. The production of experimental vitamin A deficiency in rats and mice. *Lab Anim.* 1971;5:239–250.
7. Tseng SCG, Hatchell D, Tierney N, Huang AJW, Sun TT. Expression of specific keratin markers by rabbit corneal, conjunctival, and esophageal epithelia during vitamin A deficiency. *J Cell Biol.* 1984; 99:2279–2286.
8. van Horn DL, Schutten WH, Hyndiuk RA, Kurz P. Xerophthalmia in vitamin A-deficient rabbits: clinical and ultrastructural alterations in the cornea. *Invest Ophthalmol Vis Sci.* 1980;19:1067–1079.
9. Rao V, Friend J, Thoft RA, Underwood BA, Reddy PR. Conjunctival goblet cells and mitotic rate in children with retinol deficiency and measles. *Arch Ophthalmol.* 1987;105:378–380.
10. Sommer A, Green WR. Goblet cell response to vitamin A treatment for corneal xerophthalmia. *Am J Ophthalmol.* 1982;94:213–215.
11. Hatchell DL, Sommer A. Detection of ocular surface abnormalities in experimental vitamin A deficiency. *Arch Ophthalmol.* 1984; 102:1389–1393.
12. Hassell JR, Newsome DA, De Luca LM. Increased biosynthesis of specific glycoconjugates in rat corneal epithelium following treatment with vitamin A. *Invest Ophthalmol Vis Sci.* 1980;19:642–647.
13. Gipson IK, Inatomi T. Mucin genes expressed by the ocular surface epithelium. *Prog Ret Eye Res.* 1997;16:81–98.
14. Gendler SJ, Spicer AP. Epithelial mucin genes. *Annu Rev Physiol.* 1995;57:607–634.
15. Inatomi T, Spurr–Michaud S, Tisdale AS, Gipson IK. Human corneal and conjunctival epithelia express MUC1 mucin. *Invest Ophthalmol Vis Sci.* 1995;36:1818–1827.
16. Inatomi T, Spurr–Michaud S, Tisdale AS, Zhan Q, Feldman ST, Gipson IK. Expression of secretory mucin genes by human conjunctival epithelia. *Invest Ophthalmol Vis Sci.* 1996;37:1684–1692.
17. Gipson IK, Ho SB, Spurr–Michaud SJ, et al. Mucin genes expressed by human female reproductive tract epithelia. *Biol Reprod.* 1997; 56:999–1011.
18. Moniaux N, Nollet S, Porchet N, Degand P, Laine A, Aubert J-P. Complete sequence of the human mucin MUC4: a putative cell membrane-associated mucin. *Biochem J.* 1999;338:325–333.
19. Wu K, Fregien N, Carraway KL. Molecular cloning and sequencing of the mucin subunit of a heterodimeric, bifunctional cell surface glycoprotein complex of ascites rat mammary adenocarcinoma cells. *J Biol Chem.* 1994;269:11950–11955.
20. Price–Schiavi SA, Meller D, Jing X, et al. Sialomucin complex at the rat ocular surface: a new model for ocular surface protection. *Biochem J.* 1998;335:457–463.
21. Inatomi T, Tisdale AS, Zhan Q, Spurr–Michaud S, Gipson IK. Cloning of rat Muc5AC mucin gene: Comparison of its structure and tissue distribution to that of human and mouse homologues. *Biochem Biophys Res Commun.* 1997;236:789–797.
22. Guzman K, Bader T, Nettesheim P. Regulation of MUC5 and MUC1 gene expression: correlation with airway mucous differentiation. *Am J Physiol.* 1996;14:L846–L853.
23. Jetten AM, Rearick JI, Smits HL. Regulation of differentiation of airway epithelial cells by retinoids. *Biochem Soc Trans.* 1986;14: 930–933.
24. Manna B, Lund M, Ashbaugh P, Kaufman B, Bhattacharyya SN. Effect of retinoic acid on mucin gene expression in rat airways in vitro. *Biochem J.* 1994;297:309–313.
25. Rearick JI, Deas M, Jetten AM. Synthesis of mucous glycoproteins by rabbit tracheal cells in vitro: modulation by substratum, retinoids and cyclic AMP. *Biochem J.* 1987;242:19–25.
26. Wu R, Nolan E, Turner C. Expression of tracheal differentiation functions in serum-free hormone-supplemented medium. *J Cell Physiol.* 1985;125:167–181.
27. Guzman K, Gray TE, Yoon J–H, Nettesheim P. Quantitation of mucin RNA by PCR reveals induction of both MUC2 and MUC5AC mRNA levels by retinoids. *Am J Physiol.* 1996;271:L1023–L1028.
28. Shams BKS, Hanninen LA, Chaves HV, et al. Effect of vitamin A deficiency on the adhesion of rat corneal epithelium and the basement membrane complex. *Invest Ophthalmol Vis Sci.* 1993; 34:2646–2654.
29. Gipson IK, Spurr–Michaud S, Moccia R, et al. MUC4 and MUC5B transcripts are the prevalent mucin mRNAs of the human endocervix. *Biol Reprod.* 1999;60:58–64.
30. Nakayama H, Yokoi H, Fujita J. Quantification of mRNA by nonradioactive RT-PCR and CCD imaging system. *Nucleic Acids Res.* 1992;20:4939.
31. Lee H-Y, Dawson MI, Walsh GL, et al. Retinoic acid receptor- and retinoid X receptor-selective retinoids activate signaling pathways that converge on AP-1 and inhibit squamous differentiation in human bronchial epithelial cells. *Cell Growth Diff.* 1996;7:997–1004.