SPECIAL LECTURE

NEPHRITIDES AND DYSIMMUNIZATION

With special reference to the significance of lytic process in immunologically induced experimental glomerulonephritis*

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Experimental-pathological studies have provided sufficient evidence that glomerular lesions comparable to those in human glomerulonephritis could be induced by immunologic means in laboratory animals. Notable examples may be Masugi nephritis (anti-rat-kidney rabbit serum glomerulonephritis and anti-rabbit-kidney duck serum glomerulonephritis, Masugi 1933, 1934; see also Fujimoto et al., 1964; Unanue and Dixon 1967, and others) and experimental serum sickness nephritis (for example, Ehrich et al., 1949). The immune complex nephritis experimentally induced by repeated injections of heterologous protein (Dixon et al., 1961, 1962) seems to be remarkable as well.

In the study in our laboratory (Tada and Fujimoto 1964; Fujimoto, Okada, Kondo, and Tada 1964; Fig. 1, a and b) the pathobiology of Masugi nephritis has suggested the two modes of development of glomerulonephritis produced by antigen-antibody reaction. One is the induction of proliferative glomerulonephritis caused by

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antibody produced in the host reacting with heterologous anti–kidney antibody fixed in or on the glomerular basement membranes. This is the case of classic Masugi nephritis of rabbits and the second or autologous phase of rat Masugi nephritis. The other is the induction of degenerative glomerulonephritis caused by direct interaction of heterologous anti–kidney antibody with the host glomeruli, particularly glomerular basement membranes, as in the first or heterologous phase of classic Masugi nephritis of rats.

The study of glomerulonephritis caused by circulating antigen-antibody complexes in experimental serum sickness produced by one or a few closely spaced large injections of foreign serum proteins, as well as induced by injections daily for many months of a given heterologous serum protein, has been performed by Dixon and his co-workers (19611), 19622). He (Dixon 19653) noted that the morphological and immunohistochemical characteristics of the various types of experimental serum sickness glomerulonephritis are faithful reproduction of various stages of clinical glomerulonephritis and the nephritis of lupus erythematosus in man.

At the Vth International Congress of Allergology, Montreal, 1967 where I was also in attendance, Dixon4) has pointed out that glomerulonephritis caused by anti–glomerular basement membrane antibodies and that caused by nonglomerular antigen–
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Antibody complexes show generally distinct immunohistochemical and morphological characteristics, which are distinguishable on biopsies where sufficient glomerular structure remains. The glomerular lesions which have been induced by anti-glomerular basement membrane antibodies show an uniform linear deposition of gamma globulin as well as complement along the inner aspect of the glomerular basement membrane detected by immunofluorescence. We have also confirmed such a finding in Masugi nephritis (Okabayashi 196918, Fig. 10). In a piquant contrast, circulating nonglomerular antigen-antibody complexes, plus complement, accumulate in discrete, irregular, lumpy or granular deposits along the outer aspect of the basement membrane beneath the epithelial cell which are demonstrable by either immunofluorescence or electron microscopy. In the extreme cases, they are even visible on light microscopy. In this way, Dixon and his colleagues have suggested the role of antigen-antibody complexes in serum sickness nephritis and related disorders.

With respect to the pathogenesis of glomerulonephritis, the experimental studies of prolonged sensitization with foreign proteins (egg albumin etc.) for over a year in rabbits have also accumulated much histo- and immunopathological observations on the induction of various types of glomerular lesions (see Okabayashi 196717).

We (Okabayashi 196214, 196415; Okada 196319) have been confronted not only with the abrupt onset of acute and subacute proliferative glomerulonephritis in the earlier stage of prolonged sensitization but also with an insidious onset of chronic degenerative (membranous) glomerulonephritis in the later stage in some of the experimental rabbits respectively. And there has been suggested the presence of degenerative conversion of glomerular inflammatory and reparative reactions in the glomerulonephritis occurred in late stages of prolonged sensitization (Okabayashi 196716). Furthermore, the development of florid chronic glomerulonephritis including active lupus nephritis type has also been demonstrated in a proportion of rabbits given prolonged injections of foreign protein (Okabayashi 196415).

Together with the experimental studies of nephrotoxic serum nephritis (Masugi)
and immune complex nephritis (Dixon), our research on the glomerular lesions produced in the course of prolonged sensitization (Okabayashi) has casted a light on the fundamental processes involved in the pathogenesis of immunologically induced experimental glomerulonephritis as is summarized in Table 1.

As has been well known for many years in the studies of rabbit Masugi nephritis (since Masugi 1934\(^{12}\)) and experimental serum disease nephritis (Ehrich et al., 1949\(^{31}\) and others), there takes place proliferative and inflammatory lesion in glomeruli (3 in Table 1). Degenerative-sclerosing (membranous) alteration of glomeruli with deposits along the outer and/or inner aspect of the basement membrane (4 in Table 1) has been demonstrated in chronic serum sickness nephritis in rabbits (Dixon 1961\(^{11}\)), in the first or heterologous phase of rat Masugi nephritis (Fujimoto et al., 1964\(^{4}\), Shigematsu 1968\(^{9}\) and Kobayashi 1968\(^{8}\) in our laboratory) and in glomerulonephritis or related conditions obtained in the later stage of prolonged sensitization of rabbits (Okada 1963\(^{19}\), Okabayashi 1964\(^{15}\)).

In our investigation we found the existence of an essentially similar process in the such complicated inflammatory and degenerative glomerular lesions induced by various experimental procedures, i.e., the lytic process (2 in Table 1) probably

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PMN: Polymorphonuclear Leukocyte

Okabayashi (1969)
resulting from antigen-antibody reaction with or without complement interaction. It should not be overlooked that the lytic process may be the initial basic histological event in immunologically induced experimental glomerulonephritis.

From the viewpoint of this basic process, the proliferation of glomerular cell constituents including epithelial cell element seems to succeed after the lysis of mesangial area and basement membrane. Here take place the transformation of original glomerular tuft into the irreversible enmeshed framework or nodular formation and adhesions. In glomeruli there appear, in fact, subacute histolytic-proliferative (disorganizing), lytic-hemorrhagic-necrotic-granulating (disorganizing) and other disorganizing inflammatory changes resulting in scar formation. The disorganizing inflammatory glomerular lesion resulting in scarring (3 in Table 1) is seen in the glomerulonephritis occurred under conditions of heteroimmunization such as serum sickness and autologous phase of rabbit Masugi nephritis. In the autoimmune glomerulonephritis (cf, rat Masugi nephritis caused by anti-glomerular antibody) the lytic-degenerative-sclerosing (chronic membranous) alteration (4 in Table 1) becomes manifest in glomeruli throughout the initial phase together with deposits along the inner aspect of the basement membrane.

In florid type of chronic glomerulonephritis characterized by disorganizing-degenerative glomerular change (5 in Table 1) obtained in the middle and later stages of prolonged sensitization there appeared not infrequently the lytic exacerbation of degenerative-sclerosing glomerular alteration, which was accompanied usually by strong intracapsular protein exudation. There were exhibited ladder-like or spiky changes of the thickened glomerular basement membrane in the periodic acid-methenamine silver stain (6 in Table 1).

It is of considerable interest that the phagocyte (polymorphonuclear leukocyte and monocyte) accumulating reaction in glomeruli (1 in Table 1) presenting a proliferative glomerulonephritis has been revealed by our recent electron and light microscopic investigations at the beginning either of the first heterologous phase of rat Masugi nephritis (Shigematsu et al, 19689) or of the second autologous phase of rabbit Masugi nephritis (Kondo 196910). This type of glomerular disorder is
thought more or less akin to the immunopathologic glomerular changes described above and may result also from the antigen–antibody union at glomeruli. The phagocyte accumulating reaction, however, seems to be in essence of transient nature at the very beginning of glomerulonephritis, its duration being within a few days without regard to the remained glomerular lesions. It is a circumstance worthy of being noted that, after this intravascular phagocytic reaction, the proteinuria can often disappear, while the capillaries demonstrate recirculation without their marked structural alteration only with or even without axial fibrillosis or sclerosis in glomeruli. In other words, the transient glomerular reaction in such a circumstance may grossly result in a phagocytic–reparative (cleaning out)–healing teleological process taking place in glomeruli against a nephritic attack that is probably mild in its degree. Similar transient proliferative glomerulonephritis sooner or later resulting in healing clinically and histologically may occur in experimental serum sickness or recurrently in the course of experimental prolonged sensitization with foreign protein, too.

During the experimental prolonged sensitization, as has been confirmed by us, there occurred a variety of glomerulonephritides, proliferative, disorganizing and degenerative or acute (transient), subacute and chronic (prolonged and progressive). Behind the occurrence of these various types of glomerulonephritis and other connective tissue diseases, there have been demonstrated in experimental animals characteristic physiological and pathological changes in the blood, and also in the systemic hyperactivities of antibody–forming tissues or immune system like the bone marrow, spleen and lymph nodes. In the later stage of prolonged sensitization, for example, a complex disease resembling systemic lupus erythematosus accentuated by wire-loop glomerular lesion and so on was experimentally induced, which may be a reflection of structural alteration in the immune system (Okabayashi 196415). There was revealed dysglobulinemia containing autoantibodies such as antiglobulin (rheumatoid) factors and anti–blood cell and LE factors (Hojo 19617, Tada 196320; see also Okabayashi 196415). The presence of circulating antibody against autologous
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as well as homologous kidney has also been suggested in special test animals (Tanaka, Nishimura, Tada, and Okabayashi 1964\textsuperscript{22}; see also Okada 1963\textsuperscript{19}).

The pathogenetic significance of humoral anti-kidney antibody had been postulated since Masugi’s earlier studies of nephrotoxic serum nephritis. In this connection, Dixon\textsuperscript{4} too stated in the above mentioned International Congress at Montreal in 1967:

“In retrospect, it is surprising that for 5 to 6 decades the rabbit donors of anti-rat kidney (Masugi) sera had been developing autoimmune nephritis without attracting the attention of the investigators immunizing them.”

The development of autoimmunization in the later stage of prolonged sensitization

\textbf{DYSIMMUNIZATION}

\begin{tabular}{|l|}
\hline
\textbf{IMMUNIZATION (HETEROIMMUNIZATION→)} \\
\textbf{DEVELOPMENT OF SYSTEMIC IMMUNE REACTION} \\
\textbf{Hyperproteinemia and –globulinemia} \\
\textbf{Induction of Usual Antibodies in the Physiologic or Hyperergic (Enhanced)–} \\
\textbf{Physiologic State of Antibody-Forming Tissues} \\
\textbf{Occurrence of Heteroimmune-Allergic Inflammations (Glomerulonephritis etc.)} \\
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Degenerative Conversion of Inflammatory 
and Reparative Tissue Reactions

Development of Autoimmune Diseases of the Blood and Tissues  
(Systemic Lupus Erythematosus etc.)

Induction of Unusual Antibodies Including Autoantibodies* in the State 
of Post-hyperergic Dedifferentiation of Antibody-Forming Tissues

Dysproteinemia and –globulinemia

\textbf{WASTING IN SYSTEMIC IMMUNE REACTION}  
\textbf{(AUTOIMMUNIZATION) DYSIMMUNIZATION}

* Heterophil agglutination reaction, biologic false positive reactions for syphilis, antiglobulin (rheumatoid) factors, anti-blood cell and LE factors, reactive globulins against connective tissues, etc.

Fig. 2. The development of autoimmunization in the later stage of prolonged sensitization with foreign protein—Dysimmunization. Okabayashi (1967)\textsuperscript{17}.

* Degenerative glomerular lesion was induced by means of intraabdominal administration of the own or/and homologous kidney emulsion into 2 rabbits which had been sensitized with egg white-liquid paraffin for 162 or 165 days. In this experiment fluorescent antibody technique revealed that globulin was present in the thickened glomerular loop walls with fibrinoid degeneration in the residual kidney of a test animal. Moreover, reactive globulin against homologous kidney was also demonstrated to be present in the serum of this rabbit by the same procedure.
with foreign protein in the same individual has been termed 'dysimmunization' by us (Okabayashi 1962, 1967, Fig. 2). Under conditions of dysimmunization developing in the course of prolonged sensitization there may appear in general the degenerative conversion or its tendency of inflammatory and reparative tissue reactions in various organs including glomerulonephritis. Indeed, generally (in control normal rabbits) proliferative Masugi nephritis, if it was superimposed to rabbits at the later stage of prolonged sensitization, has been found to be converted to the degenerative one in its nature (Nogiwa 1959, see also Okabayashi 1967).

From this immunopathological viewpoint, various types of experimental glomerulonephritis developing at each stage, either one-shot or recurrently, during prolonged sensitization could be called 'dysimmune nephritides'. Their modes of development may be summarized as seen in Fig. 3. In late stages of prolonged

**DYSIMMUNE NEPHRITIDES**

**Acute or Subacute**

**PROLIFERATIVE GLOMERULONEPHRITIS (ONE-SHOT OR RECURRENT)**

- phagocytic-reparative-healing
- inflammatory-disorganizing-scarring

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with deposits along the outer and/or inner aspect of the GBM
characterized by ladder-like or spiky changes of the thickened GBM

**DEGENERATIVE GLOMERULONEPHRITIS**

Prolonged or Progressive

GBM: glomerular basement membrane

Fig. 3. Various types of experimental glomerulonephritis developing in the course of prolonged sensitization—Dysimmune nephritides. Okabayashi (1969).
sensitization which were over a period of 100 experimental days, florid chronic glomerulonephritis including active lupus nephritis type was experimentally reproduced by us (Figs. 12 to 16). It seems that immunological events, local as well as systemic, are most basic processes for the full-blown development of chronic glomerulonephritis of prolonged and progressive nature, despite the facts of modification by uremia, hypertension, circulatory disturbances in the kidney and other factors. To be sure, active lupus nephritis and related conditions here obtained experimentally in rabbits may occur in chronic form, or as a series of acute attacks, under conditions of dysimmunization developing during the prolonged sensitization.

It is pointed out in the present special lecture that the immunologically induced glomerulonephritis, as well as the plasma and blood cell reactions, might be a bridgehead for the investigating the situation of immunization process as a general phenomenon of the entire body of the individual organism. These histo- and immunopathological studies of experimental glomerulonephritis including that developing during prolonged sensitization appear also contributory to understanding the mechanisms involved in pathogenesis and their connection concerning the occurrence in succession of seemingly different glomerulonephritis types in man. Further investigations are desirable.

Acknowledgment

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References


10) Kondo, Y.: Cellular aspects of proliferative glomerulonephritis; Experimental studies. To be published.


16) Okabayashi, A.: Degenerative conversion of glomerulitis in the later stage


**Explanation of Plates**

*Figs. 4 to 6. Phagocyte (polymorphonuclear leukocyte and monocyte) accumulating glomerular reaction—Proliferative acute (transient) glomerulonephritis. Electron micrographs.* *(1 Table 1.)*

**Abbreviations used for labeling the electron micrographs including Fig. 11 are as follows: Cl, capillary lumen; Dp, deposit; Edc, endothelial cell; Epc, epithelial cell; Ma, macrophage; Mc, mesangial cell; Mo, monocyte; P, polymorphonuclear leukocyte; Us, urinary space.**

Fig. 4. Intracapillary accumulation of polymorphonuclear leukocytes. Neutrophils (P) gain a direct contact with the basement membrane and dislodge the endothelial cytoplasm away from it (arrow). 2 hours after an injection of anti-rat-kidney rabbit rG antibody. ×2,900. Rat 476, female (Masugi nephritis). Shigematsu (1968).9)

Fig. 5. Intracapillary accumulation of monocytes (Mo) and macrophages (Ma). 30 hours after an injection of anti-rat-kidney rabbit rG antibody. ×3,100. Rat 475, female (Masugi nephritis). Shigematsu (1968).9)

Fig. 6. Monocyte (Mo) accumulating reaction in capillary lumens. 2 days after the onset of
proteinuria (14 days after an injection of anti-rabbit-kidney duck gammaglobulin). \( \times 3,300 \). Rabbit T206, female (Masugi nephritis). Kondo (1969)\(^{10}\).

Figs.7 and 8. Phagocyte accumulating glomerular reaction, transient in nature.....
Phagocytic-reparative-healing acute glomerulonephritis. (1 in Table 1.)

Fig. 7. This proliferative glomerulonephritis may consist of monocyte accumulation in glomerular loops as is shown electron-microscopically in Fig. 6. 2 days after the onset of proteinuria (14 days after an injection of anti-rabbit-kidney duck gammaglobulin).

Fig. 8. Recovery from the intracapillary monocyte accumulating glomerular reaction. The proteinuria disappeared within 20 days after the onset. There appears recirculation in the glomerular capillaries without their marked structural alteration. 71 experimental days.

Epon, toluidine blue stain.

Rabbit T206, female (Masugi nephritis).

Figs.9 and 10. Disorganizing inflammatory glomerular lesion resulting in scarring.....
Disorganizing-scarring subacute glomerulonephritis. (3 in Table 1.)

Fig. 9. Histolytic-proliferative (disorganizing) glomerular lesion. Note the lysis of mesangial area, as well as capillary wall, resulting in enmeshed frame work of the broken area in glomerular tufts, the histolytic tuft lesions being various in their aspects. Epon, toluidine blue stain. The day of the onset of proteinuria (17 days after an injection of anti-rabbit-kidney duck gammaglobulin). Rabbit T203, female (Masugi nephritis).

Fig. 10. Histolytic-proliferative (disorganizing) glomerular lesion (compare with the findings in Fig.9). In this staining there are distinctively demonstrated cystic transformations of glomerular tufts, one of which becomes larger and is going to rupture. Staining with fluorescein-conjugated anti-duck-gammaglobulin rabbit \( \gamma G \) (the linear deposition of the \( \gamma G \) along the glomerular capillary walls is still recognizable). The day of the onset of proteinuria (11 days after an injection of anti-rabbit-kidney duck gammaglobulin). Rabbit T201, female (Masugi nephritis).

Figs.11 and 12. Degenerative glomerular alteration resulting in sclerosis.....Degenerative-sclerosing chronic (prolonged) glomerulonephritis. (4 in Table 1.)

Fig. 11. Degenerative glomerular alteration. Note the subendothelial amorphous deposits (Dp) along the inner aspect of the glomerular basement membrane and the thickened endothelial cell cytoplasm. Electron micrograph. 24 hours after an injection of anti-rat-kidney rabbit \( \gamma M \) antibody. \( \times 7,900 \). Rat 513, male (Masugi nephritis). Kobayashi (1968)\(^9\).

Fig. 12. Degenerative glomerular alteration with fibrinoid deposits along the inner aspect of the glomerular basement membrane. The alteration may be comparable with wire-loop lesion and is accompanied by some thrombi. PAS stain. 19.5 hours after an injection of anti-rat-kidney rabbit \( \gamma G \) antibody. Rat 16, female (Masugi nephritis).

Figs.13 to 16. Florid type of chronic glomerulonephritis following a series of acute attacks demonstrated in experimentally induced systemic lupus erythematosus at the later stage of prolonged sensitization.....Experimental active lupus nephritis. (5 and 6 in Table 1.)
Fig. 13. Defect of some of the glomerular lobules following severe histolysis, in the remained lobules being seen mesangial sclerosis. Periodic acid-methenamine silver (PAMS) stain.

Fig. 14. Wire-loop glomerular lesions with some partial cellular proliferation. HE stain.

Fig. 15. Ladder-like or spiky changes of thickened basement membranes and irregular dissolutions of thickened mesangial axes resulting from a lytic exacerbation of degenerative-sclerosing glomerular alteration. Note also the strong intracapsular protein exudation. PAMS stain.

Fig. 16. A bird’s eye view of the histologic findings. The pathologic change here consists of a complex of disorders like disorganizing-scarring inflammatory lesion and degenerative-sclerosing alteration taking place in glomeruli, as well as of atrophying and fibro-sclerosing alteration occurring in tubular and interstitial tissues. This chronic progressive glomerulonephritis is characterized by a recurrence of lytic exacerbation. PAMS stain.

Rabbit R157, female (prolonged sensitization with egg-white for 210 days).
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Phagocyte Accumulating Glomerular Reaction (Figs. 4 to 6)
Phagocyte Accumulating Glomerular Reaction, Transient in Nature (Figs. 7 and 8)
Disorganizing Inflammatory Glomerular Lesion Resulting in Scarring (Figs. 9 and 10)

Degenerative Glomerular Alteration Resulting in Sclerosis (Figs. 11 and 12)
Florid Type of Chronic Glomerulonephritis (Figs. 13 to 16)