PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 146, 232-235 (1974)

Alpha-1 Globulin Trypsin Inhibitor in Canine Surfactant Protein[1] (38076)

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(Introduced by U. C. Luft)

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The observation in man of a relationship between an inherited deficiency of serum alpha-1-antitrypsin and the occurrence of pulmonary emphysema suggests that alpha-1-antitrypsin may play an important role in the normal and pathologic physiology of the lung (1). Alpha-1-antitrypsin inhibits the activity of trypsin, collagenase, elastase, and leukocytic proteases (2, 3), and therefore, may protect the lung from proteolytic damage resulting from inhalation of a variety of toxicants.

The distribution of alpha-1-antitrypsin in normal lung tissue is not known. In order to determine if alpha-1-antitrypsin may be a part of or found *in situ* with the surfactant lipid–protein complex, we have evaluated the trypsin inhibitory capacity of canine surfactant protein.

Methods. Surfactant was obtained by bronchopulmonary lavage of healthy adult Beagle dog lungs by the method of Kylstra (4) as modified by Boecker *et al.* (5). Unilateral lavage with normal saline was performed *in vivo* on nine animals under light halothane anesthesia. In order to reduce the possibility of contamination of the surfactant protein with whole blood protein, we also obtained surfactant by lavage of isolated, saline-perfused lungs. For this purpose, four animals were anesthetized with sodium pentobarbital and sacrificed by exsanguination. The heart and lungs were removed en bloc and irrigated with saline. The lungs were ventilated with a Harvard constant volume pump at the rate of 12 cycles per minute and the pulmonary vasculature was perfused with normal saline. When the lungs became white, bilateral lavage with normal saline was performed.

The lavage fluid was treated identically whether it was obtained from live anesthetized animals or from the isolated, perfused lung preparations. Cells were removed from the whole lavage fluid by slow speed centrifugation (3,000 *g*-min). Precipitation of the surfactant was then accomplished by high speed centrifugation (2×10^6 *g*-min) at 4°C. The fluffy pellet obtained by high speed centrifugation has been shown by previous studies in this laboratory (6) to contain 94–96% of the pulmonary washing phospholipid and to be highly surface-active. Following dialysis against distilled water for 48 hr, the surfactant pellet was lyophilized. Lipids were extracted from the freeze-dried material with 4:1 v/v hexane–ethanol. The hexane–ethanol insoluble fraction, hereafter referred to as surfactant protein, was suspended in normal saline.

Immunoelectrophoresis of the surfactant protein from each animal was conducted as follows. Electrophoresis was performed at 4°C in 2% agarose in barbital buffer (pH 8.6, ionic strength 0.05) poured on 1 × 3 in. microscope slides. Current was regulated at 5 mA/slide. Following electrophoresis, troughs were filled with either canine antiserum globulin (rabbit origin, Colorado Serum Co.), antidog whole serum antiserum (rabbit origin, Colorado Serum Co.), or

[1] This research was conducted under AEC Contract AT(29-2) 1013, and in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

immunoglobulin against canine IgG (rabbit origin, Colorado Serum Co.). Antisera were allowed to react with the separated proteins in a humidified chamber for 24 hr. Thereafter the slides were washed in saline and distilled water, placed in a 37°C incubator until dry, and stained with Ponceau S-II dye.

Trypsin inhibitory capacity of the surfactant protein was evaluated by the radial diffusion and electrophoresis methods described by Cawley (7). Both assays were performed on fibrinogen–agarose slides composed of 4 parts 2% fibrinogen in barbital buffer, 2 parts 0.025 *M* calcium chloride, and 9 parts 2% agarose. The slides were heated for 1 hr at 65–75°C to allow the fibrinogen to coagulate. The radial diffusion assay provided an estimate of the trypsin inhibitory capacity of a whole sample of surfactant protein. Saline, serum, or surfactant protein were placed in wells alternating with trypsin (1:250, Difco) on the slides and the samples were allowed to diffuse through the fibrinogen–agarose substrate. Inhibitory capacity was evaluated in terms of the degree to which radial trypsin digestion of the fibrinogen was blunted. In the electrophoresis trypsin inhibition assay, the inhibitory capacities of electrophoretically separated fractions of the surfactant protein were observed. Electrophoresis was conducted as it was in the immunoelectrophoresis studies except that the troughs were filled with trypsin instead of antiserum. The trypsin was allowed to digest the fibrinogen at room temperature for 16 hr at which time the reaction was stopped by immersing the slides in 10% acetic acid. The presence of trypsin inhibitor was noted at points where the fibrinogen was not digested by the trypsin solution. After the radial diffusion and electrophoresis procedures were performed, the fibrinogen–agarose slides were washed, dried, and stained in the manner described for the immunoelectrophoresis studies.

Results. Results of the immunoelectrophoresis studies of surfactant protein obtained from isolated, perfused lungs were identical with those observed with surfactant protein from live anesthetized animals. Immunoelectrophoresis of surfactant protein using canine antiserum globulin as the antiserum indicated the presence of two proteins antigenically related to serum proteins. Immunoelectrophoresis using antidog whole serum antiserum indicated the presence of three serum proteins, two of which were identical to those observed with the canine antiserum globulin and a third which migrated opposite albumin in whole serum (Fig. 1, above and middle). Studies in which antidog IgG was placed in the trough identified one of the serum proteins as IgG (Fig. 1, below). In summary, the immunoelectrophoresis studies indicate the presence in surfactant protein of albumin, IgG, and a third serum protein that remains near the point of application during electrophoresis and produces a short rounded arc upon reaction with antisera to canine serum proteins.

Both types of trypsin inhibition assays indicated the presence of trypsin inhibitor in the surfactant protein. In the radial diffusion experiments, the inhibition of trypsin digestion of the fibrinogen by the surfactant protein was clearly intermediate to the lack of inhibition by saline and the strong inhibition by whole serum (Fig. 2). The elec-

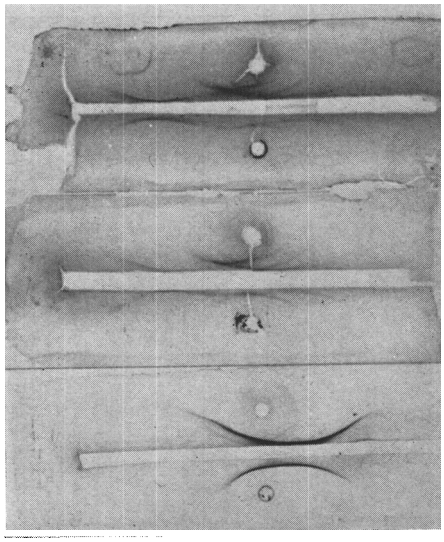

FIG. 1. Immunoelectrophoresis of canine whole serum (top wells) and canine surfactant protein (bottom wells). Antisera: antidog whole serum antiserum (above and middle); antidog IgG (below). Anode to left.

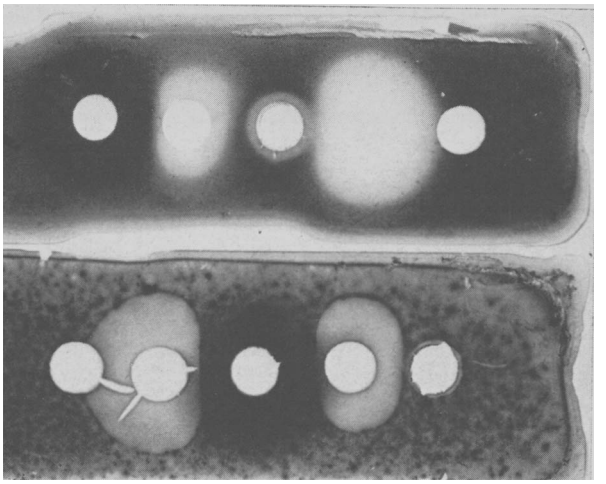

FIG. 2. Radial diffusion trypsin inhibition assays. Wells filled as follows: Top (left to right)—canine whole serum, trypsin, canine surfactant protein, trypsin, saline. Bottom (left to right)—saline, trypsin, canine whole serum, trypsin, canine surfactant protein.

trophoresis experiments demonstrated the presence of a trypsin inhibitor in surfactant protein that migrates electrophoretically in the same location (alpha-1 region) as the major trypsin inhibitor of canine whole serum (Fig. 3). In human whole serum, the major trypsin inhibitor migrates in the same manner and has been identified as alpha-1-antitrypsin (8). As in the case of the immunoelectrophoresis studies, no differences in the results of the trypsin inhibition assays were observed between surfactant protein from perfused and nonperfused lungs.

Discussion. In these studies we have immunoelectrophoretically demonstrated the presence of albumin, IgG, and a third unidentified serum protein in surfactant protein obtained *in vivo* by bronchopulmonary lavage. The same proteins were present when lavage followed perfusion of the pulmonary vasculature with saline. This lends support to the hypothesis that these serum

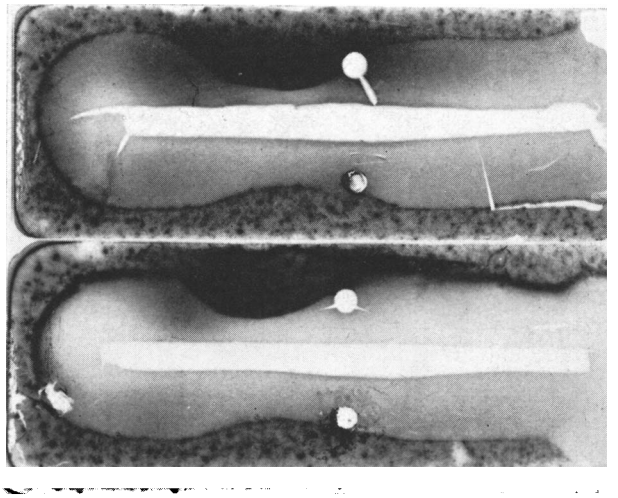

Fig. 3. Electrophoretic trypsin inhibition assays of canine whole serum (top wells) and canine surfactant protein (bottom wells). Troughs filled with trypsin. Anode to left.

proteins are not present as a result of whole blood contamination occurring during the lavage procedure. In addition, the concentration of these three proteins in the surfactant protein was of sufficient magnitude to indicate that if their presence were due to whole blood contamination, additional precipitin arcs representing the other serum proteins should have appeared during immunoelectrophoresis.

Demonstration of trypsin inhibitor in surfactant protein that migrates as an alpha-1 globulin has not, to our knowledge, been reported, although Taylor and Abrams (9) reported inhibition of fibrinolysis by pulmonary surface-active lipoprotein that migrated in the alpha-1 region. It is curious that trypsin inhibitor was observed in the alpha-1 region when immunoelectrophoresis did not indicate the presence of an alpha-1 protein. This may be explained in terms of the relatively high sensitivity of the trypsin inhibition assay when compared to immunoelectrophoresis. In human serum, a sample that produces a barely discernible alpha-1-antitrypsin arc on immunoelectrophoresis exhibits very strong inhibition in the trypsin inhibition assays.

Summary. Immunoelectrophoresis demonstrates the presence of three serum proteins in canine surfactant protein obtained *in vivo* by bronchopulmonary lavage. The surfactant protein inhibits trypsin and the trypsin inhibitor has electrophoretic mobility similar to that of human alpha-1-antitrypsin. The trypsin inhibitor is present when lavage follows saline perfusion of the pulmonary vasculature suggesting that although the trypsin inhibitor may arrive in the lung via the blood, it is not in the lavage fluid as a result of whole blood contamination.

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Received Jan. 2, 1974. P.S.E.B.M., 1974, Vol. 146.