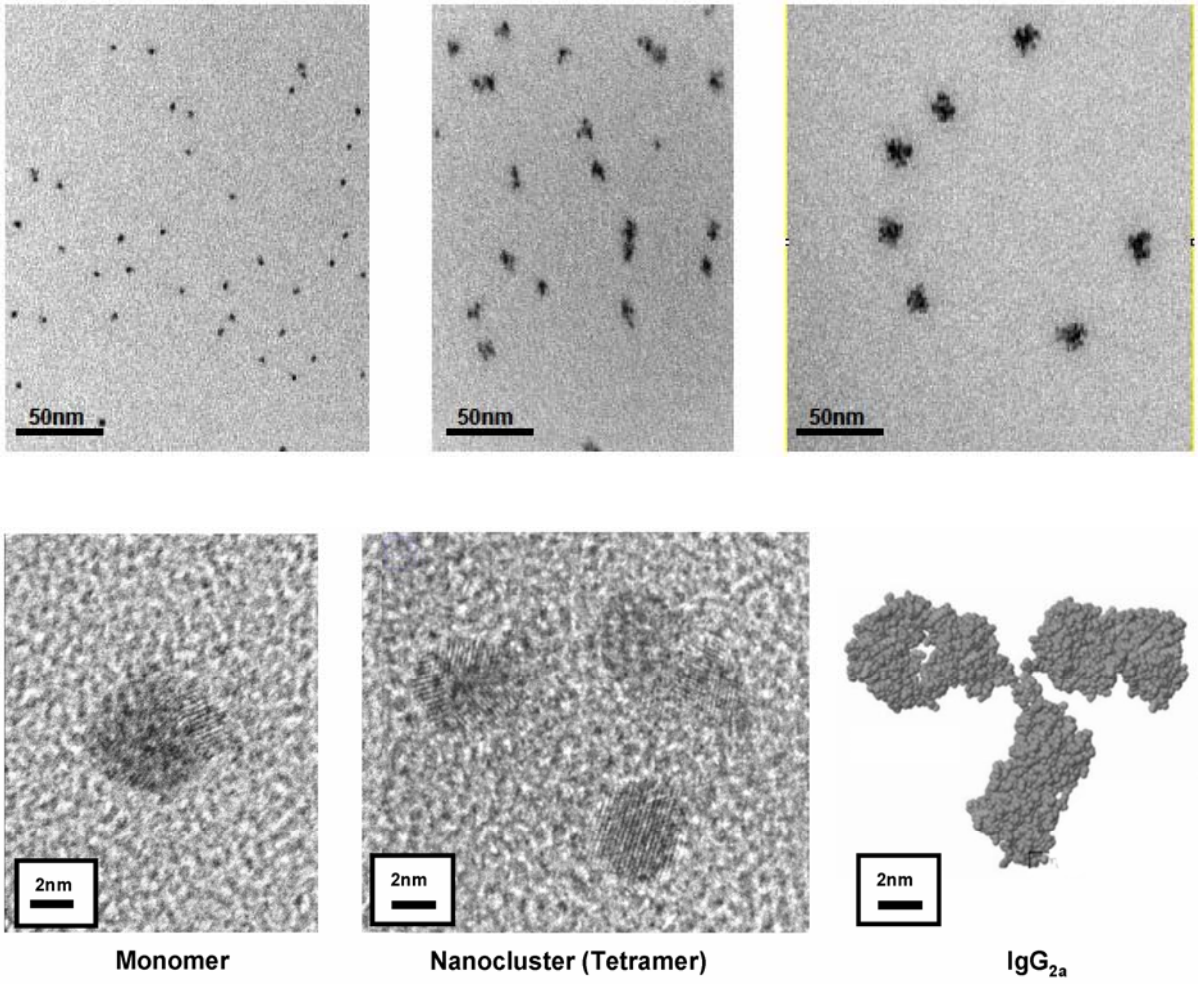
This grant had two specific aims:

Specific Aim 1 was to determine the degree to which the progression from microalbuminuria to overt albuminuria is the consequence of deterioration in the glomerulus filtration barrier, as judged by changes in the ability of the glomerular basement membrane (GBM) to exclude molecules in a graded manner inversely related to their size.

Specific Aim 2 was to determine the degree to which the progression is consequence of deterioration intubular function, as judged by changes in the removal of macromolecules from the tubular fluid.

We made excellent progress towards Specific Aim 1, starting initially by improving the method of making gold nanoparticles for use in testing GBM permeability. Thus we found that the nanoparticles we had been making (by reducing $HAuCl_4$ with $NaBH_4$ and coating the resulting particles with GSH) were unsuitable for studying permeation into the GBM of molecules greater than or equal in size to albumin because they agglutinated in plasma. Accordingly, we developed an alternative way of making them - by reducing $HAuCl_4$ with NaSCN and coating the resulting particles with GSH. Using this method allows us to prepare NaSCN/GSH nanoparticles with hydrodynamic sizes ranging from about that of ovalbumin (45 KD) to greater than IgG dimers (300KD), as illustrated in Figure 1. The upper part of the figure illustrates the size variation; the lower part of the figure shows that the small particles are crystalline monomers, while the larger particles are clusters of crystals. The number of subunits in the clusters can be varied by slight but controlled changes in their synthesis. The clusters are stable in solution for many weeks. They do not agglutinate in plasma. Indeed, they provide the tool needed for our first specific aim..

Figure 1



We then proceededed to test the new particles in mice that are easier to produce than Akita diabetic mice. Our collaborator, Jeff Miner from University of Washington in St. Louis, provided us with two suitable types of gene targeted mice: one with disruption of the gene controlling the synthesis of the GBM protein laminin, and the other with a deletion in the gne coding for type IV collagen, another GBM protein. They are, respectively, mouse models of Pierson's and Alport's syndromes, which are characterized by heavy proteinuria and marked ultrastructural changes in the glomerular capillary wall.

The particles are injected into the superior mesenteric artery and thence via the downstream renal arteries into the glomeruli followed by rapid ligation (within a second) of the renal hilum of the left kidney, and after a few minutes by ligation of the right kidney. Portions of the excised kidneys and then fixed and prepared for transmission electron microscopy.

Figure 2 illustrates the results obtained with a normal wild type mouse that received electron dense nanoparticles having a size between that of IgG (MW 150 kD) and that of its dimer (MW 300kD)

Figure 2

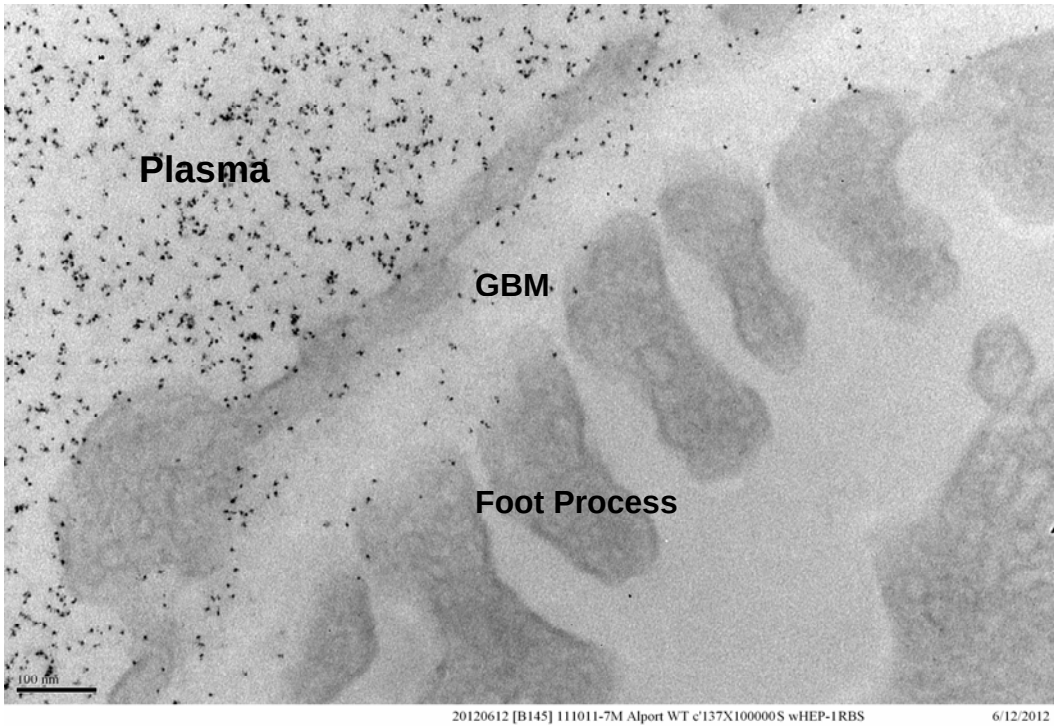


Figure 3 illustrates the results with a mouse homozygous for disruption of the Coll4A3 collagen gene (a model of Alpert's syndrome).

Figure3

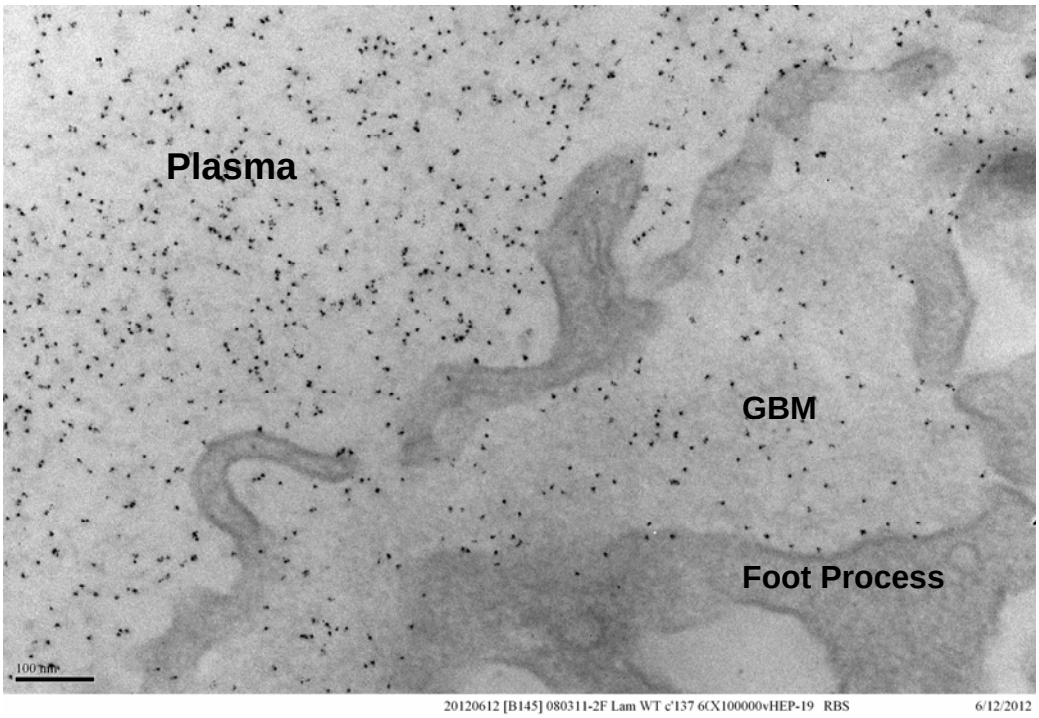


Figure 4 illustrates the results when the same nanoparticles were injected into a mouse homozygous for disruption of the Lam b2 laminin gene (a model of Pierson's syndrome).

Figure4

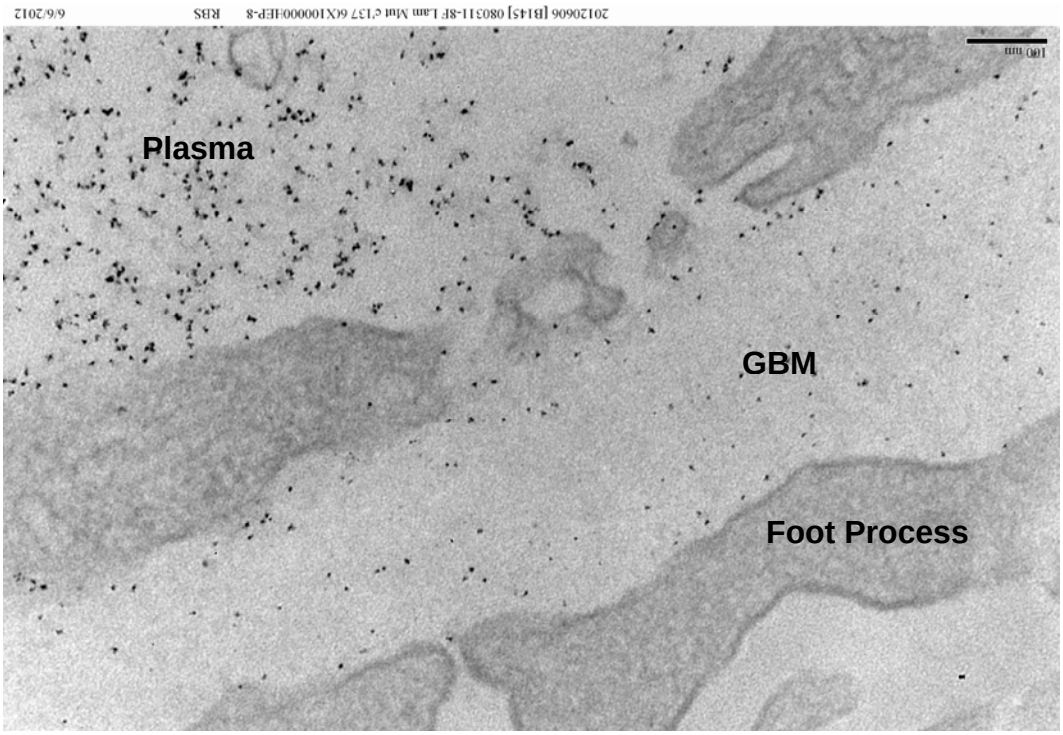
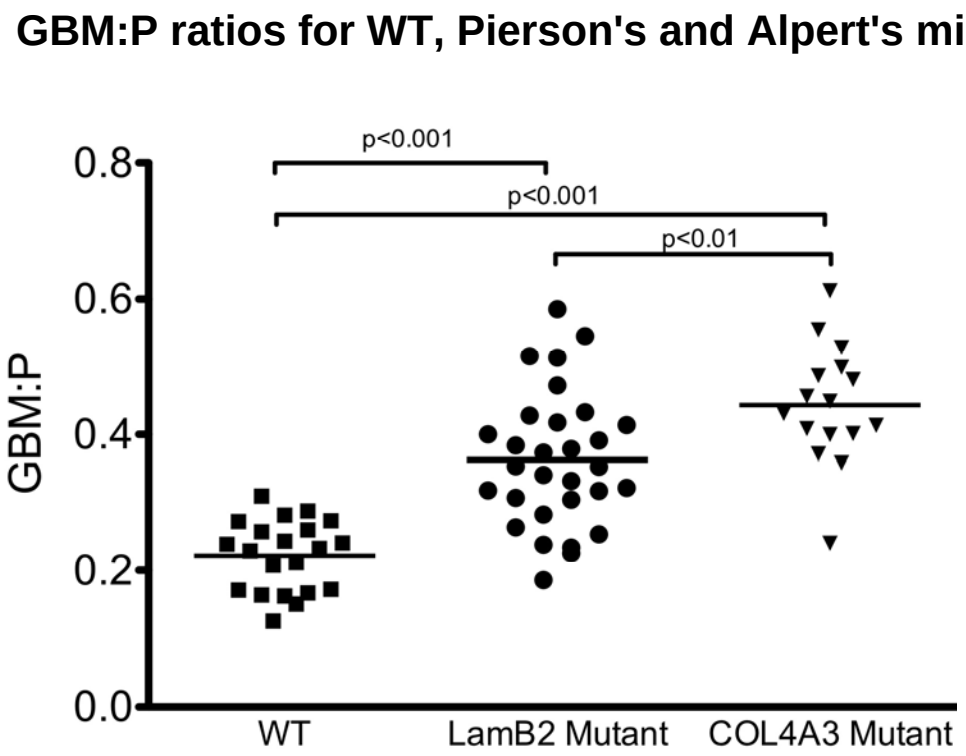


Figure 5 summarizes the GBM/Plasma ratios (GBM:P), obtained by counting the number of particles per unit area of GBM and Plasma for each type of mouse. This ratio is a measure of the permeability of the GBM.

Figure5



These data demonstrate that the GBM of both mutants has increased permeability compared to control. Further, In the Alport's model the distribution of particles within the GBM is altered; particles are distributed more uniformly throughout the GBM compared to the control where approximately two-thirds of the particles are found in the internal portion of the GBM (the lamina rara interna). These findings throw a new light on these particular diseases, and demonstrate that we have a valid new tool to use in studying other proteinuric diseases, including diabetic nephropathy.

The work is continuing, and is now at the stage of breeding to generate a mouse model of diabetic nephropathy with high fidelity to human histopathology. This model employs B6 x 129 F1 generation with genetic deficiency in endothelium nitric oxide synthase and diabetes via the Akita mutation, as we have described previously (Wang et al., 2011 . We anticipate that these mice will develop clinical and pathological findings consistent with diabetic nephropathy around six months of age and at that time we will perform experiments similar to those presented in this progress report.

We do not yet have any publications from the work, but we expect to publish several papers and to be able to continue the work under new funding.