# Lung Localizing Antibodies in Anti-Lung and Anti-Kidney Serum.*† (26435)

I. TAMANOI, Y. YAGI, R. HIRAMOTO AND D. PRESSMAN
*Department of Biochemistry Research, Roswell Park Memorial Institute, Buffalo, N. Y.*

Rabbit antibodies formed against rat lung have been shown to localize in the lungs when injected into rats. There are antibodies also in this serum which localize in other tissues such as kidney(1,2). In this aspect anti-lung serum is similar to anti-kidney serum and also shows nephrotoxic activity(3,4). Moreover, the kidney localizing or nephrotoxic components in anti-kidney serum can be removed by treatment with lung homogenate(4,5). Previous studies of anti-kidney serum by means of radiolabel technic without specific purification have generally failed to show any significant localization in lungs (6). This was due to technical difficulties involving a rather high and variable non-specific uptake of normal serum globulins in the lungs. However, the presence of such lung localizing components in anti-kidney serum, although in minor amounts, has been evidenced by *in vivo* and *in vitro* purification methods(7,8).

The present work utilizing the fluorescein-labeled antibody technic has revealed clearly the presence of lung localizing components in anti-kidney serum as well as in anti-lung serum. Although its content was significantly lower in anti-kidney serum than in anti-lung serum, the lung localizing antibody from both sera seemed to be fixed in the same regions of the lungs.

*Experimental. Antisera.* Anti-rat kidney sera (referred to as anti-RK) were pooled from rabbits injected with rat kidney homogenate as described previously(9). The saline insoluble fraction of rat lung homogenate sedimented between 1,500 and 25,000 RCF (15 min centrifugation) was used as injecting antigen for anti-lung serum. Anti-lung serum from a single rabbit with a high lung localizing activity was used (anti-RLuH). Normal rabbit serum was a commercial product (Pentex, Inc., Kankakee, Ill.)

The $\gamma$-globulin fractions of anti-lung and normal serum were obtained by ethanol fractionation(10) and are referred to as $\gamma$ G anti-RLuH and $\gamma$ GNS respectively. Globulin from the anti-RK serum was prepared by ammonium sulfate precipitation (G anti-RK) (11). Radioiodination of proteins was carried out as described previously(12,13). *Detection of localized globulin in the tissues.* Sprague-Dawley rats were used throughout this study. Since the globulins from anti-tissue serum were found to be quite toxic to rats,‡ globulin solutions with or without prior radioiodination were divided into 2 equal portions when 10 mg was administered, and into 3 portions when 30 mg was given (only G anti-RK and $\gamma$ GNS were used at the higher level). Each portion was injected intravenously at 2 hour intervals. The rats were perfused 18 hours after last injection. In those animals receiving radioiodinated globulins, the tissues were excised and radioactivity was determined as described previously (13). For the fluorescent antibody studies, 6% gelatin-saline solution (C. B. Knox Corp., Camden, N. J.) was infused into lungs *via* trachea, and bronchi of both lobes were tied with silk thread to prevent outflow of gelatin solution. This procedure was used to prevent the collapse of alveolar sacs during the manipulation. The lungs and the kidneys were frozen in dry ice immediately after excision and 4 $\mu$ thick sections were cut and stained for the localized globulin with fluorescein-labeled horse anti-rabbit globulin antibody as described previously (14). Rat liver sediment was used instead of beef liver powder to remove non-specifi-

* Supported by grant from Nat. Heart Inst.

† Part of the work was presented at 44th Annual Meeting, Am. Assn. Immunol., April, 1960, Chicago, Ill.

‡ 10 mg $\gamma$ G anti-RLuH and 20 mg G anti-RK were lethal for rats weighing 140-150 g when injected at one time, but 30 mg $\gamma$ GNS showed no toxic effect.

TABLE I. Amount of Globulin Localized in Tissues.*

| $I^{131}$-globulin inj. | Inj. dose, mg† | % of inj. dose localized (whole organ) | | | | | Amt (μg) of globulin localized (whole organ) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Lung | Kidney | Liver | Spleen | Blood‡ | Lung | Kidney | Liver | Spleen |
| γ G anti-RLuH | 10 | .81 | .26 | 1.00 | .11 | 38 | 81 | 26 | 100 | 11 |
| G anti-RK | 30 | .28 | .74 | 1.08 | .16 | 19 | 84 | 222 | 324 | 48 |
| γ GNS | 30 | .44 | .08 | .29 | .03 | 40 | 132 | 24 | 87 | 9 |

* Four rats were used for each group. Rats were perfused 18 hr after last inj. and radioactivity of the tissues determined.

† γ G anti-RLuH was inj. in 2 equal portions and γ G anti-RK and γ GNS were inj. in 3 equal portions at 2 hr intervals.

‡ Calculated assuming total blood as 1/10 of body wt.

cally staining components in labeled horse antibody preparations.

*Results and discussion.* Preliminary results indicated that localization of radioactivity in the lungs of animals one day after injection with G anti-RK or γ GNS was less than ½ the localization found in animals receiving γ G anti-RLuH (on the basis of percent of injected dose localized in the tissue). Therefore, pairs of animals were injected with γ-globulin fractions of anti-lung serum (10 mg), anti-kidney serum (10 mg and 30 mg), or normal serum (10 mg and 30 mg). The rats were killed after one day and observed for localized globulin by the fluorescein technic as described above. At the 10 mg dose level, only animals receiving γ G anti-RLuH showed positive staining in the lung. However, when the dose was increased to 30 mg, the group receiving G anti-RK became positive whereas those receiving γ GNS remained negative even at this level. Positive staining was seen in the kidney with both γ G anti-RLuH (10 mg) and G anti-RK (10 mg and 30 mg), but not with γ GNS (10 and 30 mg).

Total amounts of globulin localized in the lung were estimated by injecting the same preparations with radioiodine. Amounts of 10 mg of γ G anti-RLuH, 30 mg of G anti-RK or γ GNS were injected into 4 rats for each group (Table I). The weight of globulin localized in the lungs was approximately the same for animals receiving the anti-lung and the larger amount of anti-kidney preparations. For animals receiving 30 mg of the normal serum preparation, the weight localized was even greater.

The negative results obtained by fluorescein label technic with even 30 mg of γ GNS indicate that it is rather uniformly distributed throughout the lung tissue (or washed out during the staining procedure) whereas the lung localizing antibody in anti-RLuH and in anti-RK must be fixed tightly at local concentrations high enough to give staining (15). Indeed in the assay of globulin preparations directly for lung localizing antibodies the use of the fluorescein label technic is especially effective. The background fixation of protein from radiolabeled γ GNS is variable from sample to sample (0.3-0.7% of injected dose). Actually, the observed localization from G anti-RK falls within this range and only with the fluorescein technic is a lung localizing activity, not present in γ GNS, observed without resorting to specific purification procedures. (Radioautographs might also show the high local concentration.)

Radiolabel technics with specific purification do show lung localizing antibodies in G anti-RK(7,8) and do not require administration of the high levels of protein required with the fluorescent method(15).

The positive staining of the lung sections is primarily localized in certain areas of alveolar walls excluding nuclei (Fig. 1). Arteries, veins, bronchi and bronchioli showed no staining. Although the fragility of lung tissue in frozen state prevented us from identifying the exact location of localized globulin in finer detail, both endothelial and epithelial cells seemed to be stained. No difference in staining pattern was observed between anti-RLuH and anti-RK.

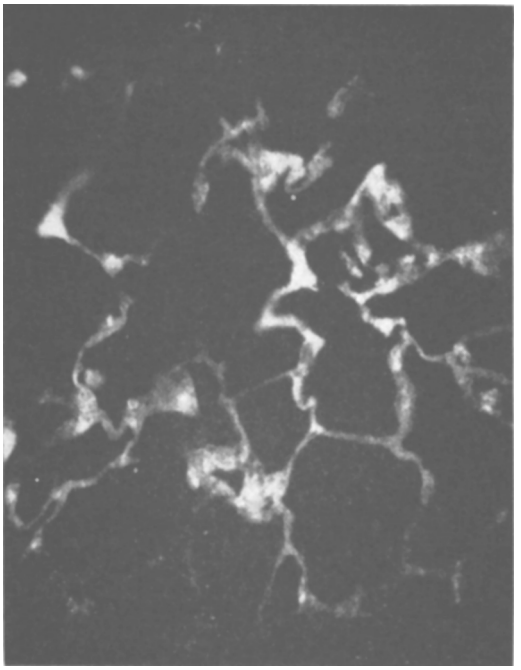

FIG. 1. Section of lung tissue of rat inj. with 10 mg $\gamma$ G anti-RLuH and stained with fluorescein labeled horse anti-rabbit globulin antibody. Although staining is confined to the alveoli, the difference in intensity in certain regions of the alveoli indicates a differential concentration of $\gamma$ G anti-RLuH at these sites (see figure). Nuclei were not stained.

It is interesting that the lung localizing component in both anti-lung and anti-kidney serum seems to be fixed in the same region. This does not necessarily mean that they are reacting with the same antigen, but it implies that the lung antigens responsible for cross-localization of anti-kidney antibody seem to be present in the same region as those responsible for fixation of anti-lung antibody. Indeed, that the responsible antigens may be different for these 2 types of lung localizing components has been suggested by previous studies on the *in vivo* purification of anti-kidney and anti-lung antibody(7). The radioglobulin recovered from the lungs of animals receiving anti-lung antibody, when reinjected, localized in the lung tissue to a high extent, but not in the kidney. In contrast, the similar preparation from the lungs of animals receiving anti-kidney antibody, localized in the kidney as well as in the lung, indicating the presence in the lung of antibodies capable of localizing in kidney.

*Summary.* Localization of antibodies in lungs was examined by fluorescent antibody technic after injection of rabbit anti-rat lung and anti-rat kidney serum into rats. Although both sera gave positive staining in lungs, anti-lung serum contained significantly higher amount of lung localizing antibodies than anti-kidney serum. The localizing components in both sera seemed to be fixed in the same region of alveolar walls.

We would like to thank Dr. J. Jurandowski, Messrs. J. Planinsek, J. Bernecky and E. Kanski for technical assistance.

1. Pressman, D., Eisen, H. N., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 143.

2. Eisen, H. N., Pressman, D., Sherman, B., *J. Immunol.*, 1950, v65, 543.

3. Chikamitsu, H., *Folia Endocrin. Japan*, 1940, v16, 85.

4. Baxter, J. H., Goodman, H. C., *J. Exp. Med.*, 1956, v104, 467.

5. Eisen, H. N., Pressman, D., *J. Immunol.*, 1950, v64, 487.

6. Pressman, D., *Ann. N. Y. Acad. Sci.*, 1957, v70, 72.

7. Pressman, D., Sherman, B., *J. Immunol.*, 1951, v67, 21.

8. Korngold, L., Pressman, D., *ibid.*, 1953, v71, 1.

9. Pressman, D., Keighley, G., *ibid.*, 1948, v59, 141.

10. Deutsch, H. F., *Methods in Medical Research (Year Book)*, 1952, v5, 284.

11. Pressman, D., Eisen, H. N., *J. Immunol.*, 1950, v64, 273.

12. Yagi, Y., Engel, K., Pressman, D., *ibid.*, 1960, v85, 375.

13. Day, E. D., Planinsek, J., Pressman, D., *J. Nat. Cancer Inst.*, 1956, v17, 517.

14. Hiramoto, R., Pressman, D., *Cancer Research*, 1957, v17, 1135.

15. Pressman, D., Yagi, Y., Hiramoto, R., *Internat. Arch. Allergy and Applied Immunol.*, 1958, v12, 125.

Received January 23, 1961, P.S.E.B.M., 1961, v106.