Role of retinoid receptors in the regulation of mucin gene expression by retinoic acid in human tracheobronchial epithelial cells

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To investigate which retinoid receptors are critical in the regulation by all-*trans*-retinoic acid (RA) of the mucin genes *MUC2*, *MUC5AC* and *MUC5B* in cultured normal human tracheobronchial epithelial (NHTBE) cells, we used pan-RAR-, pan-RXR- and RAR- isotype (α, β and γ)-selective agonists and RARα- and RARγ-selective antagonists (RAR is RA receptor and RXR is retinoid X receptor). RAR-, RARα- and RARγ-selective agonists strongly induced mucin mRNAs in a dose-dependent manner, while the RARβ-selective retinoid only weakly induced mucin gene expression at very high concentrations (1 μM). The pan-RXR-selective agonist by itself did not induce mucin gene expression, but acted synergistically with suboptimal concentrations of the pan-RAR agonist. A retinoid with selective anti-activator-protein-1 activity only marginally induced mucin gene expression. The RARα antagonist strongly inhibited mucin gene induction and mucous cell differentiation caused by RA and by the RARα- and RARγ-selective retinoids. In contrast, the RARγ antagonist only weakly inhibited RARα-selective-retinoid-induced mucin gene expression, but completely blocked mucin gene expression induced by the RARγ-selective retinoid. Our studies indicate that RARα is the major retinoid receptor subtype mediating RA-dependent mucin gene expression and mucous cell differentiation, but that the RARγ isotype can also induce mucin genes. Furthermore these studies suggest that RARβ is probably not (directly) involved in RA-induced mucin gene expression.

Key words: cell culture, differentiation, mucin secretion.

INTRODUCTION

Retinoids, analogues of vitamin A, play a pivotal role in cell growth, differentiation and programmed cell death, and are essential for normal development (for review, see [1]). In the respiratory tract they control the development and maintenance of mucociliary differentiation of the airway epithelium which lines the conducting airways.

The effects of all-*trans*-retinoic acid (RA) are mediated through two families of retinoid receptors, the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs), which belong to the superfamily of steroid/thyroid hormone receptors (for review, see [2]). The two subfamilies of retinoid receptors each consist of three receptor isoforms, designated RARα, β, γ and RXRα, β, γ (for review, see [3]). RA has been shown to bind to all three RARs, and its metabolite 9-*cis*-RA is the only known natural ligand to bind to RXRs. RAR/RXR heterodimers and RXR homodimers act as transcriptional regulators (for review, see [4]). The ligand-bound receptor dimers recognize RA response elements (RAREs) in the promoter–enhancer regions of target genes [5] and control their expression.

Mucins are high-molecular-mass glycoproteins which are the major components of mucus produced by the epithelia of the respiratory, gastrointestinal and reproductive tracts. They are responsible for the visco-elasticity and hydrophilicity of secreted mucus, and provide lubrication and protection against injurious agents for the mucous membranes which produce them. To date nine human mucin genes have been identified (for a recent review, see [6]).

We reported previously that treatment of retinoid-deprived human tracheobronchial cell cultures with RA induced the expression of the mucin genes *MUC2*, *MUC5AC* and *MUC5B* in a time- and dose-dependent manner [7]. We chose to study these three mucin genes because MUC5AC and MUC5B are major constituents of airway secretions [8,9], and because MUC2 and MUC5AC have been shown to be subject to regulation by a variety of inflammatory mediators [10–12] and bacterial lipopolysaccharides [13].

The purpose of the present studies was to determine which retinoid receptors and receptor isoforms are involved in RA-induced mucin gene expression in human bronchial epithelial cells. Using retinoid-receptor-selective agonists and antagonists, we demonstrate that RA-induced mucin gene expression and mucous cell differentiation of airway epithelial cells is mediated through RARα, and to a lesser extent through RARγ.

EXPERIMENTAL

Air–liquid interface cultures and retinoids

Normal human tracheobronchial epithelial (NHTBE) cells (strains 2002 and 17684; Clonetics, San Diego, CA, U.S.A.) from passage 2 were seeded at a density of 1×10^5 cells per insert on to 24 mm-diam. semi-permeable membrane inserts (Transwell-

Abbreviations used: RA, all-*trans*-retinoic acid; RAR, RA receptor; RXR, retinoid X receptor; NHTBE cells, normal human tracheobronchial epithelial cells; RARE, retinoic acid response element; AP-1, activator protein-1; RT-PCR, reverse transcriptase–PCR; β2M, β_2-microglobulin; the suffix 'sel' indicates a selective agonist for the preceding receptor type (e.g. RARsel); the suffix 'ant' indicates a selective antagonist for the preceding receptor type (e.g. RARαant); anti-AP-1, anti-AP-1-selective retinoid (SR11302).

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clear; Costar, Cambridge, MA, U.S.A.) in serum-free, hormone- and growth factor-supplemented media, and grown as described previously [14], except that the membranes were not coated with collagen gel (see [15]). NHTBE cells were grown in the absence of retinoids for 7 days and then treated with the indicated retinoid for 7 days, with a change of medium every 24 h.

RA was purchased from Sigma (St. Louis, MO, U.S.A.). The RAR-selective retinoid SRI-6751-84 [4-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-anthracenyl)benzoic acid; 'RARsel'] [16], the RXR-selective retinoid SR11217 {4-[1-(5,6,7,8-tetrahydro-5,5,8,8,-tetramethyl-2-naphthalenyl)-2-methylpropenyl]benzoic acid; 'RXRsel'} [17], the RARγ-selective retinoid 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalenecarboxylic acid ('RARγsel') [18,19], also designated CD437, the anti-activator-protein-1 (AP-1)-selective retinoid SR11302 [(*E*)-3-methyl-9-(2,6,6-trimethylcyclohexenyl)-7-(4-methylphenyl)-2,4,6,8-nonatetraenoic acid; 'anti-AP-1'] [20,21] and the RARγ-selective antagonist CD2665 [4-(6-methoxyethoxymethoxy-7-adamantoyl-2-naphthyl)benzoic acid; 'RARγant'] [22,23] were generously donated by Dr. Marcia I. Dawson (SRI International, Menlo Park, CA, U.S.A.). The RARα-selective retinoid Am580 [Ro40-6055; 4-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-6-naphthalenyl carboxamido)benzoic acid; 'RARαsel'] [18,24,25], the RARβ-selective retinoid Ro48-2249 ('RARβsel') [26] and the RARα antagonist Ro41-5253 {6-[1-(4-carboxyphenyl)propen-2-yl]-3,-4-dihydro-4,4-dimethyl-7-heptyloxy-2H-benzothiopyrene-2,2-dioxide; 'RARαant'} [25] were provided by Hoffmann-La Roche (Nutley, NJ, U.S.A.). RA and selective retinoids were dissolved in DMSO; the final DMSO concentration in the media was never greater than 0.1 % (v/v). Control cultures were treated with vehicle only.

Immunodetection and quantification of secreted mucin

To monitor mucous differentiation, apical secretions were analysed for mucins. Quantitative dot-blotting for detection of mucin using H6C5 antibodies and purified human mucin from cystic fibrosis sputum as a mucin standard (0.85 mg dry weight of purified mucin per ml) has been described previously in detail [14]. The data are expressed as percentages of secreted mucin of RA-treated cultures, and are means $\pm$ S.D. of triplicate cultures. Analysis of variance with a subsequent Duncan's test was used to determine significant differences in multiple comparisons [27]. Values of $P < 0.01$ were considered to be significant.

Reverse transcriptase–PCR (RT-PCR) analysis of mRNA of mucin genes and isotypes of retinoid receptors

Methods to detect MUC2, MUC5AC and MUC5B mRNA levels using RT-PCR have been reported previously in detail [7,28]. Briefly, total RNA isolated from pools of triplicate cultures using Tri-Reagent (Molecular Research Center, Cincinnati, OH, U.S.A.) was reverse-transcribed into cDNA using random hexanucleotide primers and Moloney-murine-leukaemia virus reverse transcriptase (Perkin–Elmer, Morrisville, NC, U.S.A.). PCRs for MUC2, MUC5AC and MUC5B were performed using mucin-gene-specific amplimers (see Table 1) in the presence of an internal standard, a so-called MIMIC [28,29]. A MIMIC is a double-stranded DNA with ends that are complementary to the specific target gene primers and which contains different lengths of 'stuffer' sequences having no homology with the target gene sequences. The ratio of the signal intensity of target cDNA to MIMIC was used to estimate the relative abundance of mucin mRNAs among the various samples. The details of using MIMICs as internal competitive standards in the amplification reaction to measure changes in mucin gene mRNA levels were described previously [28]. Since *MUC5AC* and *MUC5B* are usually induced concomitantly by RA or retinoids, we measured both mucin genes only in the most critical experiments.

Table 1 Primers used for PCR amplification

F, forward; R, reverse amplification primer.

Gene product	GenBank no.	Primer sequence	Product (bp)	Refs.
MUC2	L21998	F: TGCCTGGCCCTGTCTTTG R: CAGCTCCAGCATGAGTGC	438	[40]
MUC5AC	U06711	F: TCCGGCCTCATCTTCTCC R: ACTTGGGCACTGGTGCTG	680	[41]
MUC5B	Z72496	F: ACTCCAGAGACTGTCCACAC R: TACCACTGGTCTGTGTGCTA	388	[42]
RARα	X56058	F: GACCGGCTCTTGAGACATCC R: GGTGCTGGAGAGTGGTCAGA	372	[43,44]
RARβ	X07282	F: GGAACGCATTCGGAAGGCTT R: GGAAGACGGACTCGCAGTGT	383	[44,45]
RARγ	M24857	F: AGAAAGAGGTGAAGGAAGAAG R: AATGCTGAGCCCTGTAAAG	266	[46]
RXRα	X52773	F: TCCTTCTCCCACCGCTCCATC R: CAGCTCCGTCTTGTCCATCTG	169	[44,47]
RXRβ	X63522	F: TGGAACAGAAGAGTGACCAG R: TGAAAAGGAGGCAATGAG	224	[48]
RXRγ	U38480	F: TGTCCACAGGGAAGCCAATGG R: GGGTGGGGCAACCAAGTTG	194	[49]

PCRs for RARs and RXRs were performed by standard methods recommended by the RT-PCR kit supplier (Perkin-Elmer) in the presence of 1.5 mM $MgCl_2$ with 0.2 μM oligonucleotide primers designed according to the published sequences (see Table 1). The amplification conditions were as follows: 30 s at 95 °C, 1 min at 55 °C and 1 min at 72 °C, using 25 cycles for RARα, RARβ and RXRβ, 30 cycles for RARγ and RXRα, and 32 cycles for RXRγ, followed by a final extension for 20 min at 72 °C. The efficiency of PCRs using primers for the different receptor cDNAs may not be the same. Therefore preliminary experiments were conducted with RNA isolated from RA-deficient and RA-sufficient cultures to find the optimum number of PCR cycles and to establish that the amplification of products lay within the linear range of the PCR. Oligonucleotide amplimers for the β_2-microglobulin (β2M) gene, which was used as a control gene for RT-PCR, were purchased from Clontech. Amplified products were electrophoresed on a 2 % agarose gel in the presence of ethidium bromide (50 ng/ml), and the image was captured by an IS-1000 digital imaging system (Alpha Innotech Co., San Leandro, CA, U.S.A.). The intensity of the bands was analysed using IA-200 image analysis software provided by the manufacturer. Specific amplification of mucin genes and retinoid receptors was confirmed by sequencing the PCR fragments. Sequences of the amplification primers are summarized in Table 1.

RESULTS

Effects of RA on expression of retinoid receptor isotypes

Expression of retinoid receptors is developmentally regulated and varies greatly among different cell types (for review, see [3]). To determine which receptor isotypes are expressed in NHTBE cell cultures and whether their expression is altered by treatment

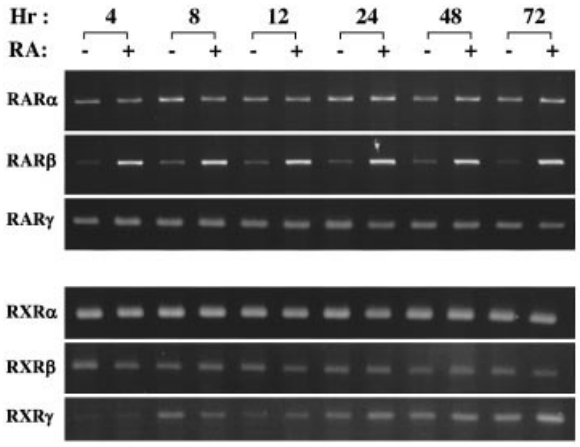

Figure 1 Expression of RAR and RXR isotypes in NHTBE cells

NHTBE cells were cultured for 7 days in RA-free media and then treated with 1 μM RA for the indicated times ('Hr'). Control cultures (—) were sham-treated. Expression of RARα, RARβ, RARγ, RXRα, RXRβ and RXRγ mRNAs was determined by RT-PCR using total RNA.

with RA, total RNA was isolated from cultures grown in the absence of RA or following treatment with 1 μM RA, and was subjected to PCR analysis. In this and all subsequent experiments, RNA from triplicate cultures was pooled. As shown in Figure 1, all major receptor isotypes were expressed in the cultures, regardless of the presence or absence of RA. With the exception of RARβ, the expression of retinoid receptor mRNAs was not measurably affected by RA treatment. RARβ mRNA was clearly increased as early as 4 h after RA treatment and remained elevated for the remainder of the experiment. The increase was approx. 2.6–7.9-fold over a 72 h period in experiment 1 and 2.9–6-fold over a 168 h period in experiment 2, as determined by image analysis of the intensity of the PCR products.

Induction of mucin gene expression and mucous differentiation by RA and RAR-selective retinoids

To determine which retinoid receptors play a major role in the induction of mucin gene expression, NHTBE cells grown in RA-free media were treated with 100 nM RA, RARsel or RXRsel. Some cultures were treated with anti-AP-1 to determine whether the induction of mucin gene expression involves RARE-dependent trans-activation or trans-repression of AP-1 activity. In this and all subsequent studies, unless indicated otherwise, the cells were grown for 7 days in retinoid-free medium; the cultures were then treated with the indicated agent, while control cultures were treated with DMSO only. After 7 days of treatment, RNA was extracted and the levels of expression of MUC2, MUC5AC, MUC5B and β2M were determined by quantitative RT-PCR. To monitor the effects of treatment on mucous differentiation, we determined the amount of apically secreted mucin. As shown in Figure 2(A), mucin mRNA levels were very low or undetectable in RA-free cultures. RA and RARsel treatment strongly induced mucin gene expression. In contrast, RXRsel did not induce mucin mRNAs, and anti-AP-1 induced mucin mRNAs only very weakly. The control gene β2M was not affected by any of the treatments. RA and RARsel also induced mucous differentiation, as indicated by increased mucin secretion (Figure 2B). Treatment with RXRsel or anti-AP-1 failed to induce mucous differentiation. The results were found to be highly reproducible (the strong induction of mucin genes by RAR, but not by RXR, is also seen in Figure 3A). These findings suggest that ligand binding to RARs alone, but not to RXRs alone, is sufficient to induce mucin gene expression and mucous differentiation, and that this does not involve trans-repression of AP-1 activity.

To determine whether RARsel and RXRsel act co-operatively

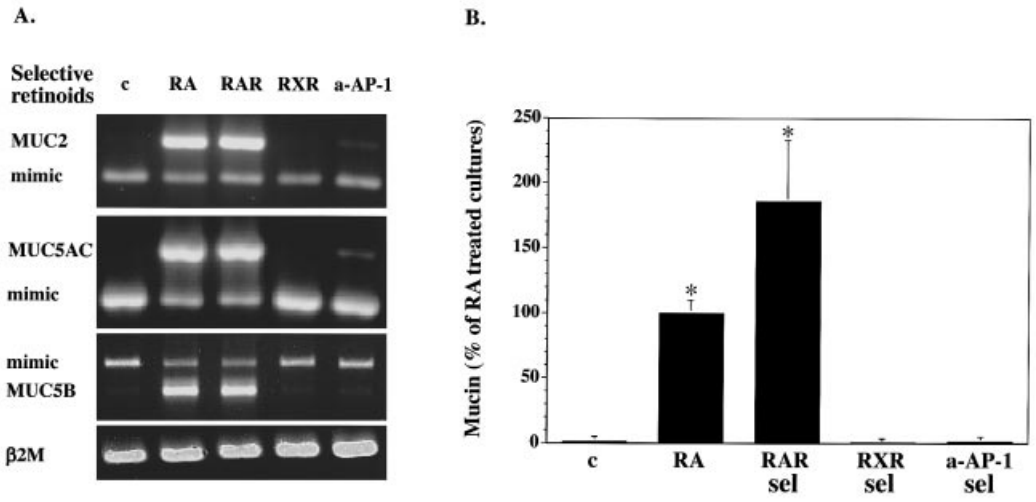

Figure 2 Induction of mucin gene expression and mucous differentiation by RAR-selective retinoids

NHTBE cells were cultured for 7 days in RA-free media and then treated with 100 nM RA, RARsel, RXRsel or anti-AP-1 (a-AP-1) for 7 days. Control cultures (c) were sham-treated. (**A**) MUC2, MUC5AC, MUC5B and β2M mRNA levels were determined by RT-PCR. The control gene, β2M, was not affected by retinoid treatment. (**B**) At 7 days after the start of treatment, apical secretions were collected and mucin was measured by immunoblotting. Data are expressed as percentages of mucin secretion by RA-treated cultures (202.1 μg of mucin per culture). The results shown are representative of two independent experiments, and are means $\pm$ S.D. of triplicate cultures (*$P < 0.01$).

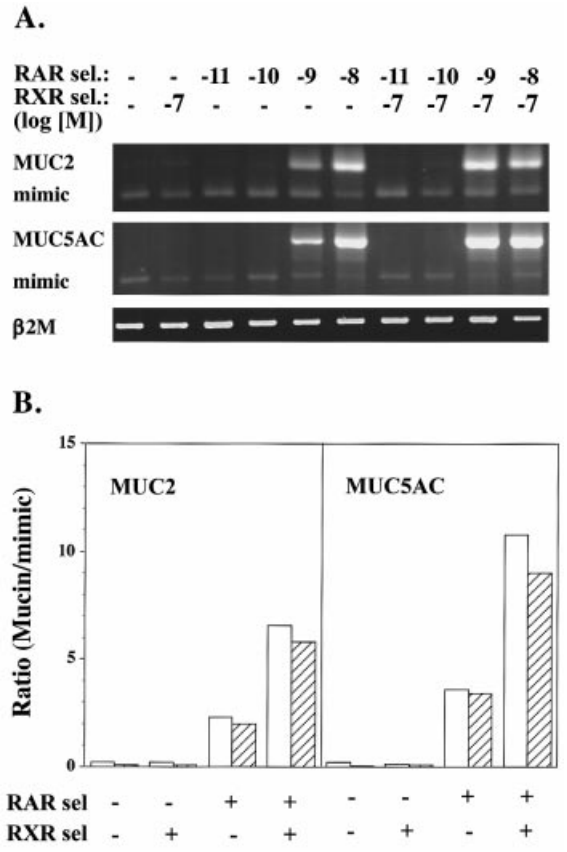

Figure 3 Synergistic effects of RAR- and RXR-selective retinoids on MUC2 and MUC5AC mRNA expression

NHTBE cells were grown for 7 days in RA-free media and then treated with either RARsel or RXRsel alone or with RXRsel together with various concentrations of RARsel for 7 days. (**A**) MUC2, MUC5AC and β2M mRNA levels were determined by RT-PCR. β2M was not affected by retinoid treatment. (**B**) Quantification of MUC2 and MUC5AC mRNA levels in two independent experiments (open and hatched bars respectively). The ratio of the signal intensity of target cDNA to MIMIC was used to estimate the relative abundance of mucin mRNAs (see the Experimental section).

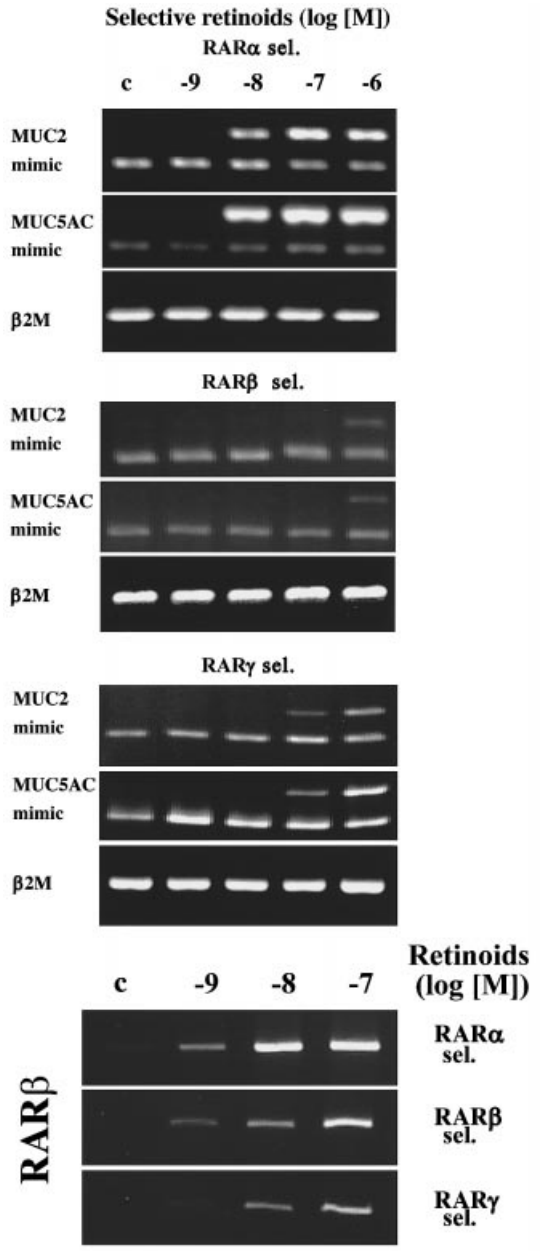

Figure 4 Concentration-dependent induction of mucin gene expression by RARα-, RARβ- and RARγ-selective retinoids

NHTBE cells were cultured for 7 days in RA-free media and then treated with various concentrations of RARαsel, RARβsel or RARγsel for 7 days (c, control). MUC2, MUC5AC and β2M mRNA levels were determined by RT-PCR. The levels of RARβ mRNA were also determined after treatment with RAR-selective retinoids (bottom panel). The results were confirmed in an independent experiment.

or synergistically, retinoid-free cultures were treated either with various concentrations of RARsel (0.01–10 nM) in the presence or absence of RXRsel (100 nM) or with RXRsel alone (100 nM). As can be seen in Figure 3(A), 100 nM RXRsel alone did not induce either MUC2 or MUC5AC mRNAs. RARsel strongly induced mucin gene expression at 10 nM. Induction was evident at 1 nM, but was clearly weaker, and at 0.1 and 0.01 nM RARsel, mucin mRNAs were not expressed. Only at the sub-optimal concentration of 1 nM RARsel did the addition of RXRsel significantly enhance RARsel-induced mucin gene expression. As shown in Figure 3(B), which summarizes the results of two independent experiments, co-treatment with RAR (1 nM) and RXR (100 nM) increased both MUC2 and MUC5AC mRNAs by approx. 3-fold compared with treatment with RARsel alone.

RARα- and RARγ-mediated induction of mucin gene expression

To determine which RAR isotypes are most critical, cultures grown in RA-free media were treated with retinoids selective for RARα, RARβ or RARγ. As shown in Figure 4, RARαsel strongly induced mucin genes at 10 nM, a concentration several-fold lower than its IC_{50} (39 nM; [25,26]). RARβsel increased mucin mRNA levels only weakly at 1 μM, a concentration much higher than its IC_{50} (28 nM; [26]). At this high concentration of RARβsel, induction could be due to binding to and activation of RARα and RARγ. RARγsel induced mucin gene expression at 100 nM, a concentration close to its K_d (77 nM; [19]). Thus, relative to the binding affinities for their respective receptors, the effectiveness with which these RAR-isotype-selective retinoids

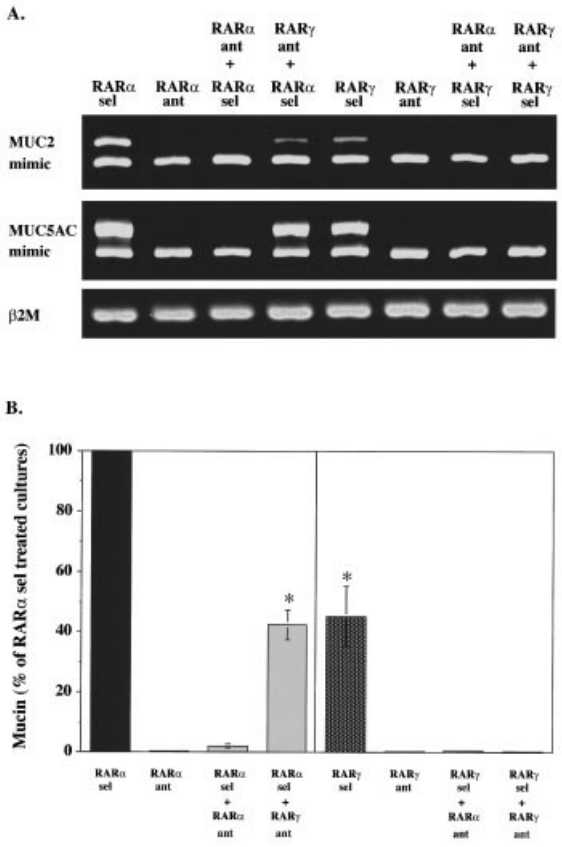

Figure 5 Inhibitory effects of RARα and RARγ antagonists on mucin gene expression and mucous differentiation induced by RAR-isotype-selective retinoids

NHTBE cells were cultured for 7 days in RA-free media and then treated with RAR-isotype-selective retinoids (RARαsel, 10 nM; RARγsel, 100 nM), alone or together with RARαant (1 μM) or RARγant (1 μM) for 7 days. (**A**) Mucin and β2M mRNA levels were determined by RT-PCR. (**B**) Apical mucin was collected and measured by immunoblotting. Mucin secretion is expressed as a percentage of that in RARαsel-treated cultures (115.5 μg of mucin per culture). Representative results of two experiments performed with triplicate cultures are expressed as means ± S.D. Significant difference compared with RARαsel-treated group: $^*P < 0.01$.

induce mucin gene expression is RARαsel > RARγsel ≫ RARβsel.

We also measured the levels of RARβ mRNA after treatment with the RAR-isotype-selective retinoids. All three RAR-selective retinoids induced RARβ mRNA in a dose-dependent manner. RARαsel and RARβsel induced RARβ expression at concentrations of 1 nM and above, while RARγsel induced RARβ mRNA at concentrations of 10 nM and above.

RARα is the major retinoid receptor involved in mucin gene expression

We further explored the role of RARα and RARγ in mucin gene expression using RARα- and RARγ-selective antagonists. RA-free cultures were treated with RARαsel (10 nM) or RARγsel (100 nM) alone or in combination with 1 μM RARαant (Ro41-5253) or RARγant (CD2665) for 7 days. The results of RT-PCR analysis of MUC2 and MUC5AC gene expression are shown in Figure 5(A). RARαsel-induced mucin gene expression was completely inhibited by RARαant, but only partially by RARγant. Both antagonists completely blocked RARγsel-induced mucin gene expression. None of the treatments had any effect on β2M expression. Mucin secretion, which was used as an indicator of mucous differentiation, closely followed the mucin gene expression pattern (Figure 5B). RARαant almost completely suppressed RARαsel-induced differentiation, whereas RARγant only partially suppressed mucous differentiation. On the other hand, both antagonists completely inhibited RARγsel-induced mucous differentiation.

Finally, we determined whether RARαant was also able to inhibit mucin gene expression and mucous differentiation by the pan-agonist RA. As shown in Figures 6(A) and 6(B), RARαant almost completely blocked induction of mucin gene expression and mucous differentiation by RA. We also measured RARβ mRNA levels in this experiment. Interestingly, RARαant, which blocked the induction of mucin genes, had only a minor effect on RA-induced RARβ gene expression. Thus RARβ expression is either not required or not sufficient for induction of mucin genes by RA.

DISCUSSION

The main objective of the present study was to obtain insight into the retinoid signalling pathway involved in the regulation of mucin gene expression and mucous differentiation. We used several retinoid-receptor-selective agonists, which have previously been shown in other cell systems to be useful tools in elucidating RA signalling pathways [21,23,25,30]. Our studies showed that RA and the pan-RAR activator, RARsel, were both highly effective in inducing mucin mRNAs and mucous differentiation. In contrast, the pan-RXR selective retinoid, RXRsel, did not by itself induce mucin mRNA or mucous differentiation. This suggests that activation of RARs is sufficient to induce mucin gene expression and differentiation of NHTBE cells. However, RXRsel enhanced the induction of mucin gene expression in cells treated with suboptimal concentrations of RARsel. Thus RARs and RXRs can act synergistically in the induction of mucin gene expression, indicating the importance of RAR/RXR heterodimeric complexes in the regulation of these genes. RARsel and RXRsel have been shown to act synergistically or co-operatively in several other test systems [31–33]. In the regulation of mucin genes, their trans-activation activity appears to be involved, rather than their trans-repression of AP-1 activity.

Several lines of evidence indicate that the control of gene expression by retinoids is mediated by both receptor-specific [26,34,35] and redundant [36,37] action of RARs and RXRs. We found that mucin gene expression can be induced by both RARα- and RARγ-selective agonists (but not by the RARβ-selective agonist), indicating that both RARα and RARγ can control the expression of these genes. However, induction of mucin genes by RARγsel occurred at relatively high concentrations (100 nM), while RARαsel was effective at 10-fold lower concentrations. We further showed that the RARα-selective antagonist Ro41-5253, a highly specific competitive inhibitor of RARα [25], completely inhibited RARαsel- as well as RARγsel-induced mucin gene expression and also severely suppressed mucin gene expression induced by the pan-agonist RA. In contrast, the RARγ antagonist CD2665 only partially inhibited RARαsel-induced mucin gene expression. We speculate that the effects of the receptor antagonists RARαant and RARγant may be explained as follows. RARαant inhibits not only RARαsel but also RARγsel activity, because the RARα–RARαant/RXR complexes bind to RAREs, blocking the access of RARγ–RARγsel/RXR complexes to RAREs. Conversely, RARγant only partially blocks RARαsel activity, since the RARγ–

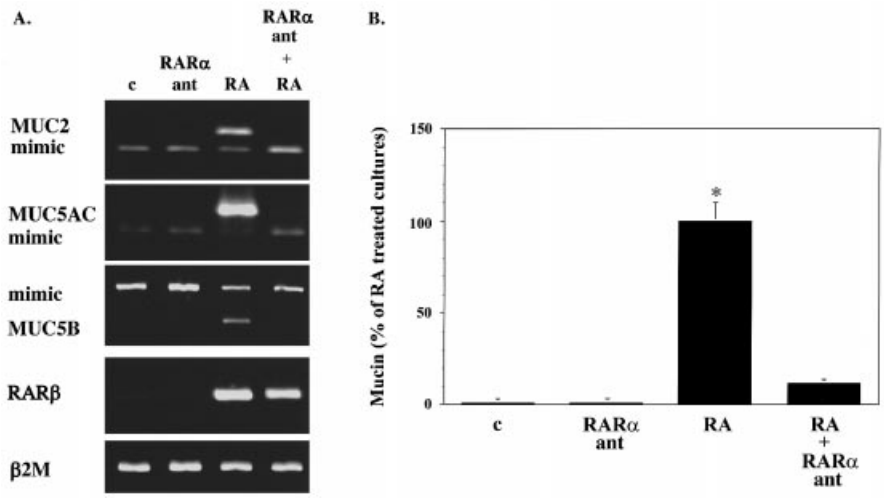

Figure 6 Inhibitory effect of RARαant on RA-induced mucin gene expression and mucous differentiation

NHTBE cells were cultured for 7 days in RA-free media and then treated with RA (10 nM) alone or together with RARαant (1 μM) for 7 days. (**A**) MUC2, MUC5AC, MUC5B, RARβ and β2M mRNA levels were determined by RT-PCR. (**B**) Apically secreted mucin was assayed by immunoblotting. Mucin secretion is expressed as a percentage of that in RA-treated cultures (41.3 μg of mucin per culture). Representative results from two experiments performed with triplicate cultures are expressed as means $\pm$ S.D. Significant difference compared with control (c): $^*P < 0.01$.

RARγant/RXR complexes, because of their low affinity for RAREs, only weakly block binding of RARα–RARαsel/RXR complexes to RAREs. Taken together, our findings suggest that RARα is the prevailing RAR isotype involved in RA-dependent regulation of mucin genes.

It has been shown that the various retinoid receptor isotypes can be differentially regulated by RA (for review, see [3]). Our studies show that, with the exception of RARβ, all RAR and RXR isoforms were constitutively expressed in NHTBE cultures, regardless of the presence or absence of RA. In contrast, RARβ mRNA levels were either very low or undetectable in RA-free cultures and, consistent with findings in other cell systems [38,39], rapidly increased following RA treatment. However, our data suggest that RARβ expression is not a prerequisite for mucin gene induction by RA, because we found that RARβsel strongly induced RARβ mRNA at a concentration (100 nM) that did not induce mucin gene expression.

In summary, our studies suggest that RA-induced mucin gene expression and mucous differentiation are mediated mainly through the RARα/RXR signalling pathway. Selective ligand binding to RARγ also can induce mucin gene expression, suggesting that the activity of RARγ overlaps that of RARα in the regulation of mucin genes by RA. We also showed that activation of RXR alone is insufficient to induce mucin gene expression, but RXR activation enhanced RAR-mediated expression. Our studies further indicate that RARβ is not involved in mucin gene regulation, but may be important in the control of other cell functions in the airway epithelium by RA. This requires further study. Unravelling of the mechanisms regulating mucin gene expression may lead to development of new therapeutic approaches to control mucus hypersecretion.

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