Collagen breakdown products and lung collagen metabolism: an in vitro study on fibroblast cultures

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Abstract

Background – In fibrotic diseases such as pulmonary fibrosis there is evidence suggesting enhanced synthesis and degradation of lung connective tissue components, including collagen. It has therefore been hypothesised that products of collagen degradation may have a role in the promotion of collagen deposition. In support of this hypothesis, it has recently been shown that intravenous injection of lung collagen degradation products in experimental animals stimulated collagen synthesis leading to increased collagen deposition and diffuse interstitial lung disease.

Methods – Rabbit and human fibroblast cultures from lung and skin were used as an in vitro model to study the responses of these cells to rabbit collagen degradation products. The effects of an acute exposure to collagen degradation products on synthesis of collagen and non-collagenous protein have been studied in confluent cultures by [³H]-proline incorporation. The effects of collagen degradation products on fibroblast proliferation and production of genetic types of collagen have also been investigated.

Results – The acute exposure of rabbit lung fibroblast cultures to collagen degradation products significantly increased collagen synthesis without affecting non-collagenous protein synthesis. This effect was dose related, specific for lung fibroblasts, and species specific. Collagen degradation products altered the rate of synthesis of genetic types of collagen with a consequent decrease of type III/I + III collagen ratio (0.26 (0.04) treated with collagen degradation products; 0.45 (0.02) controls). These effects occurred without the intervention of serum factors. In addition, collagen degradation products neither affected fibroblast proliferation nor selected specific clones emphasising one type of collagen.

Conclusions – These results suggest that collagen degradation products can influence lung collagen metabolism by stimulating collagen synthesis. The regulation of collagen mass by collagen degradation products may be of importance in lung collagen homeostasis in vivo.

good evidence that collagen turnover in normal tissues occurs at quite rapid rates.¹ It is generally accepted that collagen mass in normal tissues is dependent on the balanced relation between rates of synthesis and degradation.² Although the effects of single factors – that is, hormones, cytokines, enzymes^{3,4} – on the synthesis or degradation of collagen have been examined in detail, little is known about the mechanism(s) regulating collagen homeostasis.

The view that products of collagen degradation may take part in the regulation of extracellular matrix by stimulating collagen synthesis came initially from experimental conditions characterised by degradation of connective tissue components. In these conditions – for example, emphysema, pulmonary fibrosis, liver cirrhosis, atherosclerosis, and rheumatoid arthritis – the destruction of the extracellular matrix was matched by a significant increase in collagen synthesis. This fact was particularly evident in the experimental models of emphysema obtained by intratracheal instillation of elastolytic enzymes.⁵⁻⁷

There is now evidence that collagen peptides derived from degradation of procollagen molecules may influence in vitro collagen synthesis.^{8,9} More recently, studies from our laboratory showed that collagen degradation products derived from elastase digestion may increase in vivo lung collagen synthesis and deposition.^{10,11}

These findings support the idea that collagen degradation products may have a role in collagen homeostasis under physiological conditions or modulate collagen deposition in some pathological states.

Different culture conditions were employed in this study to evaluate: (1) whether collagen degradation products from rabbit lung can stimulate in vitro collagen synthesis by fibroblasts from different organs and species; (2) whether collagen degradation products exert their stimulatory effect without the intervention of serum factors; and (3) whether collagen degradation products enhance fibroblast proliferation and alter genetic collagen type expression.

Methods

MATERIALS

Most of the reagents used for the cell cultures were of special grade and manufactured by Sigma (St Louis, Missouri, USA); trypsin was from Gibco, [³H]-proline (Sp act 308 mCi/mg) was purchased from Amersham International (Amersham, Buckinghamshire, UK), and highly purified *Clostridium histolyticum* colla-

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The traditional view that collagen is metabolically inert is no longer tenable and there is now

genase type VII free of non-specific proteases was obtained from Sigma. The presence of contaminating non-specific proteases was excluded by assaying the collagenase on [^3H]-tryptophan-labelled proteins obtained from cell cultures by the method of Spanheimer.¹²

PREPARATION OF COLLAGEN DERIVED PEPTIDES

The various preparations of collagen derived peptides used in this study were obtained by digestion with porcine pancreatic elastase (Sigma E 127) as previously described.¹⁰ Briefly, 1 g of insoluble collagen from rabbit lung, prepared as described by Davis and Mackle,¹³ was digested with 4 mg porcine pancreatic elastase for 24 hours at 30°C in 0.2 mol/l Tris-HCl buffer, pH 8.0. After thermal inactivation of the enzyme the sample was centrifuged at 600 *g* for 30 minutes and the supernatant containing collagen derived peptides was extensively dialysed in tubing with a nominal MW cut off of 2000 at 4°C against physiological saline. The samples were then assayed for protein¹⁴ and hydroxyproline¹⁵ concentrations.

The typical electrophoretic profile of rabbit lung collagen derived peptides is shown in fig 1. As can be seen, a major band (lane A, 4) and four minor ones are evident in SDS-Page.

CELL CULTURES

Rabbit fibroblast cell lines were cultured from skin (RSF) and lung (RLF) specimens of healthy animals using the explantation method. The fibroblast lines had undergone six passages before these experiments. Human fibroblast cultures from lung (HLF) and skin (HSF) were obtained in the seventh passage from Sclavo cell bank (Biocine Sclavo, Siena, Italy).

CULTURE TREATMENT

To assess the effects of collagen derived peptides on collagen production in fibroblast cultures two different experimental conditions

were employed: (1) confluent cultures acutely exposed to collagen derived peptides; and (2) cultures chronically exposed to collagen derived peptides before and/or after confluency.

In the first experimental condition we determined the amount of cell associated collagen synthesised in the presence or absence of collagen derived peptides by confluent fibroblast cultures. For this purpose RLF, RSF, HLF, and HSF cultures were seeded at a concentration of 1×10^4 cells/cm² and grown in 75 cm² flasks in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, 5 U/ml penicillin, and 5 mg/ml streptomycin. The cultures were fed every third day after subculture. One day after confluency had been reached the fibroblast cultures (1×10^5 cells/cm²) were preincubated in serum free medium containing 50 µg/ml ascorbic acid and 50 µg/ml β-aminopropionitrile for one hour and then incubated for 16 hours in a labelling medium containing 10 µCi/ml [^3H]-proline and collagen derived peptides in a range of concentrations varying from 65 to 260 ng protein/ml. Control cultures were processed in a similar way but collagen derived peptides were omitted or replaced by 1–5 ng/ml heat inactivated porcine pancreatic elastase (70°C, 15 minutes) in sterile saline solution.

In the second experiment (chronic exposure) RLF cultures were seeded in 75 cm² flasks at a concentration of 1×10^4 cells/cm² and grown from the initial day of subculture in the standard medium with (group A) or without (group B) 130 ng/ml collagen derived peptides. After the confluency (about seven days) cultures (1×10^5 cells/cm²) from both groups were studied for their ability to produce and secrete collagen in the presence of collagen derived peptides (130 ng/ml) (groups A1; B1) or in their absence (groups A2; B2). For this purpose 10 flasks for each experimental group were employed. Cultures in serum free media were labelled for 72 hours with 10 µCi/ml [^3H]-proline in the presence of 50 µg/ml β-aminopropionitrile and ascorbic acid supplemented daily at a dose of 50 µg/ml.

RADIOACTIVE LABELLING AND DETERMINATION OF PROTEIN SYNTHESIS

Collagen synthesis was determined by measuring the amount of labelled proline in collagen and non-collagenous protein by using purified bacterial collagenase. In the first series of experiments these parameters were determined in cell associated fractions. More specifically, the labelling medium was removed, the cell layer was washed twice with phosphate buffered saline (PBS), and the cells were collected after being scraped in PBS. The suspension was clarified by centrifugation at 2000 rpm for 15 minutes, the resulting pellet was resuspended in saline, sonicated, and precipitated with trichloroacetic acid (TCA) (5% final concentration). After centrifugation the pellet was processed essentially as described by Peterkofsky and Diegelman.¹⁶ The rate of tritium incorporation into collagenase digestible protein fraction was measured¹⁷ in a liquid scintil-

Figure 1 SDS-Page of rabbit lung collagen degradation products. Lane A: collagen degradation products (20 µg in 2.3% SDS, 10% glycerol, 125 mmol/l Tris-HCl, pH 6.8). Bands 1–5 are the major cleavage products obtained by porcine pancreatic elastase digestion. The band corresponding to porcine pancreatic elastase is indicated by the arrow. Lane B: molecular weight standards. The M_r values (expressed in kDa) are shown at the right. The gel (10%) was silver stained.

lation counter (Packard, Tri-Carb 2000 CA). In this assay system incorporation of tritium into collagenase digestible protein fraction estimated the rate of collagen synthesis, while incorporation into collagenase insensitive protein fraction estimated the rate of synthesis of non-collagenous protein. Additionally, in some experiments the levels of [^3H]-hydroxyproline in collagenase digestible and collagenase non-digestible fractions were analysed by HPLC according to the method of Muramoto *et al.*¹⁸ Briefly, the two fractions were separated by precipitation with TCA and tannic acid followed by centrifugation. Aliquots of 1.6 ml of each supernatant were dialysed against 16 ml of 10 mmol/l acetic acid at 37°C for two hours. The samples outside the dialysis bag were then lyophilised and hydrolysed in 6N HCl for 20 hours at 120°C. The non-collagenous protein fraction was washed and hydrolysed in 6N HCl. [^3H]-hydroxyproline and [^3H]-proline were separated by HPLC according to Muramoto *et al.*¹⁸ on a 250 × 4.6 mm internal diameter Partisil PXS 10/25 ODS-2 column (Whatman, Pierce Chemical Co., USA) at a flow rate of 1 ml/min. The recovery rate of total radioactivity including hydroxyproline and proline for the HPLC was in the range of 96–98%.

The collagen synthesis in fibroblast cultures exposed or not exposed to collagen degradation products (260 ng protein/ml) was assessed by determining the collagen concentration in the culture media by HPLC. Collagen assays were carried out in triplicate at time 0 (taken as blank value) and 16 hours later. Hydroxyproline was essentially measured according to the method described by Monboisse *et al.*¹⁹ The values of hydroxyproline secreted into the culture medium were calculated as the difference between the amount of hydroxyproline determined at 16 hours and that present at the start of the experiment (0 time) as collagen degradation products. The amount of collagen degradation products added to experimental cultures (260 ng/ml medium) corresponds to 0.25 (0.01) nmol hydroxyproline/ml medium as determined by HPLC.

In the second series of experiments (chronic exposure) either the clarified culture medium or the acid soluble fraction derived from the cell layer was used as the starting material for subsequent procedures. In these experiments the secreted and cell associated collagens synthesised by RLF cultures in the absence or presence of collagen degradation products were isolated by limited pepsin digestion. By this technique collagens were separated by fractional salt precipitation with 0.7 mol/l (type III) and 2.0 mol/l (type I) NaCl at pH 7.6 according to Kern *et al.*²⁰ The purity of these fractions was analysed by non-interrupted electrophoresis using thioglycolic acid as a negatively charged reducer (fig 2).²¹ The amounts of radioactivity incorporated into the different types of collagen chains were determined by a liquid scintillation counter.

The specificity of the assay was checked by adding purified bacterial collagenase (type VII) to the pellets obtained after fractional salt

Figure 2 SDS-Page analysis of collagen purified by fractional salt precipitation with 0.7 mol/l (type III, lane A) and 2.0 mol/l (type I, lane B) NaCl at pH 7.6 according to Kern *et al.*²⁰ The fractions were analysed by non-interrupted electrophoresis using thioglycolic acid as negatively charged reducer (Nakamura *et al.*²¹). The gel (5%) was silver stained. Positions of globular molecular mass markers are shown at the left.

precipitation. Collagenase treatment reduced the number of counts recovered in each fraction by about 97%.

DNA SYNTHESIS AND CELL GROWTH IN CULTURES EXPOSED TO COLLAGEN DEGRADATION PRODUCTS

The effects of collagen degradation products on DNA synthesis in confluent RLF cultures was assessed by determining DNA and total protein after 24 hours exposure to collagen degradation products.²²

The effects of chronic exposure to collagen degradation products on cell growth were examined in the RLF cells grown both in the absence and in the presence of collagen degradation products (130 ng/ml medium) as described above. At selected times (two, four, and six days after subcultures) growth rate determination was assessed by measurement of total DNA and protein contents as well as by direct cell counts.

DNA levels and protein concentration were assessed in cell cultures by the methods of Taylor *et al.*²³ and Lowry *et al.*¹⁴ respectively.

STATISTICAL ANALYSIS

For each parameter the values were averaged and the standard deviation calculated. The significance of the differences between means was tested by analysis of variance (Snedecor F test). A p value of <0.05 was considered significant.

Results

In preliminary experiments we determined the maximum tolerated dose of collagen degradation products under the same conditions as those selected for the following study. Concentrations of collagen degradation products ranging from 65 to 260 ng protein/ml incubation medium (one eighth to one half of the maximum tolerated dose) were employed to evaluate the dose-response relation.

The acute exposure of RLF to collagen

Figure 3 Effects of collagen degradation products on [^3H]-proline incorporation in collagen and non-collagenous protein (NCP) fractions in fibroblast cultures (cell layer) from different organs and species. A, rabbit lung fibroblasts; B, human lung fibroblasts; C, rabbit skin fibroblasts; and D, human skin fibroblasts. Values are means (SD) of three separate determinations on eight different flasks at each concentration of collagen degradation products. NCP values are expressed as $\text{dpm} \times 10^{-1}$. The values obtained from control flasks in which collagen degradation products were replaced by heat inactivated porcine pancreatic elastase belong in the range of values reported in the figure for control flasks in which collagen degradation products were omitted. * $p < 0.05$ compared with control flasks.

degradation products resulted in a significant increase in collagen synthesis as detected by [^3H]-proline incorporation into cell associated collagenase digestible fraction (fig 3A). This stimulatory response is dose-dependent in the range of collagen degradation products concentrations reported above. The effect of collagen degradation products is specific for collagen, since they do not alter the synthesis of non-collagenous proteins (fig 3A).

The effects of collagen degradation products on synthesis of collagen and non-collagenous proteins on HLF, RSF, and HSF cultures are reported in fig 3B, C, and D, respectively. As can be seen, collagen degradation products from rabbit lung do not affect the synthesis of collagen and non-collagenous proteins in skin fibroblasts or in lung fibroblasts from other animal species. No change in collagen synthesis was observed between the control cultures in which collagen degradation products were replaced by 1–5 ng/ml heat inactivated porcine pancreatic elastase and those in which collagen degradation products were omitted (fig 3).

Similar results were obtained when the collagen synthesis in the cell layer was assessed by determining the radioactivity associated with hydroxyproline into the collagenase digestible fractions after separation with HPLC (fig 4).

Figure 4 Effects of collagen degradation products on collagen synthesis determined in cell layer as [^3H]-hydroxyproline separated by HPLC. Values are means (SD) of three separate determinations of [^3H]-hydroxyproline present in collagen fractions from eight different flasks at each concentration of collagen degradation products. * $p < 0.05$ compared with controls. RLF = rabbit lung fibroblasts; HLF = human lung fibroblasts; RSF = rabbit skin fibroblasts; HSF = human skin fibroblasts.

Figure 5 Effects of collagen degradation products (CDP, 260 ng/ml medium) on the amount of 4-hydroxyproline secreted into the culture medium after 16 hours of incubation. The reported values represent the differences between the amount of hydroxyproline determined in the medium at 16 hours and that present at the start of the experiment (0 time) as collagen degradation products (0.25 nmol hydroxyproline/ml medium). Values are means (SD) of triplicate determinations carried out on eight different flasks for each experimental condition. RLF=rabbit lung fibroblasts; HLF=human lung fibroblasts; RSF=rabbit skin fibroblasts; HSF=human skin fibroblasts.

No detectable amounts of hydroxyproline and [^3H]-hydroxyproline were found in non-collagenous protein fractions. It was apparent that collagen synthesis determined as [^3H]-hydroxyproline separated by HPLC followed a pattern identical with that determined as total radioactivity present in the collagenase digestible fraction ($r=0.94$).

Collagen synthesis was also assessed in culture media by determining hydroxyproline by HPLC (fig 5). As can be seen, a significant increase in hydroxyproline was detected in the culture media of RLF cultures after 16 hours of incubation with collagen degradation products.

These results, obtained in cell cultures at the seventh passage, were confirmed in additional trials using several cultures from the same tissues and species at various passages (8–11). Despite the large variability of the response of RLF cultures to collagen degradation products, the results obtained in these additional trials fulfilled the more stringent criteria for statistical and biological significance developed by Barile *et al.*²⁴ In particular, an increase in collagen synthesis of 1.9–3.2 times has been observed in 12 separate experiments carried out on parallel sets of RLF cultures at various passages incubated with collagen degradation products. The collagen synthesis in each of the experiments was significantly higher in cultures treated with collagen degradation products than in controls ($p<0.05$). In addition, the absolute values of the z score (difference/standard deviation) always exceeded 1.96.²⁴ However, no significant difference was observed in the mean values of the studied parameters between control cultures and those treated with collagen degradation products for HLF, RSF, and HSF at the various passages examined.

These data indicate that the stimulatory effect of collagen degradation products is specific for lung collagen, species specific, and

dose dependent, at least in the range of concentrations reported above. These results, obtained in fibroblast cultures deprived of serum, show that collagen degradation products exert their effect without the intervention of serum factors.

EFFECTS OF COLLAGEN DEGRADATION PRODUCTS ON GENETIC TYPES OF COLLAGEN SYNTHESISED BY RLF

Experiments were carried out to see whether collagen degradation products affect the expression of type I and type III collagens by altering their rates of synthesis, or by selecting specific clones emphasising one type of collagen, or both. To evaluate this possibility exposures to collagen degradation products were performed as detailed above on confluent cultures which were previously supplemented (group A) or not supplemented (group B) with collagen degradation products during RLF growth.

The data obtained in this study are summarised in table 1. As can be seen, a specific increase of collagen type I neosynthesis by RLF at confluency was observed in flasks treated with collagen degradation products in the presence or absence of pretreatment with collagen degradation products during subconfluency (groups A1 and B1). It appears that collagen degradation products caused a significant increase (about threefold) in type I collagen synthesis compared with a small increase in type III collagen. Thus a decrease in collagen type ratio (III/I+III) was observed in treated cultures (0.26 (0.04)) compared with controls (0.45 (0.02)).

However, no change in collagen synthesis was observed in control cultures (A2, B2) in which collagen degradation products were omitted during the labelling period, with or without pretreatment with collagen degradation products.

Table 1 Culture distribution of the genetic types of collagen synthesised by rabbit lung fibroblasts in the absence or presence of collagen degradation products

	^3H -proline incorporated (dpm/100 cells)	
	Secreted	Cell-associated
Type I collagen		
A1	33.2 (2.9)*	18.5 (1.2)*
A2	10.9 (1.1)	6.1 (0.8)
B1	35.1 (3.1)*	19.4 (1.9)*
B2	11.2 (1.0)	6.2 (0.7)
Type III collagen		
A1	11.9 (1.2)*	6.5 (0.6)*
A2	9.1 (0.7)	4.9 (0.3)
B1	10.9 (1.0)	6.1 (0.3)*
B2	9.3 (0.8)	5.0 (0.3)
Total synthesis		
A1	45.1 (3.7)*	25.0 (2.1)*
A2	20.0 (2.1)	11.0 (1.0)
B1	46.0 (3.8)*	25.5 (2.0)*
B2	20.5 (2.2)	11.2 (1.1)

Two series of cultures were treated (A) or not treated (B) with collagen derived peptides during cell growth; these cultures were either exposed (1) or not (2) to collagen derived peptides after confluency. Values are mean (SD) of triplicate determinations from 10 flasks for each experimental condition. The total synthesis was obtained from the combined values for the collagen type I and type III fractions.

* $p<0.05$ compared with control group B2.

EFFECTS OF COLLAGEN DEGRADATION PRODUCTS ON DNA SYNTHESIS IN CONFLUENT CULTURES AND ON RLF GROWTH IN SUBCONFLUENT CULTURES

Exposure of confluent cultures (25 cm² flasks) to collagen degradation products for a period of up to 24 hours did not result in an increase in DNA or protein content. Mean (SD) values from quadruplicate cultures for DNA content in control cultures and those treated with collagen degradation products were 31 (2) and 29 (3) µg/flask, respectively. The protein content amounted to 713 (32) and 708 (36) µg/flask for controls and cells treated with collagen degradation products, respectively.

To further assess the effect of collagen degradation products on cell growth the total DNA content and cell counts of RLF cultures, whether exposed to collagen degradation products or not, were determined at selected times (two, four, and six days) after subcultures. Table 2 shows the results obtained in parallel cultures in which eight flasks (25 cm²) were used for each time point and experimental condition. As can be seen, there was no difference in DNA content or cell number between the cultures, whether exposed to collagen degradation products or not. Similarly, evaluation of total protein at each time point indicated that exposure to collagen degradation products did not increase the total protein content of the cultures compared with control values. These results indicate that collagen degradation products do not stimulate total protein synthesis or the growth rate of RLF in subconfluent cultures.

Discussion

It has been proposed that lung synthesis of collagen and elastin can be controlled by the state of the extracellular matrix and peptides made soluble by elastase derived from that matrix. It has, in fact, been shown that conditions mimicking degradation of these interstitial proteins result in an enhanced rate of synthesis of elastin and collagen in an *in vitro*²⁵ and an *in vivo*¹⁰ model. Such regulation may have a role in reconstituting the connective tissue framework of the lung (remodelling) after elastase injury, or may also be of importance in lung collagen homeostasis under physiological conditions.

In previous papers from our laboratory we have shown that lung collagen degradation products derived from elastase digestion can enhance lung collagen synthesis when injected

intravenously into rabbits.¹⁰ In addition, the chronic stimulation of collagen synthesis by collagen derived peptides resulted in an increased deposition of collagen fibres with a relative increase of collagen type I.¹¹

In this paper we present evidence that lung collagen derived peptides can exert their stimulatory effect on collagen synthesis by lung fibroblasts under *in vitro* conditions. This effect is dose related, specific for lung fibroblasts, and species specific. Our data provide evidence that lung collagen derived peptides can enhance collagen synthesis without the intervention of hormones or effector molecules derived from inflammatory cells (for example, cytokines) and plasma protease systems (for example, complement, clotting).

The stimulation of collagen synthesis by collagen derived peptides was not matched by an increase in the synthesis of non-collagenous proteins and DNA. This conclusion comes from the experiments carried out on confluent and subconfluent cultures exposed to collagen derived peptides. In addition, the data obtained in subconfluent cultures clearly indicate that collagen derived peptides do not affect the growth rate of RLF when added to culture media.

Although an apparent increase in the number of lung fibroblasts after chronic treatment with collagen derived peptides has been reported by us previously in an *in vivo* study, the data presented here indicate that collagen derived peptides do not stimulate fibroblast proliferation. The increase in collagen synthesis by collagen derived peptides is matched by a change in collagen type ratio with a relative increase of type I collagen in respect to type III. This result is in agreement with those previously reported by us under *in vivo* conditions. It is known that in a stable extracellular environment the relative ratio of the interstitial collagen synthesised by a population of fibroblasts is constant.²⁶ It is reasonable to suppose that the change in the extracellular milieu resulting from the addition of collagen derived peptides could modify the relative proportion of collagen types produced by RLF. This change could be accounted for by either an alteration of the differentiated characteristics of each cell or by a selection of specific clones emphasising one collagen type. Indeed, the change in collagen type ratio, observed in confluent cultures in which collagen derived peptides were added during the labelling period, occurred irrespective of whether collagen derived peptides were previously included in the media during cell growth. These data are not consistent with the hypothesis that collagen derived peptides induce a selection of specific clones emphasising one collagen type. In conclusion, under the influence of collagen derived peptides RLF undergo phenotypical changes which are not sustained after the removal of collagen derived peptides.

Another interesting observation in this study is that the effect of lung collagen derived peptides was specific for lung fibroblasts and was species specific. Given the high homology

Table 2 Effect of collagen degradation products (CDP) on cell proliferation in cultures of rabbit lung fibroblasts

	Days after subculture					
	2		4		6	
	Control	+ CDP	Control	+ CDP	Control	+ CDP
Cell counts (no × 10 ⁴ /cm ²)	1.4 (0.2)	1.5 (0.3)	5.1 (0.7)	5.2 (0.5)	9.3 (0.9)	9.1 (0.8)
DNA (µg/flask)	4.7 (0.4)	4.6 (0.4)	15.6 (1.8)	15.6 (1.6)	28 (3.2)	27 (3.0)
Protein (µg/flask)	119 (7)	112 (10)	361 (17)	370 (18)	707 (38)	699 (34)

Values represent mean (SD) of eight flasks (25 cm²).

existing among the various genetic types of collagen in different mammalian species and among various organs within the same species,²⁷ the results obtained in this paper appear surprising. We cannot explain why rabbit lung collagen derived peptides exert their action only on homologous lung fibroblasts. However, we hypothesise that the specificity of the response to lung collagen derived peptides may depend on receptors to collagen fragments present on the cell surface. Another possible explanation is that the response depends either on the kind of collagen molecule or the type of enzyme generating collagen derived peptides. In this context it is of interest to note that many similarities exist in biochemical properties (for example, substrate specificity, inhibitory profile) between porcine pancreatic elastase and rabbit leucocyte elastase.²⁸ Moreover, data similar to those reported here were obtained in preliminary experiments employing lung collagen derived peptides derived by digestion with homologous leucocyte elastase.

The data presented in this paper offer an explanation for the increase in lung collagen synthesis and deposition characterising experimental animals after elastase challenge. In addition, these results suggest a role for proteinase(s) in the control of the dynamic state of extracellular matrix regulating not only the degradation of specific interstitial components but also stimulating the synthesis via breakdown products. Since collagens in normal lung tissue are exposed to a physiological burden of elastase and a complex enzyme mixture whose concentrations vary depending upon the rate of leucocatheresis,²⁹ it is likely that a regulation of collagen mass by collagen derived peptides is operative *in vivo*.

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