Immunofluorescence Studies in Paraffin-Embedded Tissue

E.	Euzenir	Nunes Sarno
L.M.	Leila M.	Machado Vieira
F.	Frederico	Ruzany

Department of General Pathology and Nephrology, State University, Rio de Janeiro, Brazil

Euzenir Nunes Sarno, Disciplina de Patologia Geral, Faculdade de Ciências Médicas, Av. 28 de Setembro, 87 4° Andar – Vila Isabel, Rio de Janeiro (Brazil)

Dear Sir,

The recent requirement to carry out immunofluorescence (IF) studies in renal biopsies justifies all attempts made to simplify this procedure. Many authors have reported successful comparative studies between frozen sections and paraffin-embedded tissue [2–5]. We have previously reported that paraffin-embedded tissue has given a high rate of false-negative results. Therefore, we continue to use frozen sections [6]. The fact is that the traditional IF method, using frozen sections, is an easy, quick and efficient method in spite of some inherent problems.

The major difficulty seems to occur in the handling of the specimen. Renal biopsies are very thin cylinders of tissue and must be divided lengthwise into two equal parts. Frequently, it is difficult to obtain equal samples for IF and light-microscopic studies and if the biopsy is not large enough to be divided, IF studies cannot be done. The other practical problem is that the biopsy must be immediately snap-frozen. In order to simplify the traditional IF study, a procedure that avoids biopsy division should be used, i.e., the same sample might be used for both studies (IF and light microscopy).

The method reported by Bolton and Mesnard [1] seems to fit in with the above criteria. The same specimen can be processed for IF, histochemical, and light-microscopic analysis.

Recently, we tried to further simplify this method by skipping several steps.

10 consecutive needle biopsies from patients with kidney disease (their histologic diagnoses are included in table I) were processed as follows: immediately after its removal, the specimen was divided into two parts: one part was placed in a small vial and snap-frozen in liquid nitrogen and the second part was fixed in formol-sucrose at 4 °C.

3-µm-thick frozen sections were obtained by cutting

Table I. Histologic Diagnosis

Case No.

Diagnosis

1

2 3 4

membranous proliferative glomerulonephritis systemic lupus erythematosus glomerulonephritis with crescents end stage

5

end stage

6

7

systemic lupus erythematosus end stage

8

membranous glomerulonephritis

9

10

acute proliferative glomerulonephritis membranous proliferative glomerulonephritis on a cryostat, air-dried at room temperature, and then processed as extensively described elsewhere [6]. The sections were incubated with commercial antisera against human IgG, IgA, C3, C1q and C4 (Behringwerke, Mannheim, FRG).

The specimen that was fixed in 4 °C formol-sucrose for 16 h was processed in the same way as all routine biopsies for light microscopic studies. For immunofluorescent studies the sections were deparaffinized and were subject to the same procedure as snap-frozen tissue.

The comparison data between frozen and fixed sections are given in table II. In relation to positivity, our results were identical (fig. 1, 2), with only a minor variation in intensity. All deposits have a granular pattern although their distribution varies. Among 60 tests, 49 showed the same intensity in both studies. However, in 7 cases the paraffin section studies produced better results and the intensity of immunofluorescence stain was higher than that obtained with frozen sections. The opposite was obtained in 4 tests, all of them with the anti-complement antisera.

These results contradict those published in our previous report and emphasize the recommendation that paraffin-embedded tissue previously fixed in 4 °C formol-sucrose can be used for immunofluorescence studies.

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Table II. Comparative IF findings in frozen and paraffin-embedded sections (formol-sucrose-fixed sections)

Fig. 1. Case 8, membranous glomerulonephritis. Frozen sections incubated with anti-human IgG. ×400.

Fig. 2. Paraffin-embedded tissue from the same case incubated with anti-human IgG. ×400.

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