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Ameliorating Effect of an Interferon Inducer Polyinosinic-Polycytidylic Acid on Bleomycin-Induced Lung Fibrosis in Hamsters

Morphologic and Biochemical Evidence

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The effects of polyinosinic-polycytidylic acid (Poly I:C), an inducer of interferons, on bleomycin (Bleo)-induced lung fibrosis was studied in hamsters. Poly I:C (10 mg/kg intraperitoneally) was administered for two days and immediately before intratracheal instillation of bleomycin (7.5 U/kg) or an equivalent volume of saline and thereafter daily for 13 days. The lung hydroxyproline in control, Poly I:C, Bleo, and Bleo + Poly I:C groups averaged 791, 752, 1177, and 766 μg/lung. As compared to control, the prolyl hydroxylase activity in the Bleo group was increased by 83% whereas in Bleo + Poly I:C group, the activity was increased by 42%. Protein in the bronchoalveolar lavage supernatant in Poly I:C, Bleo and Bleo + Poly I:C groups were 72, 286, and 206% of the control, respectively. There was no difference in total leukocyte counts between Bleo + Poly I:C and Bleo groups, but the differential cell counts were changed. The numbers of neutrophils, monocytes, lymphocytes, and eosinophils were 50, 84, 91, and 10% of Bleo group, respectively. Morphometric estimates of the volume of parenchymal lesion within the lung showed that hamsters in Bleo + Poly I:C group had significantly less volume of lesion (1.0 cucm) than the Bleo group (1.6 cucm). In addition, the fibrotic lesions in Bleo + Poly I:C group were multifocal and primarily proximal acinar in location, had fewer extracellular fibers, neutrophils and monocytes. Poly I:C treatment ameliorated bleomycin-induced lung collagen accumulation. (Am J Pathol 1988, 133:525–536)

FIBROTIC LUNG DISEASES, regardless of the cause, are characterized by an excessive accumulation of collagen in the lung.[1] These diseases are characterized by derangements of the alveolar wall comprised of accumulations of mesenchymal cells and connective tissue.[2] At present, there is no effective treatment for lung fibrosis and this is partly attributed to our lack of understanding of the regulation of collagen metabolism. However, numerous studies have demonstrated that the metabolic functions of the fibroblasts responsible for collagen synthesis and deposition are affected by various factors including inflammation,[3] pharmacologic agents,[4] proteases,[5] lipid,[6] nutritional,[7] and immunologic status[8] of the animals.

A variety of animal models have been developed to produce interstitial pulmonary fibrosis (IPF). In this regard intratracheal instillation of bleomycin, an antineoplastic drug, is frequently employed to produce experimental models of lung fibrosis.[9–11] The lung fibrosis produced in these models provide a reasonable similarity to IPF seen in humans.[9,10,12] A large number of compounds have been tested for their ability to prevent or ameliorate lung fibrosis induced by bleomy-

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cin.[10,13–15] It is unfortunate that systemic toxicity resulting from the long-term uses of these compounds have precluded their uses in therapeutic situations.

We have previously demonstrated that treatment with recombinant murine interferon gamma (IFN-γ) caused a significant reduction in the number of fibroblasts per volume of lesion in lung and collagen accumulation in the bleomycin-mouse model of lung fibrosis;[16,17] however, lack of commercial availability of IFN-γ did not allow an extensive evaluation of this lymphokine against pulmonary fibrosis in the animal model. Therefore, we have taken an alternative approach, by investigating the effect of treatment with polyinosinic-polycytidylic acid (Poly I:C), an inducer of interferons,[18,19] on the lung collagen accumulation in the bleomycin-hamster model of lung fibrosis. The data presented in this paper clearly demonstrate that treatment with Poly I:C completely ameliorated the lung collagen accumulation in the bleomycin-hamster model of pulmonary fibrosis.

Materials and Methods

Male Golden Syrian hamsters weighing 90–120 g were purchased from Simonsen, Inc. (Gilroy, CA). The animals had free access to Purina Lab Chow and water *ad libitum*. Bleomycin sulfate (Blenoxane®) was a generous gift from Bristol Laboratories (Division of Bristol Myers Co, Syracuse, NY). L-Proline, [3,4-^{3}H] (specific activity 50 Ci/mmol) was purchased from ICN Radiochemicals (Irvine, CA) and L-[^{3}H(G)] hydroxyproline (specific activity 5.0 Ci/mmol) from New England Nuclear (Boston, MA). Hydroxyproline was obtained from Cal Biochem (San Diego, CA). Sodium salt of polyinosinic-polycytidylic acid (Poly I:C) and dimethylaminobenzaldehyde (purity > 99%) were from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade and purchased from standard commercial sources.

Treatment of Animals

The hamsters were acclimated for at least 1 week in the Animal Housing Facility before starting the experiment. Poly I:C and bleomycin (Bleo) sulfate solutions were made in sterile isotonic saline just before administration. Hamsters were randomly assigned to four treatment groups as follows: Group 1 (control), intratracheal instillation of isotonic saline (5 ml/kg) and saline (5 ml/kg IP) daily for 15 days; Group 2 (Poly I:C), intratracheal instillation of sterile isotonic saline (5 ml/kg) and Poly I:C (10 mg/kg IP) in an equivalent volume of isotonic saline daily for 15 days; Group 3 (Bleo), intratracheal instillation of bleomycin (7.5 unit/kg in 5 ml) and an equivalent volume of isotonic saline (intraperitoneally) for 15 days; and Group 4 (Bleo + Poly I:C), intratracheal instillation of bleomycin (7.5 unit/kg in 5 ml) and Poly I:C (10 mg/kg IP) daily for 15 days. Poly I:C or saline was administered intraperitoneally for 2 days before intratracheal instillation of saline or bleomycin under pentobarbital anesthesia (80–90 mg/kg IP), as described in our earlier paper.[20] The dose of Poly I:C (10 mg/kg) was the same as used by Deloria et al in mice.[21]

Bronchoalveolar Lavage

All hamsters were anesthetized with sodium pentobarbital on day 14 after intratracheal instillation of saline or bleomycin. The lungs were prepared for lavage by cannulating the trachea with Teflon tubing attached to a syringe. The lung lavage was carried out with 12 ml of isotonic saline delivered in 4 ml aliquots. The fluid was transferred into a graduated tube placed on ice and its volume recorded. Recovery of bronchoalveolar lavage fluid (BALF) ranged from 85 to 90%. An aliquot of the fluid was portioned for total and differential cell count. The remainder was centrifuged at 270*g* for 5 minutes at 4 C in a refrigerated centrifuge. The supernatant was gently aspirated and stored at −20 C in several aliquots for various biochemical measurements.

Leukocyte Counts

Total leukocyte numbers in the BALF were determined by a Coulter counter (Model F, Coulter Electronics, Inc., Hialeah, FL), according to the User's Manual. A background count was made using pure Isoton II just before the actual counts were made. One hundred microliters of the remaining lavage fluid + Zapoglobin were diluted with 10 ml of Isoton II for the bleomycin treated hamsters, 300 μl of the fluid were diluted with 10 ml of Isoton II for the controls. Three more drops of Zapoglobin were added to the diluted fluid, mixed, and then run on the Coulter counter in triplicate. Cell counts were averaged and the background count was subtracted to get the adjusted average. The adjusted averages were multiplied by the dilution factor and the amount of lavage fluid recovered (in ml) to obtain total cell counts. Differential cell counts were obtained using slides prepared on a Shandon cytospin using 100 μl of lavage fluid. Slides were stained with a modified Wright's stain and coverslipped using a xylene-based mounting medium. Differential cell counts were done on a Zeiss microscope at ×630. Neutrophils, monocytes, macrophages, lymphocytes, eosinophils, and basophils were

counted, until a total of 500 cells were counted for each slide.

Morphometric Histopathology

Hamsters designated for morphometric histopathology studies were divided among the four groups (six in each) and anesthetized as described above. After bronchoalveolar lavage and thoracotomy, the heart was ligated at the base for isolation of the pulmonary vasculature. The trachea was cannulated and lungs and heart were removed *en bloc* and weighed. The lungs were fixed by airway instillation of cacodylate buffered glutaraldehyde-paraformaldehyde fixative (400 mOsm) at a pressure of 30 cm H_2O for a minimum of one hour. The cannula was removed, the trachea was tied off, and the lung and heart stored in fixative. The volume of the fixed lung was determined by its buoyant weight in saline after dissection of the heart and adjacent mediastinal tissue.[22]

Blocks of tissue were cut from at least two sagittal slabs (2–3 mm thick) from the right cranial, right caudal, and left lung lobes of each lung. Each block was cut with about a 1 sq cm face. These blocks were dehydrated in a graded series of ethanol and embedded in paraffin. Five-micron thick sections were cut from the paraffin blocks and stained with hematoxylin and eosin (H & E). Two sections for each of the three lobes were used to estimate the volume density of parenchymal lesion within the lung using point counting techniques and a square lattice grid at a final magnification of ×160.[23] All tissue on the slides was counted. The volume of parenchymal lesion in the lung was then calculated by multiplying the volume density of parenchymal lesion by the volume density of parenchyma in the lung and by total lung volume. A lesion was defined as a cluster of four or more inflammatory cells in either interstitium or airways. From selected blocks, adjacent sections were cut and stained with sirius red for specific staining of collagenous fibers.[24]

Electron Microscopy

One slab from each lobe was used to select a random block of about a 0.5 sq cm face for embedding in plastic. One-micron thick sections were cut and stained with toluidine blue. Representative lesions were selected from these sections for further observation by electron microscopy. One square millimeter regions were cut from the larger plastic blocks, mounted on BEEM capsules, sectioned at 50–80 nm thick and stained with uranyl acetate and lead citrate. These sections were examined on a Zeiss EM10 electron microscope.

Biochemical Measurement

After the bronchoalveolar lavage, the abdominal cavity was opened and the lungs were perfused with isotonic saline through the right side of the heart. All lung lobes from each animal were excised, cleaned of the extraneous tissue, washed in ice-cold saline several times (5 times) until the wash was completely free from blood. The lung lobes were quickly frozen in liquid nitrogen and stored at −20 C until assayed for the collagen content and prolyl hydroxylase activity.

Determination of Lung Collagen

Frozen lungs were thawed, then each hamster's lung was homogenized in 0.1 M KCl, 0.02 M Tris (pH 7.6) with a Polytron® (Brinkmann Instruments, Inc., Westbury, NY) in a total volume of 10 ml. An aliquot of the homogenate (1 ml) was precipitated with 50% ice-cold TCA to yield a 10% final concentration. After 10 minutes on ice, samples were centrifuged, the supernatant discarded, and the precipitate hydrolysed in 6 N HCl overnight (16–18 hours) at 110 C. To monitor the recovery of hydroxyproline, [^{3}H]-hydroxyproline (2.5×10^5 dpm) was added to each hydrolysed sample. The hydroxyproline content of each sample was measured by a technique developed by Woessner.[25] Recovery of [^{3}H]-hydroxyproline ranged from 70 to 90% and it was used to correct the amount of hydroxyproline for each sample.

Prolyl Hydroxylase Assay

Prolyl hydroxylase substrate (procollagen) was first prepared from tibias obtained from 10-day-old chick embryo and 1 mCi of L-[3,4-^{3}H] proline according to the procedure described earlier.[26] The method for prolyl hydroxylase assay was essentially the same as described by Hutton et al.[27] Briefly, an aliquot of the frozen homogenate was thawed and rehomogenized in presence 0.05% Triton X. The incubation mixture for the assay in a total volume of 2 ml consisted of ferrous ammonium sulfate (5×10^{-5} M), α-ketoglutaric acid (5×10^{-5} M), [^{3}H]-proline procollagen (250,000 dpm), homogenate (200–400 μl), ascorbic acid (2.5×10^{-4} M), and Tris-HCl buffer (0.1 M, pH 7.8) to make up the volume. The reaction was started by the addition of ascorbic acid and continued for 30 min at 37 C in a Dubnoff metabolic shaker. The reaction was terminated by adding 0.2 ml of 50% TCA. The tritiated water of the enzymatic reaction was separated by vacuum distillation of the whole reaction mixture. A 1 ml aliquot of tritiated water from each distillation was mixed in 10 ml of Ready-Solv (Beckman Instru-

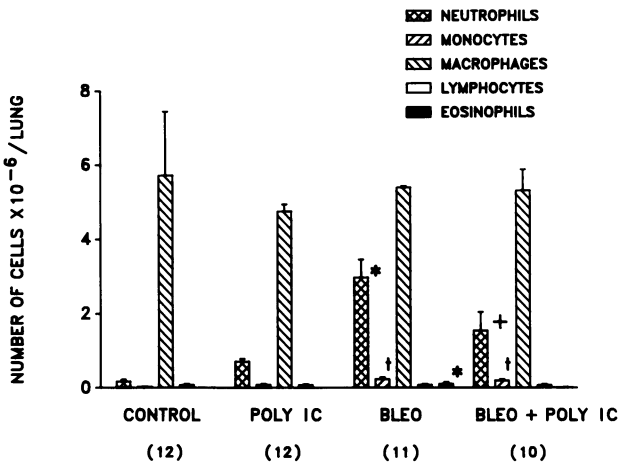

Figure 1—Absolute number of different cell types recovered in the bronchoalveolar lavage fluid of different groups of hamsters at 14 days after intratracheal injection of saline or bleomycin (Bleo). Control = saline (intratracheal) + saline (intraperitoneally); Poly I:C = saline (intratracheal) + Poly I:C (intraperitoneally); Bleo = bleomycin (intratracheal) + saline (intraperitoneally); Bleo + Poly I:C = bleomycin (intratracheal) + Poly I:C (intraperitoneally). See the text for details. Each bar graph represents the mean ± SEM. The number of animals for each group is shown in parenthesis. *Significantly increased ($P < 0.05$) over all other groups; +over control; and †over Poly I:C and control groups.

ments, Inc., Fullerton, CA) and counted in a Beckman scintillation spectrometer (Model LS 5801). The cpm of each sample was automatically converted to dpm by a quench curve programmed in the counter. The quench curve was prepared with [^{14}C]-toluene and varying amount of chloroform. The counting efficiency of the samples for tritium ranged from 37–40%. The enzyme assay under these conditions gave a linear release of product in relation to the amount of sample used and the time of incubation.

Determination of Protein in BALF-Supernatant

The protein content in the supernatant of BALF after appropriate dilutions was measured by the procedure of Lowry et al.[28]

Presentation and Statistical Analysis of the Data

The data for collagen and prolyl hydroxylase activity are expressed per total lung. Expression of the data on a per lung basis rather than per milligram wet weight, per milligram protein or DNA avoids the artifactual lowering of the values in bleomycin treated animals[29,30] because this treatment is associated with the leakage of plasma proteins and infiltration of leukocytes into the lung and they cannot be completely washed out by *in situ* perfusion of the lung.[20] Similarly, total and differential cell counts and protein in the BALF-supernatant are expressed on the basis of total lavage fluid volume recovered per lung. The values are reported as the mean ± standard error of the mean (SEM) and analyzed among appropriate groups using Duncan's multiple-range test with a $P \leq 0.05$ considered the level of significance.[31]

Results

Cell Types Recovered in the BALF

The total cell counts in the BALF at 14 days after intratracheal instillation of saline control group ($6.0 \times 10^6 \pm 1.8 \times 10^6$) or bleomycin ($8.8 \times 10^6 \pm 0.7 \times 10^6$) were different among various groups, with significantly less cells in Poly I:C ($5.6 \times 10^6 \pm 0.2 \times 10^6$) group than the Bleo group but not Bleo + Poly I:C group ($7.1 \times 10^6 \pm 0.8 \times 10^6$). The number of differential cells recovered in the BALF of hamsters following different treatments are summarized in Figure 1. The number of neutrophils in Bleo treated group was significantly higher than that of control, Poly I:C and Bleo + Poly I:C groups. The hamsters in the latter group had significantly higher neutrophil counts in the BALF than hamsters in the control group. The monocyte numbers in the BALF of control and Poly I:C groups were not different from each other but they were significantly less in both groups than Bleo or Bleo + Poly I:C group. There was no difference in the monocyte numbers between the last two groups. The number of macrophages and lymphocytes recovered in the BALF of different groups were not different from each other. The eosinophil numbers, however, were significantly higher in Bleo group than any other group.

Protein Content in BALF-Supernatant

The protein content of the BALF-supernatant following various treatments is shown in Figure 2. The protein content in the BALF-supernatant of the hamsters in control and Poly I:C groups averaged 1157 ± 60 and 840 ± 31 μg/lung, respectively. This value in the Bleo treated group of hamsters was increased by almost three-fold (3311 ± 194 μg/lung) as compared with control and four-fold compared with Poly I:C groups. The hamsters in Bleo + Poly I:C group had a significantly higher amount of protein (2383 ± 243 μg/lung) in their BALF-supernatant than control and Poly I:C groups but the value was significantly lower than that of Bleo group.

Lung Collagen

The changes in the total lung collagen content expressed as hydroxyproline after various treatments are

shown in Figure 3. The amount of hydroxyproline in control and Poly I:C groups of hamsters averaged 791 ± 44 and 752 ± 71 μg/lung, respectively. There was no difference in the hydroxyproline content between these two groups. The hydroxyproline content in Bleo treated group of hamsters was significantly increased to 1177 ± 166 μg/lung as compared with control. It is interesting that administration of Poly I:C 2 days before and 13 days after intratracheal instillation of bleomycin completely ameliorated the bleomycin-induced increase in the lung collagen content and consequently the hydroxyproline value in the Bleo + Poly I:C group of hamsters was 766 ± 75 μg/lung and this value was not different from the values obtained in control and Poly I:C groups of hamsters.

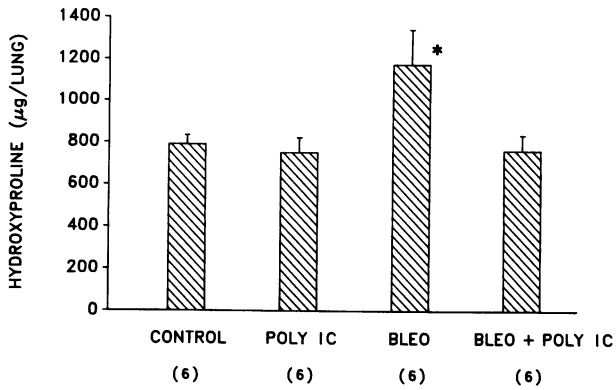

Figure 3—Lung hydroxyproline in different groups of hamsters 14 days after intratracheal injection of saline or bleomycin. See the legend to Figure 1 for explanation of treatment for different groups. Each bar graph represents the mean + SEM. The number of animals for each group is shown in parenthesis. *Significantly increased ($P < 0.05$) over all other groups.

Lung Prolyl Hydroxylase Activity

The lung prolyl hydroxylase activities in hamsters after various treatments are summarized in Figure 4. The prolyl hydroxylase activities in Poly I:C, Bleo, and Bleo + Poly I:C groups of hamsters at 13 days after intratracheal instillation of saline or bleomycin were 83, 183, and 142% of the control, respectively. The hamsters in the Bleo group had significantly higher levels of prolyl hydroxylase activity than hamsters in control, Poly I:C, or Bleo + Poly I:C groups. Although the prolyl hydroxylase activity in the latter group was significantly less than that of Bleo group, it remained significantly elevated as compared with control and Poly I:C groups.

Morphology and Morphometry

Lungs of hamsters in the control or Poly I:C groups showed normal lung structure and no lesion (Figure 5). In lungs of hamsters in the Bleo + Poly I:C group, lesions were restricted to the interstitium and comprised a significantly smaller volume as compared with the Bleo group where there was also occasional alveolar fibrosis (Table 1). Using the specific collagen stain sirius red, lesions in the Bleo + Poly I:C group showed less stainable collagen than did lesions in the Bleo group. Characteristic morphology of the Bleo group included extensive epithelial necrosis (Figure 6), fibroblast accumulation in interalveolar septa (Figure 7), and aggregations of spindle-shaped, fibroblast-like cells in alveolar spaces (Figure 8). The presence of alveolar fibroblasts was only observed in the Bleo group of hamsters. Common to all of these lesions were inflammatory cells comprised predominantly of neutrophils and alveolar macrophages. In the Bleo + Poly I:C group epithelial necrosis was observed infrequently (Figure 9), while type II epithelial cells were observed more commonly.

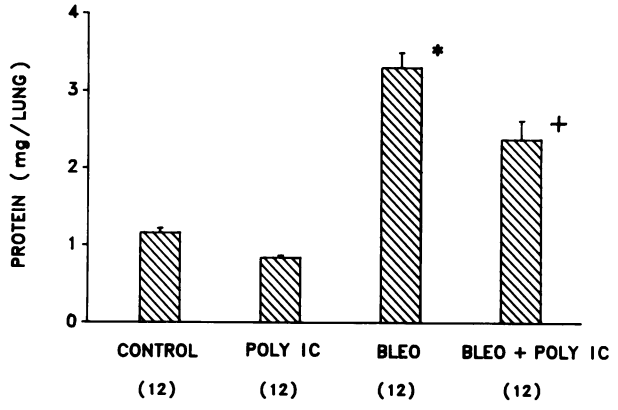

Figure 2—Protein content in the cell-free supernatant of bronchoalveolar lavage fluid of different groups of hamsters 14 days after intratracheal injection of saline or bleomycin. See the legend to Figure 1 for explanation of treatment for different groups. Each bar graph represents the mean + SEM. The number of animals for each group is shown in parenthesis. *Significantly increased ($P < 0.05$) over all other groups; +over Poly I:C and control groups.

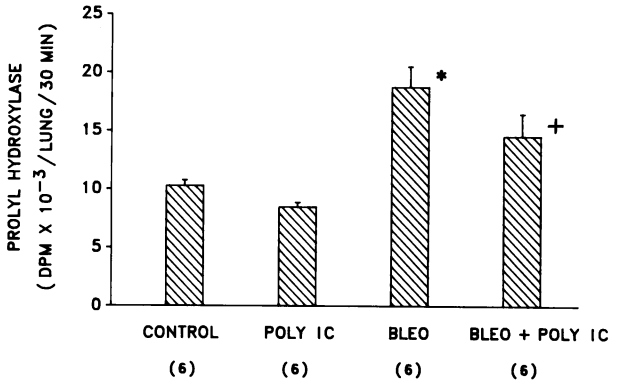

Figure 4—Lung prolylhydroxylase activity in different groups of hamsters 14 days after intratracheal injection of saline or bleomycin. See the legend to Figure 1 for explanation of treatment for each group. Each bar graph represents the mean + SEM. The number of animals for each group is shown in parenthesis. *Significantly increased ($P < 0.05$) over all other groups; +over Poly I:C and control groups.

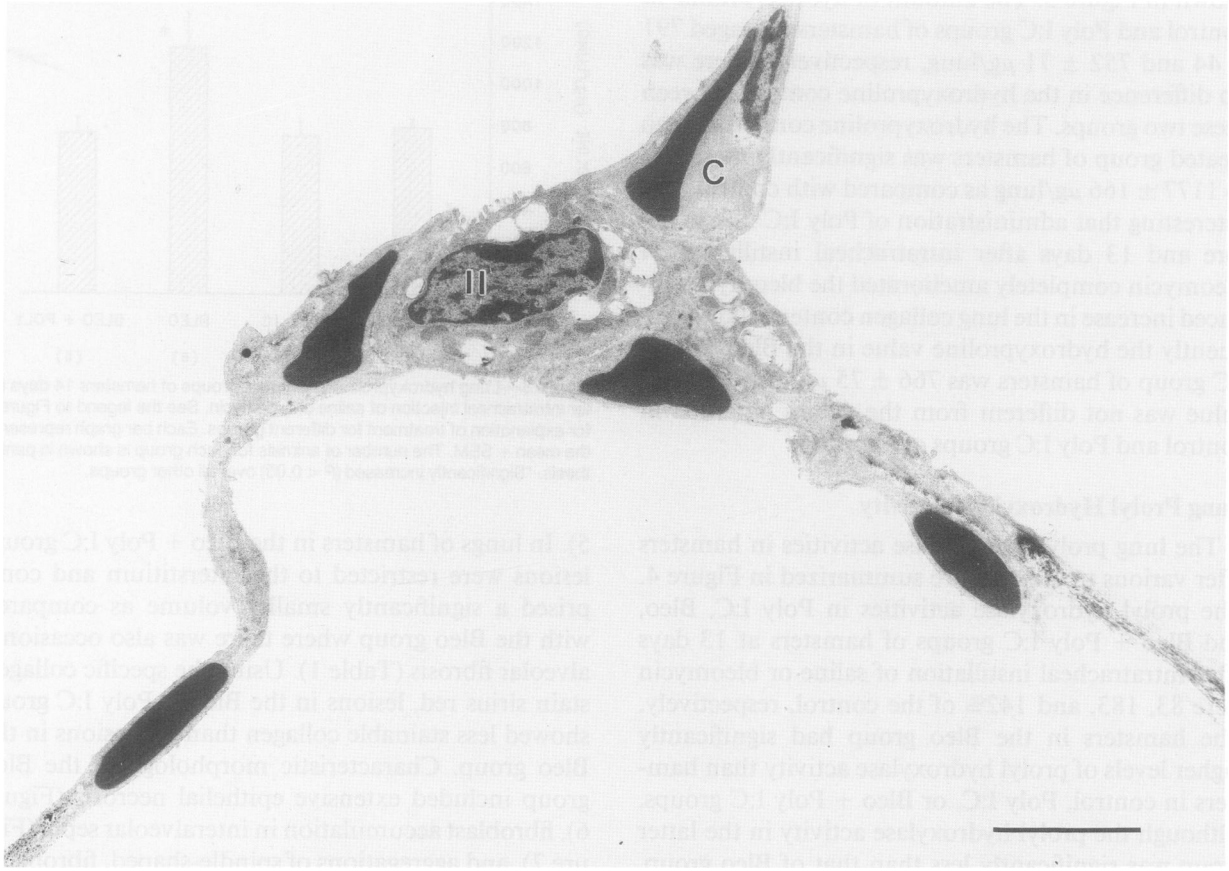

Figure 5—A transmission electron micrograph of a hamster interalveolar septum from the Poly I:C group. Note the thinness of the septum. Type II epithelial cell (II) and capillary lumen (C). Bar = 5 μ.

Discussion

Collagen, the major fibrous protein of connective tissues, constitutes the primary structural component of the tissues. In a normal healthy tissue an equilibrium exists between the rates of synthesis and degradation of collagen. If this equilibrium is shifted in the favor of synthesis it would lead to pathologic fibrosis as encountered in diseases including scleroderma,[32] keloid formation,[33] or pathologic fibrosis of internal organs such as liver and lung. The mechanisms for bleomycin-induced lung fibrosis have been attributed to increased rate of collagen synthesis and decreased rate of its degradation.[34] Using various animal models for fibrosis, investigators are actively in search of efficacious antifibrotic drugs that can reduce the accumulation of collagen from the vital organs resulting from pathologic or toxicologic consequences. The present study uses a synthetic polynucleotide, polyinosinic-polycytidylic acid (Poly I:C), as an inducer of interferon because it is the most active and readily available and therefore the most widely studied compound in the series.[18,19]

Table 1—Volume of Parenchymal Lesion in the Lung of Different Treatment Groups*

Treatment group	Lesion volume (cu cm)†
Control	ND
Poly I:C	ND
Bleo	1.61 ± 0.068
Bleo + Poly I:C	1.00 ± 0.257‡

* See the legend to Figure 1 for explanation of treatment for different groups.
† Values are mean ± SEM of six animals for each group.
‡ Significantly lower volume of lesion ($P < 0.05$) than the Bleo group.
ND, not detected.

Administration of Poly I:C in hamsters treated with bleomycin had definite effects on the course of the inflammatory process leading to lung fibrosis. The hamsters in Bleo + Poly I:C group had a significantly lower number of polymorphonuclear leukocytes (PMN) in the BALF than the hamsters in Bleo group, which had the most PMN. This effect of Poly I:C in bleomycin-treated hamsters could be due to the induction of interferon-α, which has been demonstrated to cause neutropenia[35] and would have contributed to the

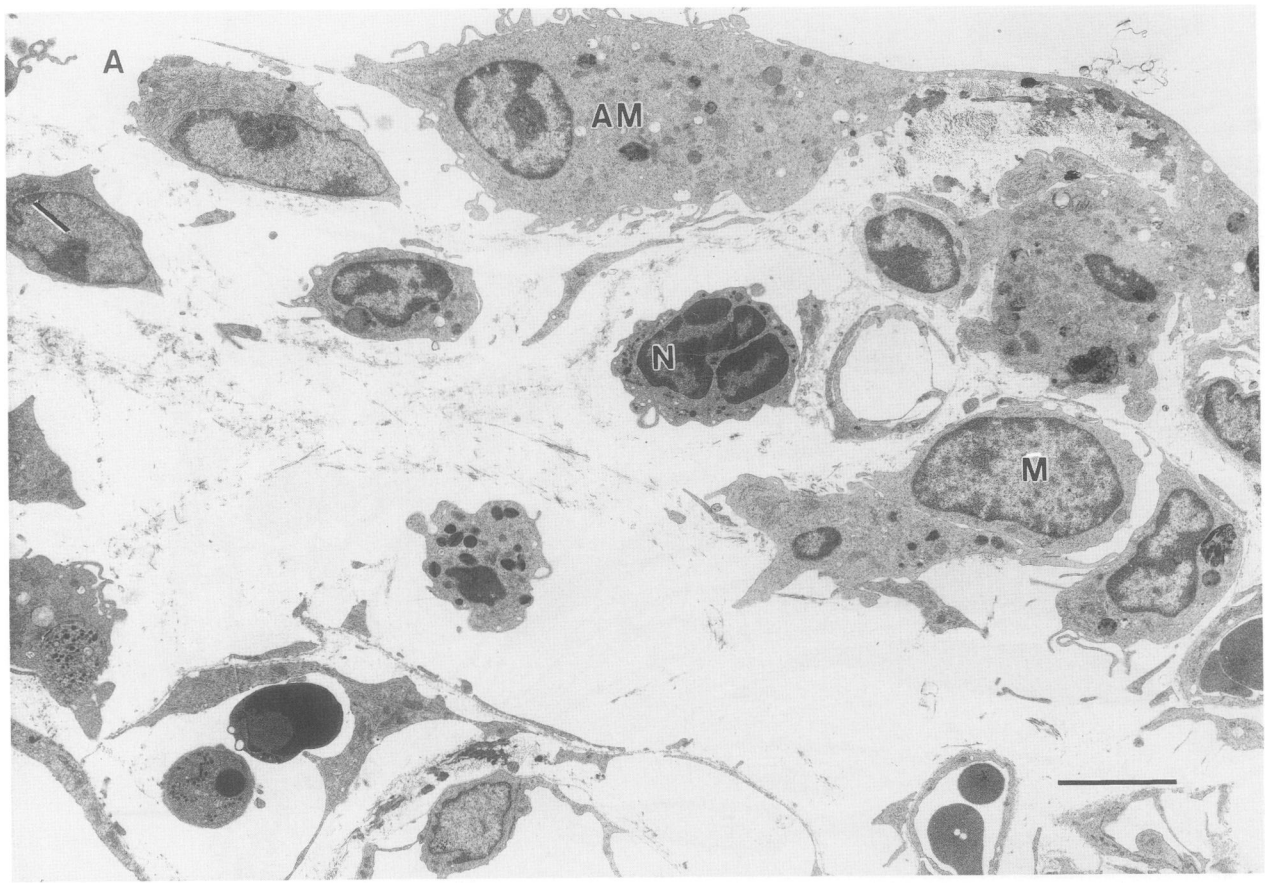

Figure 6—A transmission electron micrograph of a hamster interalveolar septum from the bleomycin group. Note the absence of epithelium at the top of the micrograph leaving the interstitium open to the alveolus (A). An alveolar macrophage (AM) partially covers this area. Note the edematous swelling and abundant inflammatory cells (mononuclear cells (M) and neutrophils (N)) in the septum. Bar = 5 μ.

lower PMN count as found in the present study. An excessive accumulation of PMN could inflict severe lung injury by several mechanisms. Reactive oxygen species produced by PMN and proteolytic enzymes contained in the azurophilic granules of these inflammatory cells would cause deterioration of the normal tissue after being exocytosed by PMN.[36,37] In addition, the lactoferrin contained in the specific granules of PMN may further promote the generation of reactive oxygen species in the lung of bleomycin-treated animals.[38] The reactive oxygen species are shown to damage lung parenchymal cells,[39] endothelial cells,[40] and oxidize polyunsaturated lipids[41,42] leading to synthesis and release of a wide spectrum of arachidonate derived products.[41–43] Some of these products are known to participate in the inflammatory and edemogenic process of the lung.[44,45] The administration of Poly I:C in bleomycin treated animals would tend to minimize the extent of lung damage by reducing the influx of PMN, as found in the present study. It has been demonstrated, however, that a reduction in PMN tends to increase the collagen accumulation[46] and collagen synthesis rate.[47] Thus, the role of PMN in bleomycin-induced lung fibrosis still remains controversial.

The protein content of the BALF-supernatant was measured as an index of pulmonary vascular permeability in different groups of hamsters.[48] The bleomycin treated hamsters had three and four times as much protein as the control and Poly I:C groups, respectively. This finding was not surprising because it has been reported previously that bleomycin is toxic to pulmonary endothelial cells administered either intratracheally[49] or subcutaneously.[50] It has also been reported that bleomycin induces a functional endothelial injury with respect to removal of norepinephrine and serotonin across the luminal endothelium membrane.[50] Thus, it is possible that bleomycin-injured endothelial lining of the capillary wall may not serve as an effective barrier against the leakage of plasma protein into the lung interstitium and air space. This would explain why hamsters in the Bleo group had several times as much protein content in the BALF-supernatant than the control, as reported earlier[20] and

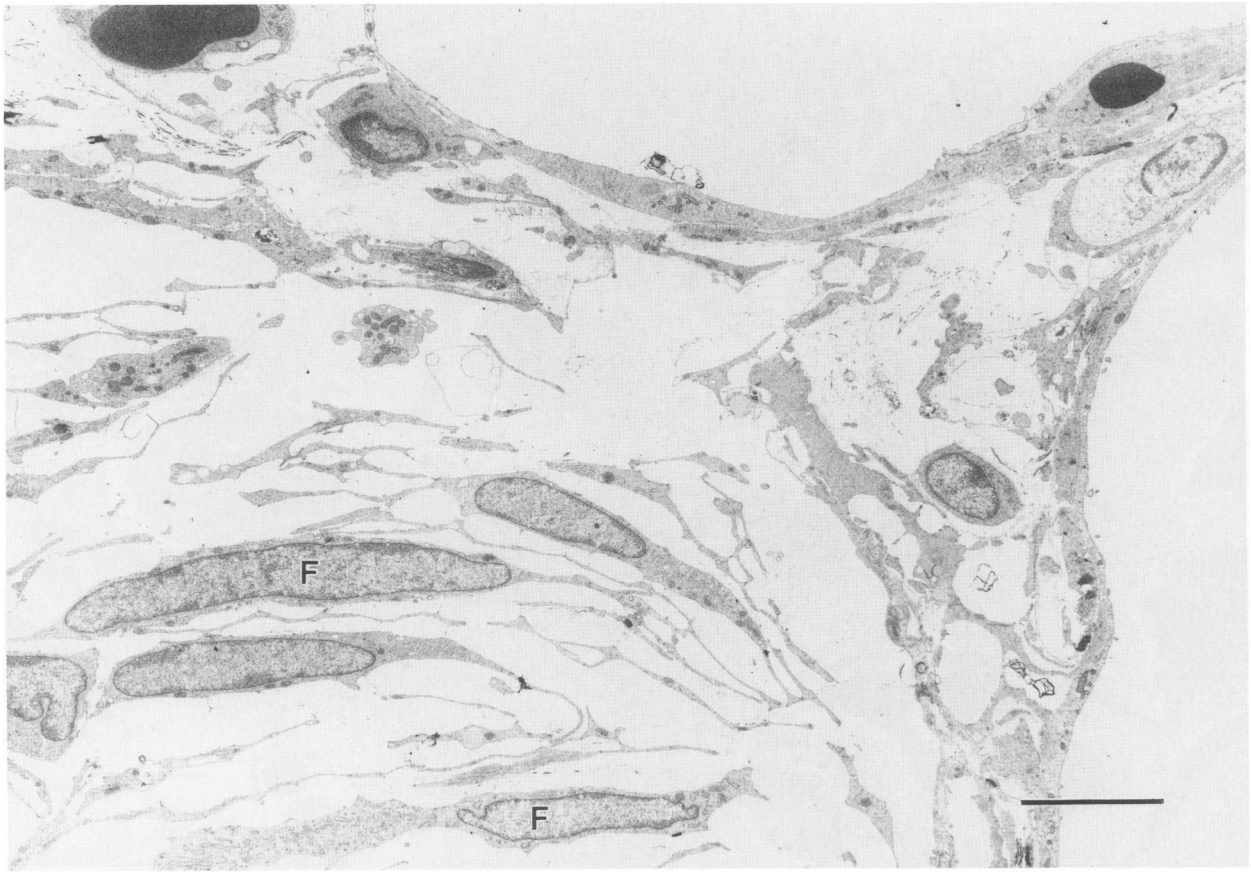

Figure 7—A transmission electron micrograph of a hamster interalveolar septum from the bleomycin group. Note the accumulation of fibroblasts (F) in the septum. Bar = 5 μ.

confirmed in this study. The interesting finding in the present study was that the administration of Poly I:C caused a significant reduction in the bleomycin-induced increased pulmonary vascular permeability. It should be noted, however, that Bleo + Poly I:C group still had an increased pulmonary vascular permeability as compared with hamsters in control and Poly I:C groups. The decreased vascular permeability in Bleo + Poly group may be attributed to inhibitory effect of Poly I:C on the bleomycin-induced release of histamine and arachidonic acid metabolites from the lung mast cells as postulated in our previous study.[20]

One of the hallmarks of interstitial pulmonary fibrosis is the accumulation of collagen in the lungs. The most exciting finding of the present study was that the administration of Poly I:C in bleomycin-treated animals completely ameliorated the accumulation of collagen in the lung. Consequently, there was virtually no difference in the lung collagen content between Bleo + Poly I:C and control or Poly I:C group.

The decreased lung lesion, infrequency of epithelial necrosis, and decreased neutrophils in lavage are all evidence that there was less lung injury in the Bleo + Poly I:C group than in the hamsters in the Bleo group. These findings were associated with less stainable collagen in lesions in the Bleo + Poly I:C than Bleo hamsters. These results imply a role for Poly I:C in decreasing epithelial necrosis and subsequent inflammatory infiltrates that eventuate in pulmonary fibrosis. The mechanisms for decreased epithelial necrosis in response to Poly I:C in the bleomycin treated hamsters may either be secondary to a decrease in the oxidant burden resulting from reduced PMN count or Poly I:C may have a direct antioxidant effect.

Prolyl hydroxylase is one of the most important enzymes in collagen synthesis. This enzyme is involved in hydroxylation of proline during the posttranslational processing of collagen.[51] The role of proline hydroxylation in the control of collagen synthesis and subsequent secretion is controversial,[52] however, several investigators have found that the prolyl hydroxylase activity is increased as collagen accumulates and this rise precedes the increased lung collagen accumulation.[53–56] Increases in prolyl hydroxylase activity after intratracheal instillation of bleomycin have been demonstrated by several laboratories including our

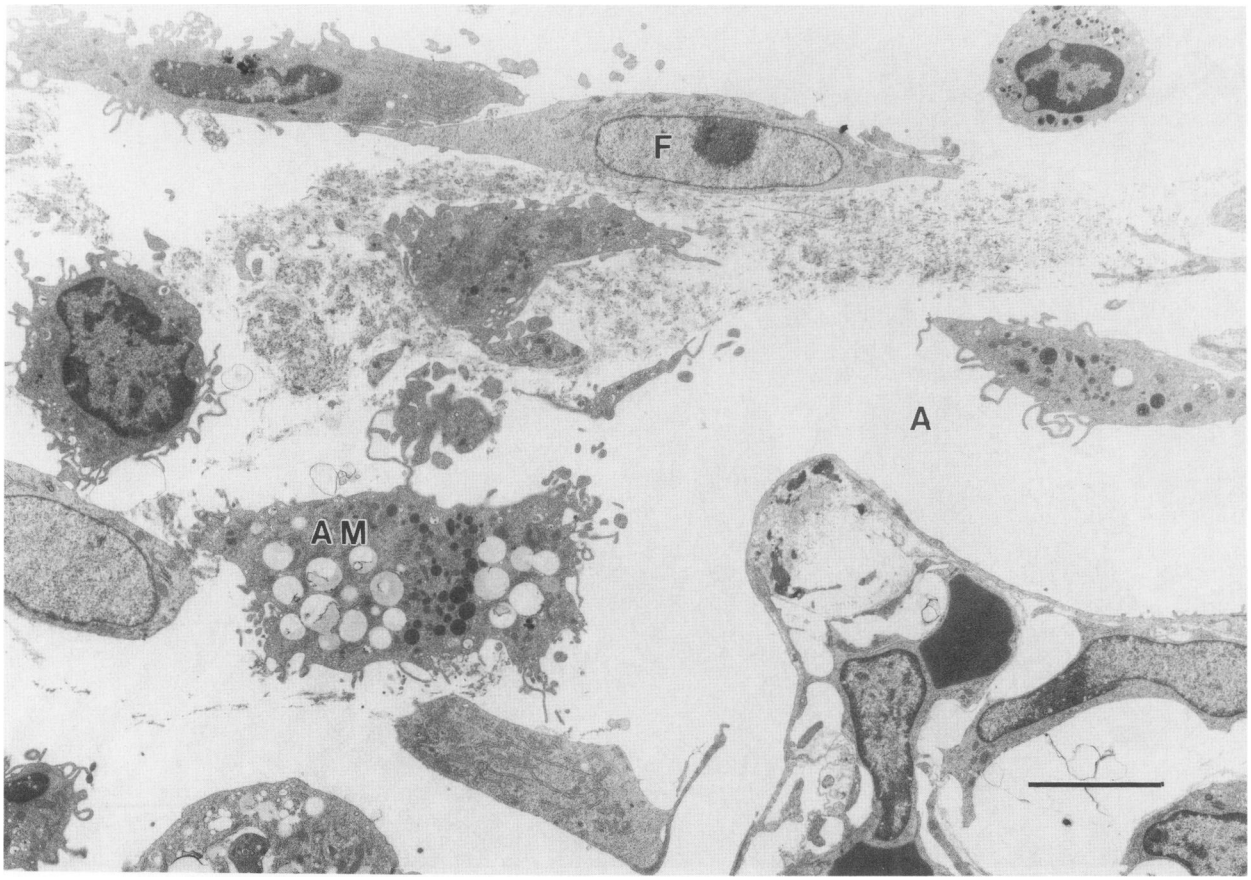

Figure 8—A transmission electron micrograph of a hamster interalveolar septal tip and an adjacent alveolus (A) from the Bleo group. Note the fibrin and collagenlike accumulations adjacent to a fibroblast (F) in an alveolus. Alveolar macrophages (AM) were always in close association with these fibroblast lesions in alveoli. Bar = 5 μ.

own.[26,56] Therefore, it was not surprising to find that bleomycin treated hamsters had increased prolyl hydroxylase activity. This observation confirmed our earlier findings and that of others. The interesting finding, however, was that the administration of Poly I:C in bleomycin treated hamsters caused a significant decrease in the lung prolyl hydroxylase activity as compared with the bleomycin-treated group, although the activity remained significantly elevated over control and Poly I:C groups. The decrease in the lung prolyl hydroxylase activity in Bleo + Poly I:C group paralleled a decreased accumulation of collagen in the lungs as compared with the Bleo group.

It has been demonstrated that increased accumulation of collagen in the lung of bleomycin-treated animals is due to increased collagen synthesis and decreased degradation.[34] Starling et al[57] have provided evidence that the mechanism for increased collagen synthesis in response to bleomycin treatment operates at the transcriptional level based on their finding of increased polysomal procollagen type I mRNA in chick skin and lung fibroblasts after bleomycin treatment. The mechanism for the ameliorating effect of Poly I:C against bleomycin-induced lung collagen accumulation is not clearly understood. It is possible, however, that the antifibrotic effect of Poly I:C is being indirectly mediated through the induction of various interferons. Presence of an excessive circulating level of interferons, particularly gamma, would have an inhibitory effect on collagen synthesis by repressing collagen mRNA synthesis. This hypothesis is based on the finding of Rosenbloom et al[58] that gamma interferon was found to inhibit the synthesis of collagen mRNA of human diploid fibroblasts. Furthermore, interferons are known to exert a potent antiproliferative effect on different cells, including fibroblasts[59,60] responsible for collagen synthesis. The antiproliferative effect of interferons on the fibroblasts would help to minimize the collagen synthesis by reducing their number, which has been reported to be markedly increased in hamster lung following intratracheal instillation of bleomycin.[61] The hypothesis that the antifibrotic effect of Poly I:C is secondary to interferon induction will remain tenuous until it can be demon-

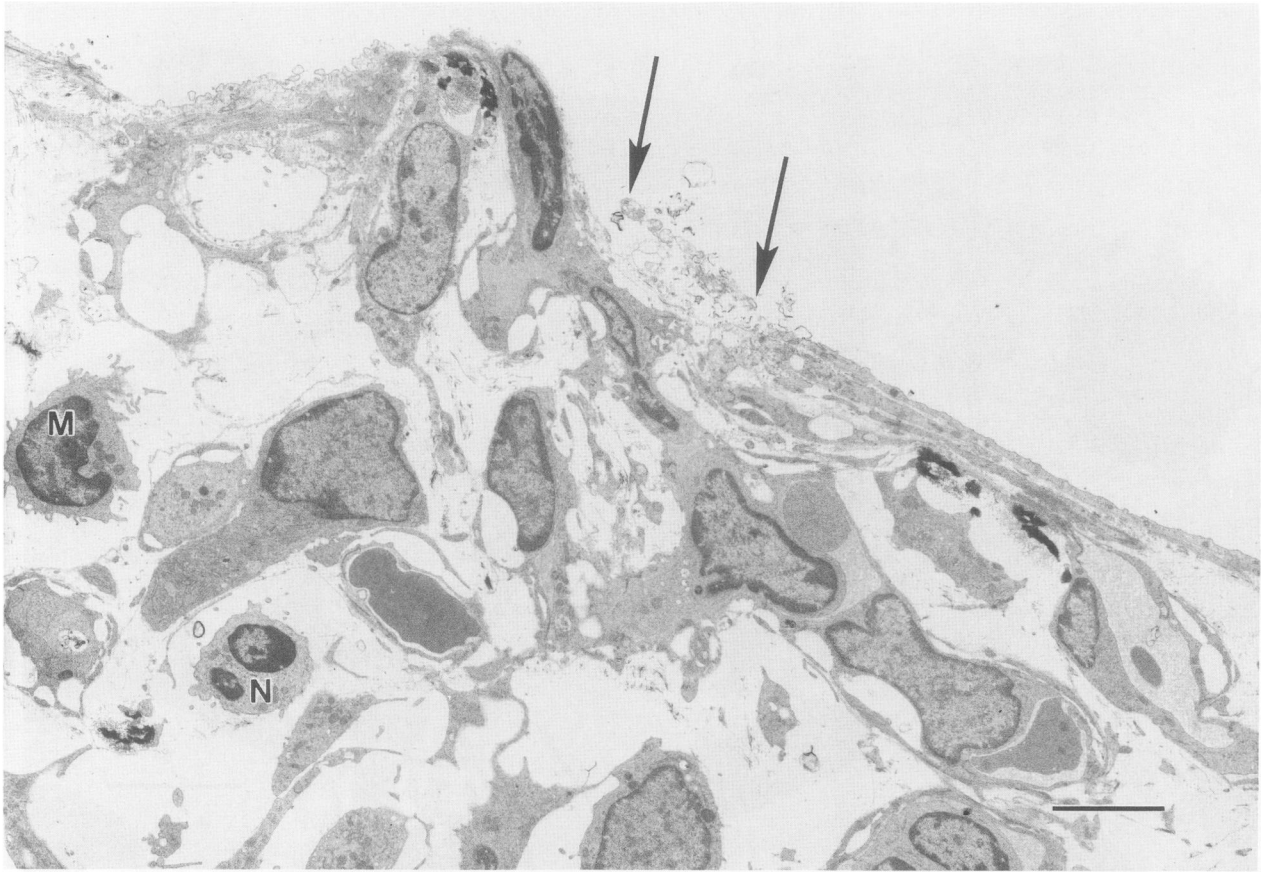

Figure 9—A transmission electron micrograph of a hamster interalveolar septum from the Bleo + Poly I:C group. Epithelial necrosis was infrequently observed in this group; however, membrane fragments (arrow) mark an area where there is epithelial necrosis. The interstitium showed much less fibroblast accumulation, but affected septa were swollen with a mixed infiltrate of neutrophils (N) and mononuclear cells (M). Bar = 5 μ.

strated that Poly I:C did indeed increase the circulating level of interferon in the present study.

Our finding that Poly I:C treatment was beneficial against bleomycin-induced lung collagen accumulation is similar to the findings of other investigators who have reported a protective effect of interferon inducers, including Poly I:C against hyperoxic pulmonary damage.[62] These findings are exciting for those who believe that the induction of the body's own interferon system may be a useful therapeutic alternative to exogenous interferon therapy. Another set of findings that must be balanced against these positive findings are the pharmacologic effects of systemically administered Poly I:C. These include provocation (but not sensitization) of the local Schwartzman reaction,[63] pyrogenicity,[64] and embryotoxicity.[65] Whether any of these other *in vivo* effects will limit the testing of Poly I:C in clinical studies is only speculative. Regardless of the clinical implications, the finding that the use of Poly I:C inhibited the bleomycin-induced lung collagen accumulation opens a new avenue to study the fundamental mechanism responsible for the genesis of the fibrotic process.

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