PART XI

NEPHROLOGY 3

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PATHOGENETIC MECHANISMS IN TUBULAR RENAL DISEASE

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Summary

Injections of heterologous tubular material into rabbits caused the formation of immune complexes deposited predominantly on the tubular basement membrane. Much fainter deposits were found on the glomerular basement membrane. Immunohistological studies revealed that the antigen involved originated from cells of the proximal tubules. In other animal experiments, purified tubular material was used for immunisation in order to analyse the antigenic structure of renal tubules. These rabbits were found to produce autoantibodies against an antigen present in the tubular as well as the glomerular basement membrane. Morphological studies of the kidneys from the immunised animals revealed alterations in the tubular epithelial cells and interstitial tissue which were characterised microscopically and electron microscopically by swelling and degeneration of the epithelial cells, and cellular infiltrates in the interstitium.

Introduction

In contrast to the extensive work on glomerular renal diseases, studies on immunological reactions associated with tubular lesions are scarce. There is, however, little doubt that in some forms of glomerulonephritis accompanied by autoantibody formation against glomerular basement membrane antigens, tubular changes may occur also. A few years ago Steblay et al reported that after immunisation with kidney preparations containing glomerular as well as tubular material, tubular necrosis could be observed in experimental animals [1]. In addition, our group found in earlier studies dealing with immunological reactions in pyelonephritis, that experimental animals produce autoantibodies against tubular antigens [2]. Following this line of investigation experiments were performed to analyse the pathogenetic mechanisms in tubular renal diseases. Immunological as well as morphological studies were undertaken to answer the following questions
1. Can immunisation with tubular preparations cause the formation of immune complexes which may be responsible for glomerular as well as tubular lesions?

2. Can humoral and cellular autoimmune reactions be demonstrated which are connected with immunological tubulopathies?

3. Which antigens are involved in these reactions and where are they located?

4. Are there pathological findings associated with tubulopathies which point to the immunological origin of these lesions?

Material and Methods

Tissue Preparations

Porcine kidneys were obtained from the local slaughter house immediately after exsanguination. All tissues were washed in physiological saline solution and either used fresh or immediately frozen and stored at −20°C. Antigenic preparations were obtained by the following procedures [3]. The tissues were homogenised in saline to make 33g/100ml suspensions. Saline extracts were obtained by exposing suspensions to supersonic vibrations of 20,000 cycles/sec for 10 min and removing insoluble material by centrifugation at 80,400g for 30 min. Renal cortex and medulla were separated by microdissection of kidney slices. The glomerular preparations were processed following the method described by Krakower and Greenspon [4] and modified by Spiro [5]. Repeated microscopic studies revealed less than 10% contamination with kidney parenchyma. After separation of the glomeruli the tissue preparations were collected; vascular material and connective tissue were removed from the remaining tubular preparations. Purified tissue preparations of basement membrane, mitochondria, microsomal fractions etc were obtained by following the procedures which have previously been described in detail [6].

Double Diffusion Gel Precipitation Tests

For this procedure all antisera were used undiluted. Plain tissue extracts and rabbit serum were adjusted to a protein concentration of 5mg/ml. The tests were interpreted 48 and 72 hours after the wells had been filled. Photographs were taken with a Polaroid MP-3 camera using indirect illumination.

Direct and Indirect Immunofluorescence Tests

Tissues were quick-frozen in liquid nitrogen, sectioned at approximately 6μ in a cryostat and treated with rabbit antisera and with a fluorescein conjugate of goat anti rabbit immunoglobulin. The conjugate had an F/P ratio of 1.6 and was used at different dilutions [7].
Microscopic and Electronmicroscopic Studies

These procedures were performed by Dr W Mönninghoff from the Lehrstuhl für Medizinische Cytobiologie, Universität Münster. The procedures used have been described previously [8].

Results

Rabbit antisera obtained after immunisation with heterologous tubular preparations were tested in double diffusion gel precipitation reactions. They combined not only with kidney preparations but also with extracts from various other tissues. After absorption of these antisera the antibodies which could be demonstrated reacted only with tubular antigens. Immunohistological studies of the kidneys from the immunised animals revealed the presence of granular deposits at the tubular basement membrane (Figure 1a). All rabbit sera contained antibodies against cytoplasmic antigens of tubular cells, and reacted with the animal’s own kidney also (Figure 1b). The suggestion that this antigen could be a component of the proximal tubules is supported by the investigations of Klassen et al [9,10] as well as Unanue et al [11] describing autoantibodies against tubular antigens after immunisation with tubular preparations. Similar granular deposits at the tubular basement membrane have been described by Andres et al [12] after kidney homotransplantation.

In view of the autoimmune nature of some glomerular renal diseases, it has been suggested that following immunisation with purified tubular material, autoantibodies may be formed which are directed against the tubular basement membrane. Immunohistological studies showed binding of these antibodies along the tubular basement membrane in a linear fashion (Figure 2a). These observations indicate that at least one autoantigen of the tubular system is located at the basement membrane. Occasionally, the antibodies reacted also with glomerular basement membrane (Figure 2b). These findings are in agreement with the analytic studies of Lehman et al [13], who demonstrated such an antigen.

The question of where the tubular antigens which cause the formation of immune complexes may be located, has not yet been finally solved. Most likely these are cytoplasmic antigens of the proximal renal tubules. In addition, the tubular system contains an antigen located in the basement membrane which causes autoantibody formation, which in turn combines in a linear fashion with the corresponding antigens of glomerular and tubular basement membrane. This antigen can be demonstrated in the basement membranes of the glomeruli as well as the tubules using direct and indirect immunofluorescence methods.

To answer the question whether or not immunisation with tubular antigenic fractions causes cellular immune reactions, migration inhibition tests were performed. The results indicated that in the course of tubular immunological processes, cellular reactions are also stimulated (Figure 3). Kinetic studies revealed that usually the cellular immune response occurs later than autoantibody formation.

The kidneys of all immunised animals were examined microscopically. These studies suggested that the immunological reactions described are associated with
Figure 1a. Direct immunofluorescence test. Note the granular deposits at the tubular basement membrane.

Figure 1b. Direct immunofluorescence test. Note the bright fluorescence of the cytoplasm of proximal tubular epithelial cells.
Figure 2a. Indirect immunofluorescence test. Note the linear fluorescence along the tubular basement membrane

Figure 2b. Direct immunofluorescence test. Predominantly linear fluorescence of the glomerular basement membrane can be seen
Figure 3. Migration indices of rabbit lymphocytes and macrophages following heteroimmunisation with tubular material.

Figure 4. Tissue section from rabbit kidney 858 showing infiltrates of lymphocytes and plasma cells as well as swelling of tubular epithelial cells.
swelling of the tubular epithelial cells. In circumscribed areas of cortex and medulla, interstitial cellular infiltrates were observed, consisting of lymphocytes and plasma cells (Figure 4). Occasionally polymorphonuclear leucocytes could also be observed. The glomeruli were only slightly altered, showing minor mesangial changes.

Electron microscopically, swelling and distension of tubular cells as well as swelling and degeneration of the mitochondria could be observed. In some instances a granular transformation was seen.

Discussion

In earlier studies dealing with immunological reactions in pyelonephritis, experimental animals were found to produce autoantibodies against kidney antigens. To analyse these reactions rabbits were immunised with purified preparations of tubular material. After several injections these animals produced autoantibodies as had been found in experimentally induced pyelonephritis. One of the antigens cross-reacted with an antigenic component of Escherichia coli strain 04: : H5 used for experimental pyelonephritis. Immunohistological studies revealed that this antigen is localised in the cells of the proximal tubular system. Another antigen was found to be present in the tubular basement membrane. These antigens also induced cellular immune reactions as demonstrated by migration inhibition tests. Microscopic studies of tissue sections from the immunised animals revealed infiltrates of polymorphonuclear leucocytes as well as swelling of the proximal tubules and degeneration of mitochondria. These observations point to an autoimmune origin of the humoral and cellular immunological reactions induced by heteroimmunisation with various tubular preparations. Comparison with results obtained in experimentally-induced pyelonephritis suggests that in chronic tubular diseases similar mechanisms may be pathogenetically important.

Acknowledgment

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References

1 Steblay, RW and Rudofsky, U (1971) J. Immunol., 107, 589
4 Krakower, CA and Greenspon, SW (1951) Arch. Pathol., 51, 629
Open Discussion

AHLMEN (Gothenburg) I would like to hear something about the purification of your antigenic preparations. Do you know anything about molecular weights or the kind of substances you were working with? And the second question: some cross reactions with Escherichia Coli have been investigated but have you studied, for example, cross reactions with Streptococci?

INTORP Answering your first question, we did several animal experiments using either crude preparations of tubular material or using partially purified material of basement membrane as well as cellular fractions like mitochondrial, microsomal or other preparations. The crude preparations of tubular material were obtained by microdissection to remove glomerular material and using the tubular preparations as heterologous substance for investigation. Partially purified tubular material was obtained by using standard procedures which had been modified in our laboratory. To obtain basement membrane material centrifugation was applied, as described by Krakower and Greenspon, modified by Spiro for glomerular basement membrane preparations. Answering your second question, in some instances cross reaction of E. Coli with kidney antigens have been described especially for E. Coli 04,014 and 022, but to our knowledge this is the first description of cross reactions between the E. Coli strain of 04: : H5 which was used in our studies. Experiments to study cross reactions of tubular antigens and Streptococci have not yet been performed.