

Hapten-Specific Immune Response Producing Glomerular Injury

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Summary. A new experimental model using a cationic hapten-carrier system was established. Attachment of the hapten, trinitrophenol (TNP) to cationic carrier proteins (human IgG; hIgG, bovine serum albumin; BSA) enables their implantation into the glomerular capillary wall. The TNP bound to the glomerular capillary wall can act as a target molecule not only for circulating antibody, leading to *in situ* immune complex formation, but also for cell mediated immune response. Epicutaneous sensitization of the TNP without carrier protein induced a TNP-specific cell-mediated immunity. When the left kidneys of these sensitized rats were perfused with cationized TNP-BSA, proliferative glomerulonephritis with proteinuria could be induced without deposition of any autologous immunoglobulins or complement. This line of approach enables us to analyse several factors involved in the glomerular injury at the cellular and molecular levels.

INTRODUCTION

Macromolecules such as high molecular weight proteins are composed of complex structural determinants. Structural requirements for antigenicity differ from those determining net charge. Histones, which are one kind of naturally occurring cationic macromolecules, are composed of two regions with unique functional polarities.¹⁾ One part is a positively charged region which is rich in arginine and lysine residues. This part is associated with binding to DNA on a charge basis. The other region of histone is hydrophobic and can interact with other histone components. Its structural specificity explains how histones are able to aggregate, in spite of the repulsion expected, between molecules with similar charge. With this in mind, we tried to make a macromolecule composed of two unique parts which would include a simple, antigenic determinant as well as a high net

charge. A hapten attached to a positively charged carrier protein can be implanted into the glomerular capillary wall, which is comprised of a filamentous network of polyanionic glycoproteins. Here we introduce two experimental glomerulonephritis (GN) models using a cationic hapten-carrier system.

Preparation of hapten-carrier proteins and their cationization

2,4,6-trinitrobenzen-1-sulphonic acid sodium salt (TNBS), and fluorescein isothiocyanate (FITC) were conjugated to bovine serum albumin (BSA) and human IgG (hIgG) as previously described.²⁾ Then, TNP-BSA, TNP-hIgG, and FITC-BSA preparations were cationized according to our routine method.³⁾ Isoelectric points of the antigenic materials used in this study were determined by isoelectric focusing. The average degree of conjugation of haptens to proteins was determined spectrophotometrically.

Immunological mechanisms of two experimental models that induce glomerular injury

(1) Hapten-specific humoral immune response producing *in situ* immune complex GN.

TNP which is implanted into the glomerular capillary wall can act as a target molecule for circulating antibody, leading to *in situ* immune complex GN.^{2,4)} Rats were immunized by injection of 1 mg of TNP-BSA in Freund's complete adjuvant subcutaneously and intramuscularly. Twenty-one days later the left kidney was perfused with 500 μ g of cationized TNP-human IgG (low and high valency antigens). In rats receiving high valency antigen, endocapillary proliferative GN with proteinuria developed. Diffuse capillary deposits of rat C3 were also observed. In contrast, no significant abnormalities in renal im-

munohistology or urinalysis were observed when low-valency antigen was injected. Electronmicroscopically, there was a significant difference in the sizes of subepithelial electron dense deposits between these groups of rats; namely, the kidneys of rats given high-valency antigens showed marked subepithelial deposits with foot process retraction. In contrast, kidneys of rats given low-valency antigens showed only small subepithelial electron dense deposits beneath the slit membrane. Lattice formation is important in both the complement activation and deposition of immune complexes within the glomeruli. Complement activation and the lattices of immune complexes are influenced not only by the degree of antigen excess, but also by the antigen valence.

(2) Hapten-specific cellular immune response leading to glomerular injury.

It is possible that the hapten bound to the glomerular capillary wall can also act as a target determinant for cell-mediated immune response. Recently, we reported a new experimental GN in which a delayed-type immune reaction predominated.⁵⁾ Cationized TNP-BSA conjugates were deposited in the glomerular capillary walls of rats which had been sensitized with TNP hapten 7 days beforehand. Histologically, marked exudative and proliferative changes were noted in their glomeruli. These changes were accompanied by transient proteinuria. Immunofluorescent studies showed no deposition of any autologous immunoglobulins or complement within the glomeruli

during the examination period. In this model, there were many inflammatory cells within the glomeruli. In order to characterize and quantify these cells, we examined cell surface markers of infiltrating cells within the glomeruli. Isolated glomeruli were incubated with monoclonal antibodies according to the method described by Schreiner et al.⁶⁾ There was a significant number of cells positive for leukocyte common antigen and Ia antigen in the glomerulus from the hapten-perfused, diseased kidney (Fig. 1a). In contrast, only a few cells with these markers were seen in the unperfused kidney (Fig. 1b). In general, Ia-negative cells are observed in exudates induced by inflammatory, non-immunological stimuli, such as mineral oil thioglycollate broth. On the other hand, Ia-positive macrophages predominate in the exudates induced by interaction of antigen-stimulated T cells and macrophages.⁷⁾ Taken together, we believe that a T-cell mechanism can induce glomerular injury without the participation of humoral antibody in our experimental GN model.

Concluding Remarks

Mechanisms for the initiation and progression of human glomerulonephritis remain unclear at the molecular and cellular levels. Attachment of a hapten to cationic carrier proteins enables us to analyse several factors involved in the glomerular injury including: hapten-specific immune responses (cellular and humoral), hapten density (valence), size of carrier molecule, affinity of an antibody, and characteriza-

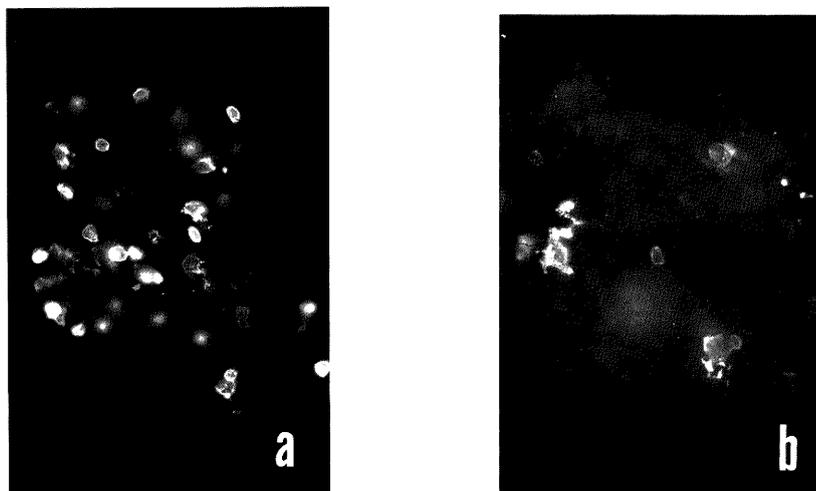


Fig. 1. Fluorescent micrographs of isolated glomeruli on day 1, stained for cells bearing the rat Ia antigen using mouse monoclonal antibody against rat Ia antigen (MCA 45). **a.** A glomerulus from the perfused, left kidney, stained with MCA 45. ($\times 200$) **b.** A glomerulus from the unperfused, right kidney, stained with MCA 45. ($\times 200$)

tion of inflammatory and immune cells. We believe that the experimental models described above using the hapten-cationic carrier protein systems broaden our understanding of the immunological mechanisms responsible for induction of glomerular inflammation and accumulation of immune deposits.

Acknowledgements. This work was supported by research grants (59570145, 60570153, 63570155) from The Ministry of Education, Science and Culture, Japan.

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