NOTE

Immunofluorescence for Feline Infectious Peritonitis Virus with Trypsin-Treated Paraffin Sections

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(Received 6 September 1982/Accepted 26 October 1982)

Immunofluorescence (IF) with paraffin sections which was proposed by Sainte-Marie [10], is widely used for revealing more exact localization of specific antigen than with frozen sections. Using formalin fixed paraffin sections, the usefulness of pronase digestion was verified by Hung [5] in detection of hepatitis B antigen without risk of infection. The utility was further extended to other virus antigens such as those of herpes simplex, measles [11], cytomegalosis and varicella zoster [4], and rabies [7]. Herpes simplex virus antigen was demonstrated in tissues which had been embedded in paraffin since 20 years [4], suggesting possible retrospective examination for other viral antigens [5–7, 11]. Very recently, the method was also applied to mouse hepatitis virus, a member of coronaviruses [1].

This brief communication is to describe IF demonstration of feline infectious peritonitis (FIP) virus antigen on feline and murine tissue sections, which were fixed in neutral buffered formalin and embedded in paraffin.

Three naturally occurring FIP cases were of female cats from Tokyo area, which were clinically, pathologically and serologically diagnosed as FIP. As shown in Table 2, tissues from these cases were put into formalin within 12 hr postmor-

tem. One week later tissues from Case A were embedded in paraffin while those of Cases B and C after several months. Paraffinized tissue blocks of Cases A, B and C were stored at room temperature for 1, 15 and 18 months, respectively.

On the other hand, tissues were sampled from two experimentally infected cats, Cases D and E, both having apparent clinical signs after inoculation with FIP virus, Yayoi strain [2, 9], and they were put into formalin shortly after death. The tissues of Case D was fixed in formalin for 7 days and embedded in paraffin for 8 months, whereas those of Case E was in formalin for about 2 months and in paraffin for 2 days. Besides, newborn ICR mice had been injected intracerebrally with Yayoi strain virus having experienced 31 passages in mice [2, 3], and the brains were sampled at 3 days postinoculation and fixed in formalin for 7 days. Then paraffinized tissues were stored for 1 day to 8 months.

From the formalin fixed and paraffin embedded samples 2 to 4 μ m-sections were made and placed on a slide treated with 0.1% Neoprene in toluene [4]. After drying at 37°C for a few days, sections were passed through xylol and a series of graded ethanol. After washing in PBS they were treated with 0.25% trypsin (Trypsin 1:250, Difco) in PBS at 37°C for

3 hr and then washed in three changes of PBS for 30 min. For comparative studies 6 μ m sections were freshly prepared from the same tissues, which had been frozen in cold n'-hexane dry ice acetone. The frozen sections were fixed with acetone at room temperature for 10 min before IF testing.

For direct IF, antibody from a naturally infected cat was labelled with fluorescein isothiocyanate (FITC) according to Kawamura [8] and it was used at a dilution showing an indirect staining titer of 1:640. For indirect IF, the primary

antibody was from a naturally infected cat and it was used at a dilution showing staining titer of 1:2,560. The secondary antibody was FITC-conjugated anti-cat IgG (Cappel Lab., Cochranville, Pa., U.S.A.) rabbit serum, which was used at a dilution showing a direct staining titer of 1:4. In some experiments the secondary antibody was absorbed twice with cat liver acetone powder [8] before use.

Tissue sections were treated with the primary and then with the secondary antibodies at 37°C for 60 min each, and they were washed in PBS and mounted

Table 1. Effect of trypsin digestion on immunofluorescence with paraffin sections

Tissue	Direct-IF		Indirect-IF	
	Trypsin(-)	Trypsin(+)	Trypsin(-)	Trypsin(+)
Suckling Mouse Brain	—*	+	+	++
Cat: Various organs	—	—	—	++

* +~++: Number of positive cells (+: A few, ++: Moderate, +++: Abundant)

Table 2. Immunofluorescence with frozen sections or formalin-fixed and trypsinized paraffin sections from FIP cases

Case (Age)		Sample	Section	
			Frozen	Paraffin
Natural	A (2.5M)**	Stomach	+	—
		Jejunum	+	+
		Mes. lymph node	+	—
	B (5M)	Cecum	+	—
		Colon	++	++
		Rectum	++	++
	C (6–7Y)	Duodenum	++	—
		Mes. lymph node	++	+
		Omentum	+	—
Experimental	D (6–8W)	Liver	++	++
		Jejunum	+	+
		Omentum	+	+
	E (6–8W)	Duodenum	++	++
		Ileum	++	+
		Cecum	+	++

* See Table 1. ** W: Week, M: Month, Y: Year.

with 0.5 M carbonate-bicarbonate buffered glycerin (pH 9.5) for fluorescence microscopy. For light microscopy the same or adjacent sections were stained with hematoxyline and eosin (HE).

With trypsin-treated paraffin sections from infected suckling mouse brain, direct as well as indirect IF revealed a granular pattern of FIP virus specific-fluorescence within the cytoplasm of neurons (Figs. 1 and 2). Without trypsin treatment no specific fluorescence was seen by direct IF, while very weak fluorescence was demonstrated by indirect IF (Table 1). Such pattern of fluorescence was never seen with sections which had been pretreated with non-labelled FIP antibody before treating with labelled FIP antibody.

From natural and experimental feline cases, paraffin sections with trypsin digestion gave clearly positive results by indirect IF as well as frozen sections by direct IF, while no specific fluorescence was revealed by direct IF with paraffin sections. Specific fluorescence was demonstrated within enterocytes and mononuclear cells in and around necrotizing lesions of most cases examined (Table 2 and Figs. 3 and 4). Such fluorescence was not seen using non-FIP feline serum.

As described above, IF with formalin fixed and paraffin embedded tissue sec-

tions with trypsin digestion is shown to be useful for detection of FIP virus antigen especially in experimentally infected cases. In natural cases, however, the detection rate of virus antigen was much lower, probably because of unfavorable effect of usually longer time period between sampling and examination.

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EXPLANATION OF FIGURES

Fig. 1. Direct IF showing granular fluorescence in neurons of an inoculated suckling mouse (A) and the HE-stained same area on an adjacent section (B). Bar=25 μ m.

Fig. 2. Indirect IF showing granular fluorescence in hippocampal neurons of an inoculated suckling mouse (A) and the HE-stained same area on an adjacent section (B). Bar=20 μ m.

Fig. 3. Indirect IF showing FIPV antigen posi-

tive monocytes in a focal liver lesion of an inoculated kitten (A) and the HE-stained same area on an adjacent section (B). Bar=25 μ m.

Fig. 4. Indirect IF showing FIPV antigen within a degenerated large mononuclear cell (arrow) in a small vein of the tunica albuginea of the testis of an inoculated kitten (A) and the same area on the HE-stained section (B). Bar=10 μ m.

要 約

トリプシン処理パラフィン切片免疫蛍光法によるネコ伝染性腹膜炎ウイルス抗原の検出(短報): 柳沢利彦・林 俊春・藤原公策(東京大学農学部家畜病理学教室)——ネコ伝染性腹膜炎ネコ自然例・実験感染例および実験感染乳のみマウスのホルマリン固定——パラフィン切片について, トリプシン処理——免疫蛍光法により病変部におけるウイルス抗原の検出を試みた。ネコ例では間接法で, 実験感染乳のみマウス脳では間接法, 直接法で特異蛍光をみとめ, 本法の有用性が明らかにされた。