

CONDITIONS FAVORING ANALYSIS OF IMMUNODEPOSITS IN MOUSE KIDNEY BY CLS MICROSCOPY

Alla S. Andryushchenko, Marina S. Krasilshchikova, *Jurek W. Dobrucki, and Olga V. Zatsepina

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

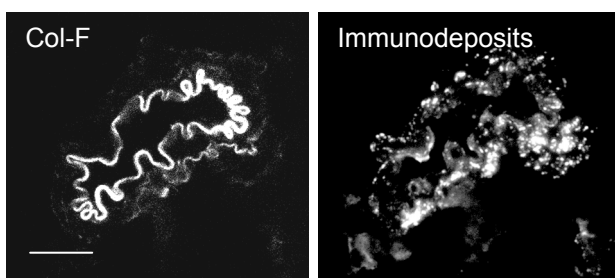
E-mail: zatsepina@ibch.ru

*Jagiellonian University, Krakow, Poland

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A characteristic feature of autoimmune diseases is appearance of glomerular immunodeposits – irregular aggregates of immunoglobulins that impair renal functions [1]. However, it still remains unclear whether the deposits are formed within all glomerular cells, whether they are accumulated in the nephron tubes, and what is the deposit topology in walls of renal vessels. To answer these questions, we used SJL/J mice which are known to develop autoimmune response against nuclear antigens following regular administrations of sublethal doses of HgCl₂ for 3-4 weeks [2]. For conventional immunocytochemistry, kidneys of autoimmune and control females were cut onto cryosections of 5-10 μm in thickness, fixed under various conditions or air-dried [1]. Then, sections were incubated with fluorochrome-conjugated antibodies against mouse immunoglobulins and studied using an Axiovert 200 epifluorescence and a confocal LSM510 microscopes (Carl Zeiss, Germany). To determine the proximal and distal convoluted nephron tubes, fixed sections were incubated with falloidin-FITC, a dye that preferentially decorates microvilli-covered surface of the proximal tube cells. To examine blood vessels, an original protocol for simultaneous staining of kidney with the dye Col-F (a marker for collagen and elastin fibrils in native tissues) and for immunolabeling of the deposits was elaborated. Kidney pieces were immersed into Col-F for 10 min and cut onto cryosections, which were then processed for immunolabeling with antibodies against mouse IgG as described above. Noteworthy, fixation and embedding of sections into Mowiol did not quench fluorescence of Col-F as observed by confocal and conventional microscopy.

The results obtained using an optimized protocol for cryosection fixation and confocal image processing analysis showed that in autoimmune and control animals immunodeposits were present almost in all glomerular cells, but their fluorescence intensity (i.e., the deposit titer) was several folds higher in autoimmune animals. In addition to glomeruli, immune deposits were also seen in the proximal nephron tubes, but were absent from other renal tube regions thus indicating that autoantibodies circling in blood are filtered not only in glomeruli, but also by the proximal tube cells. However, the most prominent difference between kidneys of control and autoimmune mice was presence of immunodeposits in renal blood vessels. The discrete aggregates and deposit clumps were seen across entire vessel walls that were intensely stained with Col-F (Figure, bar - 50 μm). We concluded that confocal image processing analysis of cryosection is a promising approach to study renal immunopathomorphology in autoimmune patients and animals.



References:

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