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INCREASED COLLAGEN SYNTHESIS IN MYELOBLASTOSIS-ASSOCIATED VIRUS-INFECTED CHICKEN EMBRYO FIBROBLASTS

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Summary: Chicken embryo fibroblasts infected with a non-transforming derivative of avian myeloblastosis virus [MAV-2(0)] showed a threefold increase in the biosynthesis of collagen compared to values in normal counterparts. In contrast, non-collagen protein synthesis was unchanged.

Myeloblastosis associated virus -2(osteo), MAV-2(0), is a derivative of avian myeloblastosis virus that causes 100% osteopetrosis when injected into 11-day old chicken embryos (12). The virus is capable of replicating in many avian tissues; recent evidence indicates that bone is a target organ in which complementary virus DNA integrates covalently into host chromosomal DNA (4-5 virus copies/bone cell) (13). Infected chickens develop a growth and development disorder detectable as early as 7-10 days after hatching (1). Although many physiological processes are perturbed in the infected bird (1), the nature of the primary defect in the disorder has not been identified.

Recently, a defect in the cross-linking of bone has been identified in avian osteopetrotic bone (Banes et al., manuscript in press). The ratio of the reducible, bone collagen cross-links dihydroxylysinonorleucine to hydroxylysinonorleucine was unusually high by 4 weeks after hatching in tibia samples from osteopetrotic birds compared to the ratio in age-matched, control tibiae. The latter observation suggests that MAV-2(0) infection of chickens may specifically alter collagen metabolism. We have therefore investigated the effect of MAV-2(0) infection on collagen metabolism in vitro.

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We have measured the amounts of hydroxylated collagen and non-collagen protein produced in uninfected and MAV-2(0)-infected secondary, chicken embryo fibroblasts, and found that virus infection is associated with an increase in the level of collagen synthesis.

Materials and Methods

Virus. An endpoint-purified derivative of avian myeloblastosis virus, MAV-2(0), was used in these experiments (13). Virus was propagated by serial passage in chickens. Sera were prepared from birds manifesting heavy osteopetrosis, pooled and stored at -70C in 0.1 ml portions.

Cell Culture. Primary chicken embryo fibroblasts were prepared from fertile white Leghorn chicken eggs certified free of avian leukosis group-specific antigen and chick helper factor (SPAFAS, Inc. Storrs, Conn.). Cells were cultured in Ham's F-10 medium (Flow Laboratories) supplemented with 5% calf serum and antibiotics (50 units penicillin/ml; 50 µg streptomycin sulfate/ml). This medium was optimal for virus production (11) but not necessarily optimal for collagen production. Culture plates were seeded at 10^7 cells per plate. Cells were infected at a multiplicity of 0.01 at the time of plating. Cells were incubated at 37C for 72 hours then trypsinized and cultured for an additional 10 days. Cell counts were performed on duplicate cultures of trypsinized cells using a standard hemocytometer.

Collagen and Non-collagen Protein Determinations. Cells were washed twice with a proline-free medium [Joklik's modified MEM (Flow Laboratories), pH 7.4, 10 minutes wash at 37C] supplemented with 1 mM ascorbate. Medium was aspirated from the plate and 4 ml of proline-free medium containing 2 µCi of ^{3}H-proline were added (G-^{3}H-proline, 1-5 Ci/mole, New England Nuclear, Boston, Mass.). Plates were incubated at 37C for 2 hours. After incubation, each plate was placed on ice and 0.4 ml of ice-cold 50% trichloracetic acid and 0.025% tannic acid (TCA-TA) was added to stop isotope incorporation. Cells were scraped from each plate, added to the supernatant fluid and 5% TCA-TA washes, transferred to a conical tube and sedimented at 4C. Material was homogenized using a tight-fitting glass homogenizer and reconstituted to 2.0 ml with 5% TCA-TA. Two 0.5 ml portions were used for determination of deoxyribonucleic acid content (2) and the remaining 1.0 ml was transferred to a conical tube and washed 5 times with 5% TCA-TA, 12 ml/wash. The washed pellet was transferred quantitatively to a hydrolysis tube and lyophilized. After addition of 0.2 ml of 3N toluene sulfonic acid to the sample, the tube was flushed with nitrogen, evacuated, sealed under vacuum and placed in an oven at 110C for 18 hours. Samples were then filtered (sintered glass filter pyrex M 306060), transferred quantitatively and chromatographed on a 0.9 x 15 cm cation exchange column equilibrated in 0.2 M citrate buffer pH 2.69 at RT (modification of (10)). Complete separation of hydroxyproline and proline was effected. Radioactivity in a portion of each column fraction was determined automatically (7); appropriate corrections were made for efficiency of counting, fraction volume counted, loss of tritium during hydroxylation, volume of homogenate hydrolyzed and amount of hydroxyproline in collagen (12%). To calculate the amount of non-collagen protein present, 10% of the DPM in collagen (proline DPM) were substracted from total proline DPM and that value was divided by 0.041 to yield the value for actual DPM of non-collagen protein present (9). Relative collagen synthesis was calculated using the equation:

$$\frac{\text{DPM collagen}}{\text{DPM collagen + DPM non-collagen protein}} \times 100 = \text{relative collagen synthesis.}$$

TABLE 1

LEVELS[a] OF COLLAGEN AND NONCOLLAGEN

PROTEINS PRODUCED IN NORMAL OR

MAV-2(0)[b]-INFECTED CHICK EMBRYO CELLS

VIRUS[b]	DPM COLLAGEN[c] / µg DNA	DPM NONCOLLAGEN PROTEIN[c] / µg DNA	% RELATIVE[d] COLLAGEN SYNTHESIS
-	424 ± 51	62271 ± 7852	1.1 ± 0.4
+	1336 ± 97	54976 ± 9272	2.1 ± 0.3
$p^a <$	0.001	0.02	0.0025
$n^a =$	8	7	7

(a) Collagen and noncollagen protein levels are based on DNA content (DNA assayed by the method of Burton (2)). Data are expressed as mean ± standard error with appropriate values for n and p (Students' 2-tailed t test).

(b) MAV-2(0) is myeloblastosis - associated virus -2(osteo). Cells were infected with a multiplicity of infection = 0.01. Minus denotes uninfected cultures; plus denotes infected cultures.

(c) Disintegrations per minute in collagen and non-collagen protein were determined after complete separation of hydroxyproline and proline by cation exchange column chromatography (See Materials and Methods).

(d) Relative collagen synthesis represents the amount of hydroxylated collagen relative to the amounts of all proline-containing proteins produced in the cells during the same period (2 h incubation at 37C with radioactive proline 2µCi/plate (see materials and methods).

Results

The synthesis of hydroxylated collagen is specifically increased in secondary chick embryo cells infected with MAV-2(0) (Table 1). Relative collagen synthesis increased 2-fold in infected cultures compared with that of normal cultures. Absolute collagen synthesis (Table 1, column 2) was increased more than 3-fold in virus-infected cells compared to synthesis in normal counterparts. In contrast, synthesis of non-collagen protein was unchanged.

Discussion

MAV-2(0) is a subgroup B, non-transforming retravirus that replicates in chicken embryo cells (12), but also produces a slight cytopathogenicity, manifested by the appearance of plaques in a specialized assay (3,13). Infection in vivo produces a striking proliferative bone disorder, resulting in enlarged long bones (1). In the present investigation, infection of cultured chicken embryo fibroblasts resulted in increased synthesis of collagen (3-fold increase in infected cultures, $p<0.001$), with no significant change in synthesis of non-collagen protein (Table 1, columns 2,3). Other investigators have demonstrated changes in collagen synthesis in virus-infected cells: Polyoma (4) and Kirsten sarcoma virus-transformed cells exhibit an increase in collagen synthesis (8); Rous sarcoma virus-transformed cells exhibit a decrease in collagen synthesis (5,6).

The observed increase in collagen quantity in MAV-2(0)-infected cells, represents the first report of the effect of a non-transforming virus on collagen synthesis.

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