Retinoic acid modulation of glutathione and cysteine metabolism in chondrocytes

Cristina C. TEIXEIRA, Irving M. SHAPIRO*, Masashi HATORI, Ramesh RAJPUROHIT and Cameron KOCH†

Department of Biochemistry, School of Dental Medicine and † Department of Radiation Oncology, School of Medicine, University of Pennsylvania, 4001 Spruce St., Philadelphia, PA 19104-6003, U.S.A.

The major objective of this investigation was to determine the thiol status of chondrocytes and to relate changes in the level of glutathione and cysteine to maturation of the cells as they undergo terminal differentiation. Chondrocytes were isolated from the cephalic portion of chick embryo sterna and treated with all-*trans* retinoic acid for one week. We found that the addition of 100 nM retinoic acid to the cultures decreased the intracellular levels of glutathione and cysteine from 6.1 to 1.6 and 0.07 to 0.01 nmol/μg DNA respectively; retinoic acid also caused a decrease in the extracellular concentration of cysteine. The decrease in chondrocyte thiols was dose and time dependent. To characterize other antioxidant systems of the sternal cell culture, the activities of catalase, glutathione reductase and superoxide dismutase were determined. Activities of all of those enzymes were high in the retinoic acid-treated cells; the conditioned medium also contained these enzymes and the cytosolic isoenzyme of superoxide dismutase. We probed the specificity of the thiol response by using immature caudal chondrocytes. Unlike the cephalic cells, retinoic acid did not change intracellular glutathione and extracellular cysteine levels, although the retinoid caused a reduction in the intracellular cysteine concentration. Finally, we explored the effect of medium components on chondrocyte thiol status. We noted that while ascorbate alone did not change cell thiol levels, it did cause a 4-fold decrease in the extracellular cysteine concentration. When retinoic acid and ascorbic acid were both present in the medium, there was a marked decrease in the level of glutathione. In contrast, the phosphate concentration of the culture medium served as a powerful modulator of both glutathione and cysteine. Results of the study clearly showed that there is a profound decrease in intracellular levels of both cysteine and glutathione and that thiol levels are responsive to ascorbic acid and the medium phosphate concentration. These findings point to a critical role for thiols in modulating events linked to chondrocyte maturation and cartilage matrix synthesis and mineralization.

INTRODUCTION

The increase in tubular bone length is mediated by the activities of cells contained within the epiphyseal growth cartilage. Chondrocytes that populate this transitory structure exist in a spatial gradient that permits inspection of selected stages of cell maturation and cartilage calcification. As chondrocytes achieve their terminally differentiated hypertrophic state, alterations in phenotypic expression include: increased activity of plasma membrane alkaline phosphatase; synthesis of type X collagen and down regulation of type II collagen; secretion of osteonectin; expression of vitamin D receptors; release of matrix vesicles and deposition of mineral in and around these particles [1–5]. Accompanying these phenotypic changes is a concomitant shift in energy metabolism such that the hypertrophic cells generate almost all their energy through glycolytic processes [6–9]. Once hypertrophy is complete, the calcified cartilage is replaced by bone.

Factors that promote phenotypic and metabolic changes during development have not been clearly defined. While there is good evidence to support the view that these shifts reflect expression of the chondrocyte genomic program, other studies indicate that the local micro-environment, especially the availability of oxygen, influences cell maturation [10]. In the chick, the blood supply to mature post-proliferative chondrocytes is limited; however, terminally differentiated hypertrophic cells are provided with an abundant vascular supply [11,12].

The relationship between cell maturation and oxygen metabolism by cells of the growth cartilage was recently examined using a chondrocyte culture system in which retinoic acid and ascorbic acid, in the presence of β-glycerophosphate, were used to drive terminal differentiation. Cultured in the presence of these agents, it was reported that chondrocytes express phenotypic characteristics similar to those observed in the growth cartilage [13]. Importantly, the cells exhibit selected shifts in energetics that are characteristic of the tissue *in vivo* [6–9]. Whether cultured chondrocytes exhibit a change in their reductive reserve has not been determined.

The objective of this investigation is to explore the relationship between retinoid-dependent development of sternal chondrocytes and cellular thiol status. We have focussed on cellular thiols for the following three reasons. First, as retinoic acid modulates energy metabolism in the maturing hypertrophic chondrocyte, there should be significant alterations in the metabolism of glutathione and other reduced thiols. Secondly, recent evidence suggests that developmental changes in phenotypic expression are linked to cellular energetics through a thiol redox system [14–18]. Accordingly, as chondrocytes undergo significant alterations in phenotype, a major change in cellular thiols should be apparent. Thirdly, cells in the growth plate are exposed to zone-dependent changes in oxygen supply, and there is some evidence to show that chondrocytes may be hypoxic; these conditions would be expected to enhance the generation of free radicals [9]. In this case, glutathione and cysteine could promote the dispro-

Abbreviation used: SOD , superoxide dismutase.

* To whom correspondence should be addressed.

portionation of reactive oxygen species [19]. Experiments described in the present paper address each of the issues discussed above.

MATERIALS AND METHODS

Cell culture

Chondrocytes were isolated from the cephalic portion of day 18 chick embryo sterna using the method described by Iwamoto et al. [13]. The freshly isolated chondrocytes were plated in 12-well plates and grown continuously without subculturing for 2–3 weeks in monolayer. During the first two days, cultures received 4 units/ml of testicular hyaluronidase to minimize cell detachment. Cultures were fed every other day with Dulbecco's modified high glucose Eagle's medium (GIBCO) containing 10 % defined fetal calf serum (Hyclone Labs), 2 mM L-glutamine and 50 units/ml each of penicillin/streptomycin. For some studies, chondrocytes were isolated from the caudal portion of day 18 chick embryo sterna as previously described [13,20–22]. These cells were plated and grown in conditions identical to those used for the cephalic sternal cells (see above). After confluence the cultures were treated daily with 0.1 % (v/v) aliquots of 1000 × stock solution of all-*trans* retinoic acid in 95 % ethanol for 1 week. Control cultures were treated with an equal volume of ethanol. In some studies, cells were treated with freshly solubilized ascorbic acid, β-glycerophosphate or P_i (as orthophosphate). The development of the mature chondrocyte phenotype in culture was followed morphologically by phase-contrast microscopy.

Cell cultures were washed with Hanks Balanced Salt Solution and then harvested. For some experiments, the conditioned culture medium was collected. To measure glutathione and cysteine, cells were permeabilized and macromolecules precipitated with 0.1 M 5-sulphosalicylic acid/1 mM EDTA/0.1 mM diethylenetriaminepentaacetic acid/0.1 mM diethyldithiocarbamate; after 15 min, an equal volume of water was added to each sample. As soon as possible (within 24 h), the glutathione and cysteine levels were determined by HPLC. To assess maturation, chondrocytes were solubilized in Triton X-100 and aliquots were used for alkaline phosphatase activity and DNA measurements. Finally, information on enzyme activity was obtained by harvesting the cells in water and then freeze/thawing the cell pellet two or three times. For studies of superoxide dismutase (SOD), endogenous oxidant activity was first removed from the freeze/thawed pellet by dialysis against distilled water for 24 h at 4 °C.

Measurement of glutathione and cysteine

Cellular and conditioned-medium cysteine and glutathione levels were measured by HPLC. Samples were injected on to a reverse-phase column (Alltech Adsorbosphere HS C18 7U; 250 mm × 4.6 mm) at a flow rate of 0.9 ml/min. The solvent was 0.1 M phosphoric acid/3 mM heptane sulphonic acid/5 % methanol (pH 2.0); the system was helium sparged. Thiol peaks were measured using an electrochemical detection system. For details of the methodology, see Koch and Skov [23].

Other enzymic and chemical analyses

SOD (EC 1.15.1.1)

SOD activity was measured spectrophotometrically at 340 nm [24]. Briefly, superoxide was generated at a constant rate and used to oxidize NADPH. The change in the rate of NADPH oxidation in the presence and absence of the sample was then determined. Highly purified SOD (from bovine erythrocytes) was used as a standard for this assay. Measurement of activity was expressed as a percentage of the mean absorbance change in the absence of the enzyme. 1 unit of SOD activity is equivalent to 50 % inhibition.

Chondrocyte SOD was characterized by isoelectric focusing at pH 3.0–9.0 using the Phastsystem (Pharmacia). Following electrophoresis, the gels were stained with riboflavin–Nitro Blue Tetrazolium in the presence of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine and then allowed to reoxidize in air. Since SOD decreases the rate of formation of blue insoluble formazan, absence of stain indicated the presence of the protein. To separate the CN-sensitive Cu–Zn isoenzyme from the Mn isoenzyme, the gels were stained in the presence or absence of cyanide (20 mM). In contrast to Mn, the Cu–Zn isoenzyme was no longer active and the focused protein bands could not be distinguished from the background stain [25].

Catalase (EC 1.11.1.6)

We measured catalase activity by following the decomposition of hydrogen peroxide. As the hydrogen peroxide (30 mM) is converted into water, there is a linear decrease in absorbance at 240 nm [26]. The activity of the enzyme was calculated using the molar extinction coefficient of 0.43×10^2.

Glutathione reductase (EC 1.6.4.2)

Glutathione reductase activity was determined using the procedure described by Goldberg and Spooner [27]. Aliquots of sample were used to reduce glutathione disulphide in the presence of NADPH. The decrease in absorbance, due to the oxidation of NADPH, was followed spectrophotometrically at 339 nm. The activity of the enzyme was calculated using the molar extinction coefficient of 6.27×10^3.

Alkaline phosphatase (EC 3.1.3.1)

Alkaline phosphatase activity was assayed using *p*-nitrophenylphosphate as a substrate in 1.5 M Tris/HCl (pH 9.0)/1 mM $MgCl_2$/1 mM $ZnCl_2$ [4]. Hydrolysis of *p*-nitrophenylphosphate was monitored as a change in absorbance at 410 nm. Alkaline phosphatase activity was expressed as nmol of product/min per 10^6 cells; 1 absorbance unit = 64 nmol of product.

DNA analysis

Samples were treated with Hoechst 33258 dye, and DNA levels were determined using the method described by West et al. [28] modified by Teixeira et al. [29]. Fluorescence was measured in a spectrofluorometer (Photon Technology International) with the excitation and emission wavelengths set at 365 and 460 nm, respectively, and with a 10 nm slit.

Statistical analysis

All experiments were repeated three–five times, and the mean and S.E.M. values were determined. Significant differences between sets of values for control and test groups were assessed by the Student's *t* test. A *P* value refers to a comparison of a measured parameter in the experimental group with that of the appropriate control.

RESULTS

To study the cysteine and glutathione concentrations, as well as antioxidant enzyme activities of cells and of the conditioned culture medium, confluent monolayers of chick cephalic sternal chondrocytes were grown in culture for up to 7 days in the presence of retinoic acid [13]. This agent caused a dose-dependent elevation in alkaline phosphatase activity. At a concentration of 35 nM, which is in the physiological range, the retinoid increased alkaline phosphatase activity nearly 20-fold. Thus the alkaline phosphatase activity of the untreated culture is 1.98 ± 0.24 nmol/min per μg of DNA; in the presence of 35 nM retinoic acid, the activity increased to 37.46 ± 0.42 nmol/min per μg of DNA. When the retinoic acid concentration was 100 nM, the activity was 145.1 ± 9.2 nmol/min per μg of DNA. The elevation in activity is time dependent, and maximum values are observed after 6–9 days in culture (results not shown). It should be noted that, when treated with retinoic acid, immature caudal chondrocytes exhibit minimal changes in alkaline phosphatase activity and there is no evidence of mineral formation (results not shown).

We measured catalase, SOD and glutathione reductase activities of cephalic chondrocytes. The activity of cell glutathione reductase was 0.54 ± 0.02 unit $\times 10^{-3}/\mu$g of DNA. The activity of this enzyme in the medium was 0.65 ± 0.08 unit $\times 10^{-3}/\mu$g of DNA. The catalase activity of chondrocytes was 0.055 ± 0.00 unit/μg of DNA. In the medium, the activity is 0.049 ± 0.01 unit/μg of DNA. Although cell and medium catalase and glutathione reductase levels appeared to decrease with increasing retinoid concentrations, these changes are not significant. With respect to SOD, cellular activity was 1.45 ± 0.00 units/μg of DNA; in contrast, the activity of the conditioned medium was 6.37 ± 0.21 units/μg of DNA. In the presence of retinoic acid, there was some inhibition of dismutase activity in both cells and medium; however, the decrease in activity was not dependent on the retinoic acid dose. We also determined the intracellular and extracellular activities of the three enzymes in terms of the length of time the chondrocytes were maintained in culture with the retinoid. No significant time-related changes were observed (results not shown).

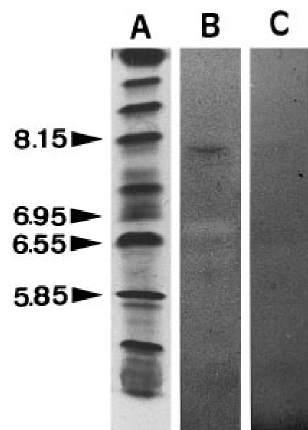

Figure 1 Isoelectric-focussed gel showing the presence of SOD in sternal chondrocytes

Cephalic chondrocytes were treated with retinoic acid (35 nM) for 1 week and then homogenized by two–three freeze/thaw cycles. The homogenate was dialysed for 24 h against distilled water at 4 °C and then lyophilized. Proteins in the lyophilized extract were separated by isoelectric focussing using the Phast system. Gels were stained with riboflavin–Nitro Blue Tetrazolium dye. Lane A, protein standards with pI values ranging from 3–9. The pH gradient from 5.2–7.35 is indicated. Lane B, stained gel showing the two unstained SOD bands at pH 6.4 and 6.7. Lane C, gel stained with riboflavin–Nitro Blue Tetrazolium in the presence of 20 mM CN. The loss of the two unstained bands indicates that the proteins shown in lane B are the Cu–Zn SOD isoenzyme.

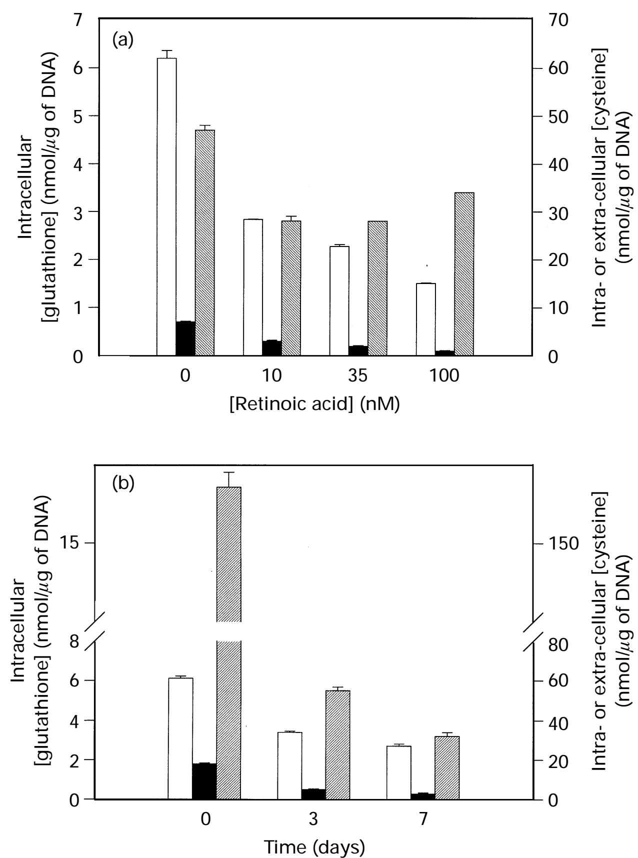

Figure 2 Effect of retinoic acid on glutathione and cysteine levels in cells and conditioned medium

Chick cephalic sternal chondrocytes were maintained in culture until confluent and then treated with physiological doses of retinoic acid (10–100 nM). At selected times (0–7 days), cells were harvested and medium was collected. Cells were analysed for glutathione and cysteine using HPLC; the medium was analysed for cysteine. (**a**) Effect of retinoic acid dose on cell glutathione (open bars), cell cysteine (filled bars) and medium cysteine (hatched bars) levels after 7 days of culture. (**b**) Effect of 35 nM retinoic acid on cell glutathione (open bars), cell cysteine (filled bars) and medium cysteine (hatched bars) levels at 0, 3 and 7 days. *, Significantly different from untreated cells ($P < 0.05$).

Since SOD can exist as the Cu–Zn and Mn isoenzymes, we further characterized the enzyme using isoelectric focussing (Figure 1). The isoenzyme focussed as two unstained bands with pI values at pH 6.7 and 6.4 respectively (Figure 1, lane B). The sensitivity of the isoenzymes to CN (Figure 1, lane C) indicates that the Cu–Zn isoenzyme is the predominant molecular species.

To assess whether the retinoid modulated the level of intracellular thiols, we measured the concentrations of glutathione and cysteine in chondrocytes using HPLC. Figure 2(a) shows that there was a dose-dependent decrease in chondrocyte glutathione levels. Indeed, 35 nM retinoic acid decreased the thiol level from 6.2 to 2.3 nmol/μg of DNA; in the presence of 100 nM retinoic acid, the glutathione level was decreased 4-fold. The rate of decrease was rapid; indeed, by 3 days, the intracellular

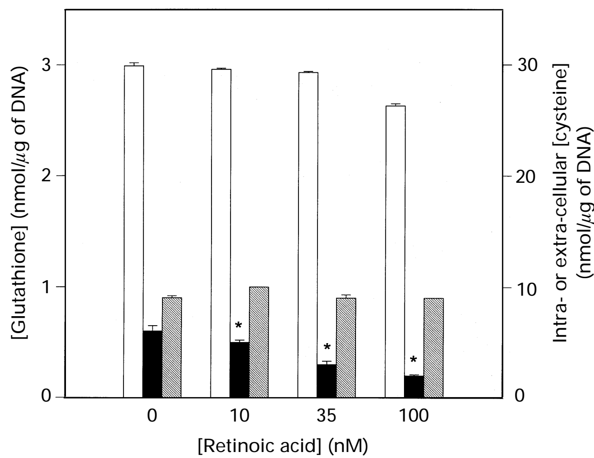

Figure 3 Effect of retinoic acid on glutathione and cysteine levels in caudal chondrocytes and conditioned medium

Caudal sternal chondrocytes were maintained in culture until confluent and then treated with physiological doses of retinoic acid (0–100 nM). At 7 days, the medium was collected and cell glutathione (open bars), cell cysteine (filled bars) and medium cysteine (hatched bars) levels were determined. *, Significantly different from untreated cells ($P < 0.05$).

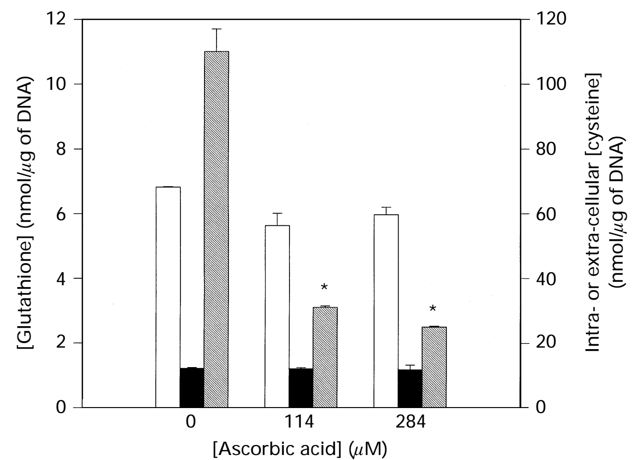

Figure 4 Effect of ascorbic acid on glutathione and cysteine levels in cephalic chondrocytes and conditioned medium

Cephalic sternal chondrocytes were maintained in culture until confluent and then treated with physiological doses of ascorbic acid (114–284 μM). At 7 days, cells were harvested, medium collected and cell glutathione (open bars), cell cysteine (filled bars) and medium cysteine (hatched bars) levels were analysed. *, Significantly different from untreated cell ($P < 0.05$).

glutathione concentration had decreased by 40 %. A smaller but nevertheless significant change in glutathione levels occurred during the following 4 days (Figure 2b). Glutathione was absent from the conditioned medium of the cephalic chondrocytes.

Cysteine was present in the cells and conditioned medium. In concert with glutathione, the concentration of intracellular cysteine was dependent on the retinoic acid concentration (Figure 2a) and length of treatment (Figure 2b). As was noted with glutathione, the effect of the retinoid on cysteine metabolism was very fast. Thus, a 3-fold decrease in the thiol level was seen after 3 days of treatment (from 0.18 ± 0.01 to 0.05 ± 0.00 nmol/μg of DNA); after 7 days the cysteine levels had dropped to 0.030 ± 0.00 nmol/μg of DNA. The effect of retinoic acid on the cysteine content of the conditioned medium is also shown in Figures 2(a) and 2(b). Treatment of the cells with the retinoid and the subsequent lowering of the cellular cysteine levels decreased the medium cysteine content; however, a dose–response relationship was not seen. Thus the effect of 10 nM retinoic acid was similar to 35 nM; when 100 nM retinoic acid was used, the extracellular cysteine level actually increased. Complete medium (no cells) contained undetectable quantities of cysteine or glutathione.

Since retinoic acid had a profound effect on both cysteine and glutathione metabolism, it was important to ascertain if the changes that we observed were specific for terminally differentiated chondrocytes. Accordingly, using immature caudal chondrocytes, we measured the glutathione and cysteine concentrations of caudal cells after treatment with the retinoid. Figure 3 shows that while these cells had a lower concentration of glutathione than did untreated cephalic cells, the retinoid had no effect on caudal chondrocyte glutathione levels; in addition, glutathione was absent from the caudal cell-conditioned medium. With respect to cysteine, the concentration of this amino acid was similar in both caudal and cephalic chondrocytes, and its concentration was dependent on the presence of retinoic acid. However, analysis of the conditioned medium indicated that while cysteine was secreted by these cells, its concentration in the conditioned medium was not dependent on the presence of the retinoid, nor on the length of treatment (results not shown).

While both glutathione and cysteine levels in cephalic sternal chondrocytes are sensitive to retinoic acid, the possibility also exists that these effects may be further modulated by redox-sensitive medium components. Indeed, there is considerable evidence to show that glutathione and ascorbate can form a redox couple, in which ascorbate oxidation can serve to maintain the levels of reduced glutathione [19,30,31]. To explore this possibility, we measured thiol levels in the presence and absence of ascorbate. Figure 4 shows that when cells are treated with 0–284 μM ascorbate, the intracellular glutathione level was maintained at 5.5–6.8 nmol/μg of DNA; thus, ascorbate does not elevate the glutathione levels above the untreated values. The effect of ascorbate on intra- and extra-cellular concentrations of cysteine is shown in the same Figure. Ascorbic acid failed to raise the cysteine concentration of the chondrocytes, although a decrease in the extracellular cysteine levels was apparent. In a parallel study, we decreased chondrocyte glutathione concentration by culturing the cells in the presence of 35 nM retinoic acid. The addition of ascorbate to the retinoid-treated cells served to further decrease cell glutathione levels (Table 1).

Finally, we examined the effect of agents that promote mineralization on cell and medium thiols. We noted that P_i dramatically decreased cellular glutathione and cysteine levels (Figure 5). At a medium concentration of 5 mM, the glutathione concentration had fallen 6-fold, while the extracellular cysteine levels had decreased nearly 3-fold. Intracellular cysteine levels were also significantly depressed by the P_i. β-Glycerophosphate, a common medium supplement, exerted little effect on cell glutathione or cysteine levels (results not shown).

DISCUSSION

The results of the study provide new information on chondrocyte function, survival and maturation in monolayer culture. First, we show that chondrocytes possess a number of enzyme systems that are required for the removal of reactive oxygen species. The

Table 1 Effect of ascorbic acid and retinoic acid on glutathione levels in cephalic chondrocytes

Cephalic sternal chondrocytes were maintained in culture until confluent and then treated with physiological doses of ascorbate (284 μM), retinoic acid (35 nM) and retinoic acid (35 nM) plus ascorbic acid (284 μM) (Asc + RA). At 7 days, cells were harvested and glutathione levels were determined. *, Significantly different from control ($P < 0.05$).

Treatment	Control	Ascorbate	Retinoic acid	Asc + RA
Glutathione (nmol/μg of DNA)	6.82 ± 0.015	5.97 ± 0.226	4.66 ± 0.206*	3.18 ± 0.064*

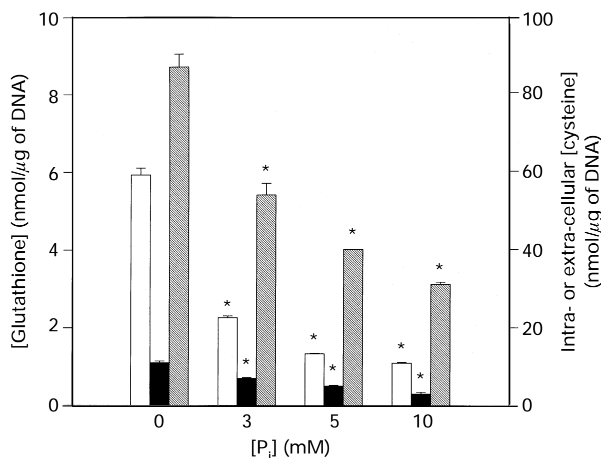

Figure 5 Effect of P_i on glutathione and cysteine levels in cephalic chondrocytes and conditioned medium

Cephalic sternal chondrocytes were maintained in culture until confluent and then treated with physiological doses of P_i (3–10 mM). At 7 days, cells were harvested and medium was collected. Cell glutathione (open bars), cell cysteine (filled bars) and medium cysteine (hatched bars) levels were determined. *, Significantly different from untreated cells ($P < 0.05$).

presence of these enzymes serves to protect cell and extracellular matrix components from oxygen radical attack [32–34]. Secondly, we found that intracellular glutathione levels are regulated by retinoic acid. Thus in the presence of physiological concentrations of the retinoid, there is a profound decrease in both glutathione and cysteine levels. It is probable that the concomitant decrease in both cellular and extracellular cysteine concentrations results from the change in intracellular glutathione metabolism. Thirdly, we noted that caudal chondrocytes contain half as much glutathione as do the cephalic cells and the level of the thiol is not influenced by retinoic acid. The profound differences in thiol metabolism between cephalic and caudal chondrocytes suggest that the retinoid effects are cell specific, possibly related to the acquisition of receptors by cephalic cells. Finally, we show that components of the chondrocyte culture medium, including retinoic acid, ascorbate and P_i, modulate the cell thiol status. This observation is of some physiological importance, as these agents are required for activities that relate to chondrocyte gene expression, macromolecule synthesis and mineral deposition [21,22].

Considering the control of cellular thiol levels, the investigation clearly showed that the retinoid caused a dose-dependent decrease in the intracellular concentrations of glutathione and cysteine. Synthesis of the thiol tripeptide is probably reduced by limitation in the energetic state of the cephalic chondrocyte. Retinoic acid inhibits anaerobic energy metabolism; accordingly, a reduction in glycolysis would limit pentose phosphate shunt activity and lead to a drop in the level of NADPH, a required co-enzyme for glutathione synthesis [22,25]. We confirmed that the change in thiol levels is related to the energy state using cells (caudal chondrocytes) that were only marginally affected by the retinoid [22]. Since there was no observed change in the glutathione status of these immature chondrocytes, these findings added considerable strength to the notion that thiol status is dependent on cellular energy metabolism.

A second mechanism that could serve to decrease the intracellular concentration of glutathione is thiol–disulphide exchange. If this system is activated by the retinoid, then there would be a marked decrease in the intracellular glutathione level, a profound change in the thiol redox ratio and a concomitant increase in the concentration of glutathione disulphide. That this exchange could account for the profound decrease in cellular glutathione levels that we report herein is unlikely; in most tissues, the glutathione disulphide values are micromolar, ranging from one-tenth to one-hundredth of the glutathione values. Indeed, we have estimated glutathione disulphide concentrations in chondrocytes using a procedure that provides a measure of both oxidized and reduced glutathione [36]. Data generated in this way indicate that the oxidized glutathione concentration is low.

Nevertheless, while thiol disulphide exchange cannot account for the low glutathione concentration of retinoid-treated cells, it is noteworthy that if there is a slight increase in glutathione disulphide levels, this change could alter the thiol redox status of the maturing chondrocyte. The effect of the change in thiol redox is of some importance, in terms of the regulation of transcription of genes encoding matrix proteins. For example, binding of the transcription factors Fos and Jun to the transcriptional regulatory element AP-1 is thiol redox sensitive; moreover there is differential expression of c-*fos* and c-*jun* during chondrocyte maturation. Relevant to the expression of these genes, a considerable number of genes that code for extracellular matrix proteins contain the AP-1 regulatory motif [14,17]. In terms of cartilage formation, it was shown that chemical depletion of glutathione caused a significant change in proteoglycan and type II and X collagen synthesis [18]. Since these changes are also characteristic of terminal differentiation of retinoic acid-treated cephalic chondrocytes, the results of our study suggest that a change in glutathione metabolism may provide one mechanism to regulate gene expression in the growth cartilage.

From a functional point of view, the investigation revealed that chondrocytes contain catalase, glutathione reductase and the cytosolic isoenzyme of SOD; moreover, the cells release all of these enzymes into the extracellular medium. It is most likely that these agents serve to protect cells from reactive oxygen species that are generated by the cells themselves, the culture system and possibly some of the medium supplemental agents that are used

to promote expression of the mature chondrocyte phenotype. Of these supplements, probably the most active pro-oxidants are retinoic acid, ascorbate and transitional elements chelated to phosphate ions. Whether these compounds significantly alter chondrocyte glutathione concentration is doubtful, since radical attack would be rapid and result in evidence of acute toxicity. No evidence of toxicity was observed during the 5–7 day culture period. Indeed, the only reported effect of the added phosphate ions was in terms of accelerated mineral deposition. In addition, it should be noted that the activity of alkaline phosphatase, an ectoenzyme that is sensitive to oxidative attack, rises rather than falls during treatment of cells with ascorbate and phosphate ions.

The observation that the conditioned medium contained cysteine and dismutating enzymes confirmed an earlier study by Tschan et al. [37]. These workers commented on the need for these agents for chondrocyte survival, and observed that in the absence of dismutating agents, cells become sensitive to oxygen radicals that form spontaneously in the culture medium. The impact of oxygen radicals on chondrocyte maturation has been noted by other workers. For example, Vincent et al. [38] reported that free radicals cause a dose-dependent decrease in cell growth, while Ishizaki et al. [39] found that, as long as the chondrocyte culture medium contained antioxidants, cells plated at low density do not leave the cell cycle. These investigators emphasized the specific need for cysteine if, during the maturation phase of the cycle, cell death is to be avoided. Indeed, while the ultimate fate of the mature chondrocyte is the subject of vigorous debate, histological and biochemical studies suggest that the terminal cells may eventually undergo programmed cell death [40–44]. Since it is known that a decrease in intracellular thiols promotes apoptosis, the argument is here advanced that a critical thiol level activates the terminal event in the sternal cells. Experiments in progress are aimed at evaluating the import of both glutathione and cysteine in terms of chondrocyte maturation and the regulation of programmed cell death.

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