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Research Note

Detection of the Parenthesis-like Body on Cyst Wall of *Pneumocystis carinii* by a Modified Toluidine Blue O Staining

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Although some recent investigations demonstrated that indirect immunofluorescent antibody test utilizing a monoclonal antibody (Kovacs *et al.*, 1986) and polymerase chain reaction (PCR) technique (Wakefield *et al.*, 1990) were potentially useful for the diagnosis of *Pneumocystis carinii* pneumonia, one of the major opportunistic infections associated with acquired immunodeficiency syndrome (AIDS), staining procedures of the causative organism are still of significant importance. Among the available techniques, toluidine blue O staining (TBO) (Chalvardjian and Grawe, 1963) has been proved to be one of the effective methods particularly for staining the cyst of *P. carinii*. However, this method has been handicapped by the fact that it does not give conclusive data especially when patient's specimen contains only a few cysts, not in a cluster, it was difficult to distinguish *P. carinii* from fungi in such cases. On the other hand, Grocott's modified Gomori's methenamine silver nitrate staining (GMS) has also been found useful, as the parenthesis-like body on cyst wall of *P. carinii* first described by McNeal and Yaeger (1960), which is absent from virtually all of the yeasts and fungi, is stainable by this technique. Indeed, even when only a few cysts are present in patient's specimen, they can be detectable by the presence of this structure (Balachandran *et al.*, 1990; Kim *et al.*, 1990). In other words, TBO, in some cases, should be followed by GMS for conclusive identification of *P. carinii* cyst. However, since GMS needs lots of manipulations even in the modified simple procedure, new methods to easily detect *P. carinii* cyst are of apparent significance. The present communication deals with our trial on the modified TBO to detect the parenthesis-like body on cyst wall of *P. carinii,* since this structure is scarcely stainable by the conventional TBO.

Our modified TBO was based on the decreased concentration of sulfuric acid in the sulfonating reagent. In the conventional method, 80 ml of diethyl ether saturated with distilled water is slowly mixed with the same volume of concentrated sulfuric acid. We diminished the volume of sulfuric acid to 55 ml, and mixed it with 80 ml of the water-saturated diethyl ether in the same manner. Other reaction conditions were exactly the same as in the conventional method.

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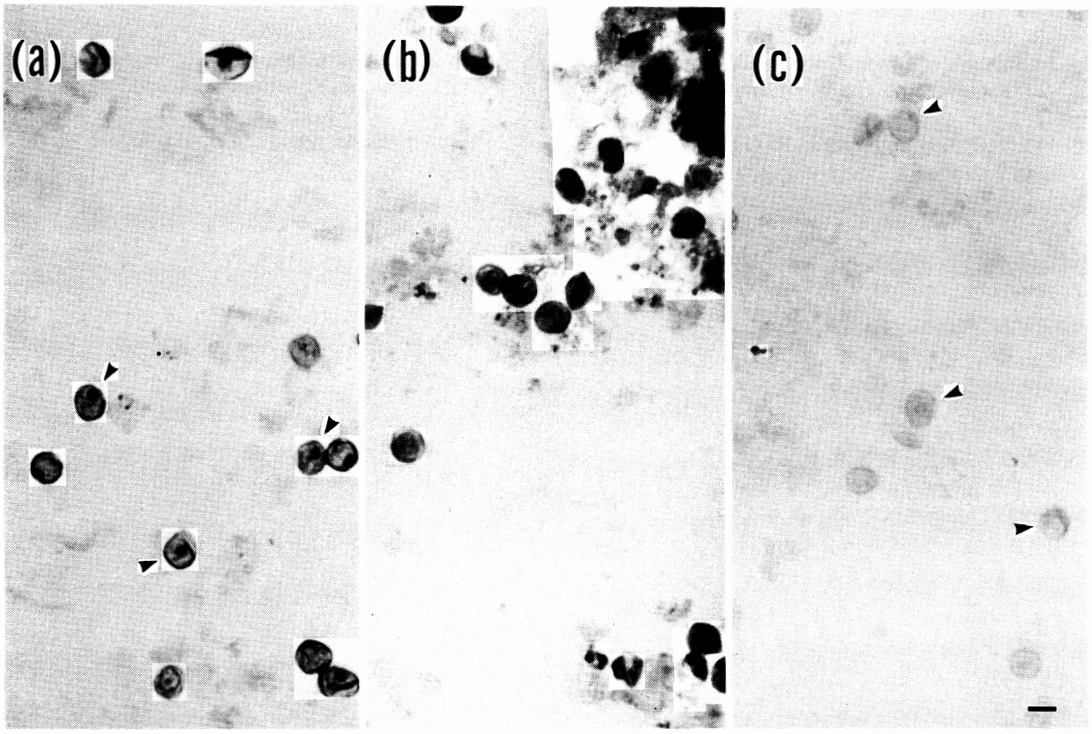

Fig. 1 A lung of Wistar male rat, which developed *Pneumocystis carinii* pneumonia by subcutaneously injecting 3.75 mg prednisolone acetate per head twice a week for 3 months, was removed and cut into small pieces, which were homogenized gently in 4–5 ml of Dulbecco's phosphate-buffered saline for approximately 1 min at 4°C. This homogenate was stained by three different methods; (a) GMS, (b) conventional TBO, (c) modified TBO. The parenthesis-like body can be detected by GMS and the modified TBO (arrows), but scarcely by the conventional TBO. Bar in (c) stands for 5 μm, and the other pictures were also taken at the same magnification.

Fig. 1-a, b and c show *P. carinii* cysts stained by GMS, the conventional TBO and the modified TBO, respectively. The parenthesis-like body was readily demonstrable by GMS and the modified TBO, but not by the conventional TBO. Although the color of *P. carinii* cyst became faint in the preparation stained by the modified TBO, it was not difficult to detect the body, since the color of background also became faint. There was little difference in the number of *P. carinii* cyst with detectable parenthesis-like body between stained preparations by GMS and by the modified TBO, whereas the number was much less concerning the conventional TBO. Virtually none of similar structures have been detected in yeasts and fungi stained with this method.

Accurate and easy methods to detect *P. carinii* cyst in patient's specimens are evidently important, as the number of AIDS patients has been increasing. Our modified TBO may be applied to overcome one of the major difficulties with the original method if it is conducted with the controls.

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