stimulus for release of posterior pituitary hormones is invasion of the neurosecretory terminals by impulses discharged down the hypothalamo-hypophysial tract (*16*); and, according to some authors (*17*), propagated impulses in excitable tissues cause the release of ATP from the plasmalemma. On this view, impulses in the neurosecretory terminals would make endogenous ATP available to interact with the adenosine triphosphatase of the secretory granules. Similar events could be involved in linking depolarization to release of transmitter in conventional neurons for there are reports that granules containing synaptic transmitter substances also show adenosine triphosphatase activity (*18*).

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Autoimmune Glomerulonephritis Induced in Sheep by Injections of Human Lung and Freund's Adjuvant

Abstract. *Sheep immunized with human lung and Freund's adjuvant develop progressive glomerulonephritis with deposition of autoantibodies and complement in the glomerular basement membrane. This nephritis appears to be the first autoimmune disease induced by antigens that are not organ-specific. By clinical and immunopathologic criteria, this nephritis appears identical to the nephritis induced in sheep by human glomerular basement membrane and greatly resembles certain nephritides of man, in particular, the nephritis in Goodpasture's disease.*

Sheep injected with heterologous glomerular basement membrane (GBM) and Freund's adjuvant (FA) invariably develop progressive, usually fulminating glomerulonephritis and form antibodies against the injected antigenic determinants (*1*). Though kidney shares antigens with other organs (*2*, *3*), our attempts to induce progressive glomerulonephritis in sheep by injections of various nonrenal tissues, such as human placenta (*1*), heart, synovia, and tonsil together with FA, have been unsuccessful.

By contrast, injections of human-lung basement membranes and FA have induced progressive glomerulonephritis in sheep. This finding is contrary to what would be expected as a result of previous efforts to induce experimental "autoimmune" disease (*4*) and may provide insight into the pathogenesis of Goodpasture's nephritis and other human diseases.

Shavings (60 μm) of human lung were cut on a freezing microtome (*2*), refrozen, and cut twice more. The shavings were washed with normal saline and centrifuged at 1800*g* for 10 minutes. This process was repeated twice. The sediment, suspended in saline, was disrupted in a sonic oscillator for 20 minutes and then washed and centrifuged (as above) three times. Microscopically, the sediment consisted predominantly of refractile plates of basement membranes and fibrillar material. The sediment was suspended in saline containing merthiolate (1:10,000) and homogenized by sonication; suspensions [50 mg of sediment (wet weight) per milliliter of saline] were emulsified with equal volumes of complete FA. Equal volumes of saline (without lung) and complete FA were emulsified for injection into control sheep. A similar emulsion containing 2 mg of heat-killed, lyophilized group A hemolytic streptococci per milliliter was injected into two sheep (*5*).

Every 2 weeks each sheep was injected with 5 ml of emulsion: 3 ml was given intradermally in the neck, axillary, and inguinal regions; 1 ml was given intramuscularly; and 1 ml was given subcutaneously. Periodic urinalyses and blood urea nitrogen determinations were made on all animals. Repeated urinalyses in untreated sheep rarely showed more than an occasional trace or 1-plus proteinuria. Hematuria was seen only in sheep with autoimmune nephritis.

Renal tissue was obtained by open-wedge biopsy or when the animals were killed and prepared by conventional methods for light and fluorescent microscopy. Fluorescent-conjugated rabbit antiserum to sheep immunoglobulin G (IgG) (*6*) and antiserum to sheep complement (β_{1C}-globulin) were prepared (*7*).

All five sheep injected with human lung and FA developed glomerulonephritis. One sheep that had received 15 ml of emulsion in 4 weeks developed proteinuria with azotemia on day 38 and died in uremia 8 days later. At autopsy, the kidneys were swollen and covered with many petechiae. The glomeruli had exudative, hemorrhagic, necrotizing, intra-, and extracapillary proliferative changes morphologically identical with those previously described (*1*). There was interstitial edema with mononuclear and polymorphonuclear cell infiltration but no fibrosis. Nearly all glomeruli were involved. A second sheep, receiving 25 ml of emulsion in 8 weeks, developed proteinuria on day 42 and azotemia on day 47 and died in uremia on day 91. Renal biopsy showed a progression from acute to subacute to chronic changes. At autopsy, only a few petechiae were seen on the kidneys. Interstitial fibrosis was diffuse, and most glomeruli were hyalinized. A third sheep received 30 ml of emulsion in 75 days, developed proteinuria on day 59 and azotemia on day 66, and died in uremia on day 126. Morphologic findings were similar to those of the second sheep. The remaining two sheep each received 60 ml of emulsion within 22 weeks and had hematuria, proteinuria, petechiae, and acute glomerular lesions on day 89. Both sheep were alive on day 160. Six controls (each receiving 50 ml of

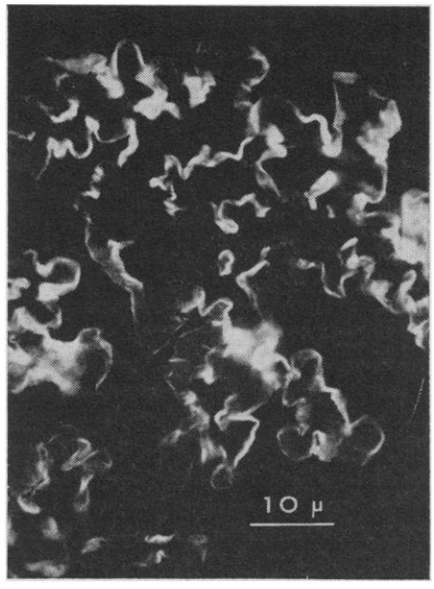

Fig. 1. Characteristic patterns of sheep IgG and β_{1C}-globulins, as shown by immunofluorescence, in glomeruli of sheep injected with human lung and FA. Kidney obtained on day 89, 58 days after onset of nephritis. There is striking linear, continuous deposition of IgG along the GBM. There is slight widening and increased intensity of staining along the mesangial GBM.

emulsion in 126 days) and two sheep (each receiving 50 ml of emulsion containing 100 mg of group A streptococci in 126 days) had no evidence of nephritis.

All glomeruli of nephritic sheep showed *intense* fluorescence when stained with fluorescent-conjugated antiserum to sheep IgG or antiserum to sheep β_{1C}-globulin. The pattern of deposit of sheep IgG along the GBM was linear, continuous, and uniform in all parts of all glomeruli (Fig. 1). A few glomeruli of one sheep showed fluorescence in segments of Bowman's capsule. All nephritic sheep had linear staining of segments of the basement membranes of the proximal tubules, but the number of involved tubules in each sheep varied widely. Staining usually stopped at the junction of tubular basement membranes and Bowman's capsule and at the junction of GBM and Bowman's capsule in the hilar region. Droplets in glomerular and tubular epithelium and casts showed fluorescent staining. Crescents did not stain. Fluorescent staining for complement was similar to that for IgG. In marked contrast to the characteristic diagnostic staining in kidneys of nephritic sheep, the staining in kidneys from untreated or control sheep was characterized by: (i) prominent granular staining in the hilar area and faint, variable irregular staining in other parts of the glomerulus; (ii) absence of staining of Bowman's capsule and tubular basement membranes.

Autoantibodies against sheep GBM were eluted from kidneys of nephritic sheep as follows: GBM prepared from each kidney (*1*) was washed four times with phosphate-buffered saline (PBS), *p*H 7.1, and then added to 60 ml of citric acid buffer, *p*H 3.2. The suspension was agitated for 4 hours at room temperature and then centrifuged. The supernatant was dialyzed against cold water, centrifuged, and lyophilized. The powder was reconstituted to form a 1-percent solution in PBS (2 percent for controls) and centrifuged to remove insoluble materials. Sheep IgG was identified in nephritic eluates by immunodiffusion, and on immunoelectrophoresis it was found to migrate in the fast γ_1G-region.

Autoantibodies were identified in nephritic eluates or serum by indirect immunofluorescence (*8*). Nephritic eluates or serums were layered over unfixed cryostat sections (4 μm) of kidney and lung from sheep and man. The sections were washed with PBS and then stained with fluorescent-conjugated rabbit antiserum to sheep IgG. A sharp continuous linear staining of GBM, Bowman's capsule, and tubular basement membranes was seen in all kidneys and basement membranes of human lung (Fig. 2). Sheep lung stained poorly. However, absorption of eluates with lung homogenates or GBM of sheep or man, but not with human red cells or mouse liver powder, eliminated or greatly decreased fluorescence. Absorption of serums with lung and GBM from sheep abolished fluorescence against sheep tissue but not human tissue. Absorption of serums with human lung removed antibodies against sheep and human tissues. Thus, human and sheep, lung and GBM, share closely related antigenic determinants. The faint staining of sheep lung was unanticipated and is probably due to fewer shared antigenic determinants per unit area of sheep-lung basement membranes. Autoantibodies or γ-globulin were not detected in any of the control kidney eluates.

These results indicate that sheep injected with human GBM and FA (*1*) or human lung and FA develop an identical nephritis. The common link in these differently induced nephritides is the presence of closely related antigenic determinants in sheep GBM and the injected antigens (human GBM or lung).

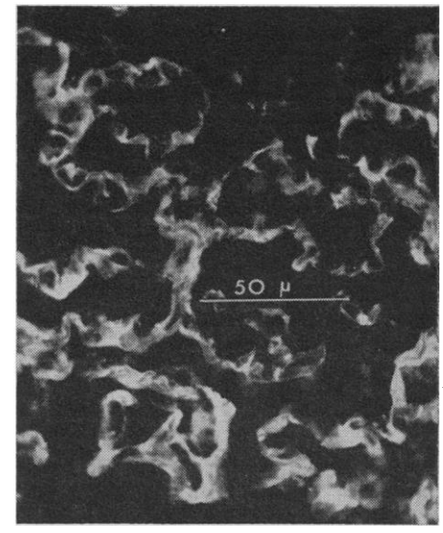

Fig. 2. Cryostat section of human lung, overlaid with eluate from a kidney of a nephritic sheep, and then stained with fluorescent-conjugated rabbit antiserum to sheep IgG. There is linear, continuous deposit of eluted autoantibodies along the lung alveolar and capillary basement membranes. This fluorescence is abolished or decreased by absorption of the eluate with GBM or lung homogenates from sheep or man, but not by mouse liver powder or human red cells. This is direct proof that the eluted autoantibodies were formed against antigenic determinants common to sheep GBM and human lung.

This is the first example in which an autoimmune disease has been induced by a heterologous antigen that is not organ-specific. Autoantibodies, eluted from kidneys in the nephritis induced by heterologous GBM (*1*), or in the nephritis described here are neither organ- nor species-specific. Thus, the concept that only organ-specific antigens can induce autoimmune disease must be abandoned (*4*).

The use of human lung tissue in this model (*9*) could explain the presence of nephritis in Goodpasture's disease, a human disease characterized by pulmonary lesions (*10*) preceding the onset of progressive kidney disease (*11*, *12*). Immunofluorescent studies of kidneys from Goodpasture's disease indicate that IgG and complement are deposited in a linear, continuous pattern along the GBM (*12*) in a manner morphologically identical with the patterns seen in this experimental model.

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A Cytoplasmically Transmitted, Diet-Dependent Difference in Response to the Teratogenic Effects of 6-Aminonicotinamide

Abstract. *The frequency of congenital cleft palate produced by maternal treatment with 6-aminonicotinamide during pregnancy is lower in the C57BL/6J than in the A/J inbred mouse strain. In the C57BL/6J strain the frequency is lower when the mothers are maintained on Purina Lab Chow than when they are on Breeder Chow. A/J females do not show this effect of diet. There is a matroclinous reciprocal cross difference in frequency of induced cleft palate which persists in the back-cross when the F_1 mothers are maintained on Lab Chow, but not on Breeder Chow.*

The nicotinamide analogue, 6-aminonicotinamide, has been shown to produce clefts of the secondary palate (as well as other kinds of malformations) in the offspring of pregnant mice injected with it (*1*). The analogue also caused a transient paralysis of the mother several hours after treatment, and an increase in the frequency of resorptions. Concurrent administration of nicotinamide prevented both the teratogenic and maternal effects. When the nicotinamide was given 2 hours after the 6-aminonicotinamide, cleft palates were produced, but there were no signs of maternal distress and no increase in resorption rate. This approach had the additional advantage that the embryo was exposed to the teratogen for a precisely defined period (*2*).

The highest frequency of cleft palate was obtained following treatment 13½ days after conception (*3*). At that time, the inbred A/J strain gave higher frequencies of cleft palate after treatment than the C57BL/6J strain, and there was a matroclinous reciprocal cross difference in the frequency of induced cleft palate in the F_1 offspring of crosses between the two strains. That is, the offspring of A/J females mated to C57BL/6J males and treated on day 13½ of gestation had more cleft palates than those of C57BL/6J females mated to A/J males. F_1 females from A/J mothers and C57 fathers (AC) and from C57 mothers and A/J fathers (CA) were back-crossed to A/J males and treated with 6-aminonicotinamide. The frequency of cleft palates was higher in the offspring of AC females than in those of CA females. This suggested that part of the difference between the strains in response of the palate to this teratogen stemmed from factors that are transmitted through the cytoplasm (*3*). During subsequent study of this question the difference between the two types of back-cross surprisingly vanished. In retrospect, the disappearance of the effect seemed related to a change in diet from Purina Lab Chow to Purina Breeder Chow. Lab Chow is said to have about twice as much niacin as Breeder Chow (*4*).

The results of further experiments are presented in Table 1. The back-crosses described above were repeated on the two diets. Details of the methods used are presented in the publications cited. The cleft palate frequency induced by maternal treatment with 6-aminonicotinamide was the same in the offspring of the AC (42.9 percent) and CA (45.9 percent) females maintained on Breeder Chow and of AC females on Lab Chow (44.5 percent), but was significantly decreased in the offspring of CA females on Lab Chow (25.8 percent). There is therefore a difference which results in a lower frequency of cleft palate induced by 6-aminonicotinamide in the offspring of CA females than in those of AC females. This difference is manifested when the treated mothers are maintained on Lab Chow but not on Breeder Chow. It is not clear whether the difference resides in the response of the mother to the teratogen, or in that of the embryo. In either case, since the nuclear genes of the two types of F_1 females are presumably identical, the difference must be transmitted through a cytoplasmic factor.

Such a factor ought to be present in the inbred strains as well, and this ap-

Table 1. Frequencies of cleft palate following injection into female mice on day 13½ of gestation of 6-aminonicotinamide (6AN) dissolved in distilled water in a concentration of 225 mg/100 ml followed 2 hours later by nicotinamide (NIC) in a concentration of 85 mg/100 ml. Abbreviations: B.C., Breeder Chow; L.C., Lab Chow.

Cross		Diet	6AN (mg/kg)	NIC (mg/kg)	Number of females	Number of offspring	Cleft palate	
♀	♂						No.	%
AC	A	B.C.	19	7.3	16	133	57	42.9
CA	A	B.C.	19	7.3	18	157	72	45.9
AC	A	L.C.	19	7.3	23	200	89	44.5
CA	A	L.C.	19	7.3	30	295	76	25.8
A	A	B.C.	14.25	5.48	14	102	57	55.9
A	A	L.C.	14.25	5.48	14	82	53	64.6
C	C	B.C.	19	7.3	18	117	79	67.5
C	C	L.C.	19	7.3	15	108	12	11.1