A New Model for Autoimmunity and the Kidney

G. Lubec

Department of Paediatrics, University of Vienna, Austria

G. Lubec, MD, Department of Paediatrics, University of Vienna, Währinger-Gürtel 18, A-1090 Vienna (Austria)

In a recent publication, Oliveira and Peters [1] published a summary of the knowledge on autoimmunity and the kidney. In their updating they paid attention to the mechanisms of the development of autoimmunity: ‘...firstly, the failure of the mechanisms by which tolerance to self antigens is maintained, and secondly the process on the effector side of the immune system that lead to tissue damage once tolerance is broken...’.

Their excellent review and updating reflects the present thoughts and views of renal autoimmunity. No attention, however, is being paid to the role of the antigen. Why not incriminating antigenic changes for the development of renal autoimmunity?

In order to reduce basement membrane collagen synthesis we used the proline analogue principle [2, 3]: a proline analogue (L-azetidine-2-carboxylic acid, cis-4-hydroxyproline, etc.) is fed for a period of months to experimental animals. The tRNA, which was originally supposed to act specifically on proline, incorporates the proline analogues into the polypeptide chains of collagen. The incorporation of those substances interferes, however, with the folding of the polypeptide chains to the triple helical conformation. This in turn leads to rapid intracellular degradation of the nonhelical collagenous material which is highly susceptible to proteolytic cleavage: a net negative collagen synthesis results. This was proposed for a treatment of excess collagen accumulation.

In order to rule out nephrotoxic alterations, we have performed direct and indirect immunofluorescence studies on animal sera and kidneys. No immunoreactivity in the sera of experimental animals could be revealed, nor was any fluorescent staining present in the kidneys. Searching for the determinants responsible for the regulation of incorporation into collagen, a series of proline analogues was used: several groups were intro-

Fig. 1. Thin-layer chromatographical demonstration of the incorporation of the analogue.

Column A, the lowest band (2,3-dehydroproline); column B, hydrolyzed collagen without incorporated analogue; column C, the lowest band presenting the incorporated analogue; column D, identical to column A.

duced and several radicals were exchanged and finally, reduced proline analogues, presenting with double bonds, were applied.

Positions 3 and 4 on the proline ring could be substituted by aliphatic residues but the tRNA did not principally differentiate between proline and the analogues. Aromatic substitution, however, prevented the incorporation. In contrast to cis-trans isomers the D-conformation (D-proline) was recognized and the D-isomer was not incorporated.

The introduction of a double bond between C3 and C4 did not prevent the incorporation of 3,4-dehydroproline. The double bond between C1 and C2 (L,2-dehydropro-130
Fig. 2 Direct immunofluorescence on a frozen kidney section of 5µm from an animal treated with 2,3-dehydroproline. Leitz.

(line) was not found in the hydrolyzed collagen, in contrast to 2,3-dehydroproline (fig. 1). The incorporation could be shown by thin-layer chromatography and HPLC after the principle of Lindblad and Diegelman [4].

The kidneys of the proline analogue-fed animals were examined by immunofluorescence. With the exception of the 2,3-dehydroproline-treated animals (fig. 2) no positive staining could be observed. Glomerular but not tubular basement membranes showed linear fixation of IgG in all the 10 animals fed 2,3-dehydroproline.

Elution of the IgG bound in the experimental animals’ glomeruli by acidity and subsequent testing against collagen of glomeruli of normal/control animals showed negative results, but clear immunoreactivity against collagen of 2,3-dehydroproline-fed animals.

Collagen type IV was eluted from the kidneys of all animals treated with proline analogues with double bonds introduced and controls. Antisera (polyclonal) against glomerular basement membrane collagen IV showed different reactivity in a commercially available ELISA indicating altered antigenicity in the 2,3-dehydroproline-treated animals in contrast to the 1,2- and 3,4-dehydroproline-fed rats.

Those preliminary findings of changed antigenicity by the incorporation of a proline analogue which was fed orally to rats could give us new insights into the complex autoimmunity mechanisms in terms of changes of the antigen, not of the immune system.

The problems given in the short report of our preliminary results remain the permanent questions: why only autoimmunity against glomerular basement membrane collagen, not against tubular basement membrane collagen?

The incorporation of proline analogues must have taken place evenly distributed. Its explanation is vague like our general knowledge of many immunological recognition processes of that kind: masking of the antigen, accessibility?

References