IMMUNOHISTOLOGICAL STUDY OF IMMUNE DEPOSIT DISEASE

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At present time two types of pathogenetic mechanisms are proposed by Dixon and others for glomerular nephritis:

(1) anti-glomerular basement membrane antibody disease, where glomerulonephritis is mediated by anti-glomerular basement membrane antibody; and

(2) immune complex disease, in which circulating antigen-antibody is caught by the Kidney. In the present study the authors examined the distribution, appearance, and properties of immune complexes in diseases in which immunoglobulin is seen as a lump. This study showed the immune complex in membranous nephropathy, which is thought to be the purest form of immune complex disease, is not related to glomerular basement membrane where the complex is situated. Therefore the authors next produced experimental nephritis using glomerular basement membrane alone and the immune complex as antigens, and analyzed histological appearance of resulting nephritis, the circulating antigen and antibody, and compared these with the nephritis we produced in the past using antigens not containing glomerular basement membrane.

CLINICAL CONSIDERATIONS

As is stated above, the immune complex disease is a collective name for those diseases in which immune complex is found deposited as a lump within the glomerulus. In many common renal diseases immune complexes are found in basement membrane, mesangium, or on the vascular pole. These diseases are briefly reviewed.

I) Acute Nephritis
Immunohistological Study of Immune Deposit Disease

In this disease, immune complex is found on epithelial side of the basement membrane, and Kimmelstiel called the complex as a 'hump'. Some authors proved streptococcal antigen in this complex, and this complex is thought to perform some function in the pathogenesis of acute nephritis.

2) Chronic Nephritis

According to clinical types of the disease (latent type, nephrotic type, hypertensive type, progressive type, etc.), the immunohistological appearance are varied, and the presence of different types of antibodies are assumed. Especially characteristic are the deposition of Ig-M antibody on vascular pole in hypertensive type, and the deposition of fine granular immune complex of effective filtrating surface in lobular nephritis. The presence of immune complex is not detected in latent type.

3) Other Associated Diseases

The presence of immune complex is seen in various collagen diseases (especially S.L.E.), purpura nephritis, diabetic nephropathy, hepatorenal syndrome, and toxemia of pregnancy. These diseases were exhibited on slides during the symposium.

4) Membranous Nephropathy

While in above-mentioned diseases, antibodies, in addition to immune complexes, are found on basement membrane and the mesangium and suggest the complexity of immune mechanisms, in membranous nephritis fine granular immune complex alone is found on basement membrane appearing like a string of beads. In order to know the relationship between this immune complex and the Kidney and the glomerulus, antiglomerular and anti-renal tubular antibodies were made and were labelled by F.I.T.C. By this process the immune complex is not attained, and thus the immune complex is found to be non-renal and non-glomerular, and membranous nephropathy is thought to be the purest form of immune complex disease.

EXPERIMENTS

Noting immune complex in membranous nephropathy is non-glomerular, experimental nephritis was produced by following two methods.

1) Experimental nephritis by non-renal, and non-glomerular antigen. Immune
complex disease was produced by single or repeated injections of foreign protein (acute serum sickness or chronic serum sickness), autologous antigen, or renal tubular antigen. Especially in chronic serum sickness a similar immune complex as in membranous nephropathy is found, and this appears to give some insight into the pathogenesis of membranous nephropathy.

2) Experimental nephritis using glomerular basement membrane antigen. The antigen was made by the following method. Glomerular basement membrane was extracted by the method of Greenspon and Korakower, made soluble by the collagenase, and the component was obtained by ammonium sulfate fractionation (GBMα). The rabbits were sensitized by G.B.M.α antigen and from these anti-G.B.M. serum was obtained. When anti-G.B.M.α serum and G.B.M. were made to react, two electrophoretic bands were formed, and these were named G.B.M.α₁ complex and G.B.M.α₂ complex from the side of the antiserum, and these complexes were washed out with saline solution. The rabbits were then inoculated in the foot pad with GBM⁻, GBMα₁ complex, and GBMα₂ complex as antigens, together with Freund's complete adjuvant. The former may be produced the complex in the body of the host, and the latter two produced complex in vitro and produced experimental nephritis. As the result, in one animal out of nine a quite localized fine granular deposit was found. However, this took a proliferative form centering around the mesangium, and no fine beaded deposit was found as in membranous nephropathy.

Therefore we next performed an analysis of circulating antigen and antibody in these experimental animals (see Fig. 1). The bands α₁ and α₂ are the result of reaction between GBMα and anti-GBMα, and these represent α₁ and α₂ complexes respectively, which were used as antigens. The bands II' and II'' are fused with α₁' and α₂', and thus are thought to be related with the GBM antigen. When II' and II'' are extracted and are used as antigens to sensitize rabbits, a nephritis develops and an antibody is found in circulating serum which reacts against GBMα. Thus it is shown that the antigen II' and II'' contained GBMα component. This fact shows that the antigen GBMα coexisted within the animals were sensitized with GBMα and GBMα₁ (α₂) complex. The bands I' and I'' are due to the production of an.
antibody against homologous \( \gamma \)-globulin within the animal sensitized by GBM\( \alpha_1, (\alpha_2) \) complex: these bands were identified by rabbits \( \gamma \)-globulin.

**CONCLUSIONS**

1) While immune complexes are seen in various renal diseases, the purest form is found in membranous nephropathy. The immune complex in this case is non-renal and non-glomerular.

2) Noting the fact that immune complex in membranous nephropathy is non-renal and non-glomerular, the GBM complex was produced both in vivo and in vitro, and experimental nephritis was produced. While the presence of circulating antigen and antibody was found in these experimental nephritis, the histological picture showed mostly proliferative inflammation. Thus, although circulating antigen-antibody may be present, the resulting nephritis is not likely to take a pattern similar to membranous nephropathy when the GBM component is concerned with the disease process.
Membranous Nephropathy

Nephritis immunized by
(Anti GBMα₁ complex + Ant GBMα)