Parasitol Res (1991) 77:351–352
004432559100047N

Parasitology Research

An identical epitope in *Pneumocystis carinii* and *Toxoplasma gondii* causing serological cross reactions*

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Accepted January 17, 1991

Abstract. A monoclonal antibody raised against membrane proteins of *Toxoplasma gondii* with molecular weights of 35 and 21 kDa also reacts strongly and "specifically" with surface antigens of *Pneumocystis carinii* with molecular weights of 394.2 and 69 kDa when used in a direct immunofluorescence antibody test, on the one hand, and in a immunoblot after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), on the other. Whether or not this observation might have any phylogenetic relevance remains open.

In the course of continuous surveillance of HIV-positive Austrians for opportunistic parasitic infections as described by Aspöck and Hassl (1990), we incidentally discovered a distinct reaction of the anti-*Toxoplasma* monoclonal antibody (mAb) used in our *Toxoplasma* detection system for sputum and bronchoalveolar lavage (BAL) screening using *Pneumocystis carinii*. To our surprise, this unusual observation turned out to reflect a strong, reproducible and evidently "specific" reaction of *Pneumocystis* trophozoites with an anti-*Toxoplasma* antibody. Therefore, we tried to characterize some biochemical properties of this possibly common antigen.

Materials and methods

Monoclonal antibodies against *T. gondii* were raised by Müller et al. (1988). The antibody 5B10, an IgG_{2a}, recognizes antigens with molecular weights of 35 and 21 kDa (Koch et al. 1989). The antibody was processed to a fluorescein isothiocyanate (FITC) conjugate and was used for the detection of *Toxoplasma* trophozoites in sputa and BAL in a direct immunofluorescence antibody test (DIFT).

Due to the observation that *Pneumocystis carinii* in BAL samples reacted with the mAb, two sediments of BAL containing large numbers of *Pneumocystis* cells as determined by homologous DIFT (Genetic Systems Corp., Seattle, WA) were lysed in reducing sample buffer and, together with marker proteins, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, PhastGel 10–15) in a PhastSystem (Pharmacia LKB Biotechnology, Vienna) according to the manufacturer's recommendations. Thereafter, the proteins were transferred to a PVDF membrane (Millipore, Bedford, Mass.) in a PhastTransfer system as described by the manufacturer, and immunoblotting with a non-conjugated 5B10 mAb was performed according to Hassl and Aspöck (1990). The staining patterns, which had a distinct and strong appearance, were transferred to an image and evaluated in a PhastImage system. For controls we tested our system with human lung cells (Table 1) as well as with the conjugate alone; in these cases no staining reaction was observed.

Results and discussion

When tested against >200 sputum and BAL samples, 60% of them containing *Pneumocystis carinii*, the antibody reacted strongly with trophozoites and, more weakly, with cysts of *P. carinii* in all cases. The FITC-conjugated mAb showed a bright staining of the surface of *Toxoplasma gondii* as well as of *P. carinii* (Fig. 1). However, although the antigens recognized by the mAb in both parasites seem to be membrane components, their molecular weights are totally different: 35 and 21 kDa in *Toxoplasma* vs 394.2 and 69 kDa in *Pneumocystis*. For this reason and because of the negative controls, any misinterpretation of the results due to a non-

Table 1. Results of the immunoblot after SDS-PAGE of different lysates using an anti-*Toxoplasma* monoclonal antibody

	Lysates of		
	Toxoplasma trophozoites	*Pneumocystis* cells	Lung tissues
Mol. wt. of the bands stained by the 5B10 monoclonal antibody	35 kDa 21 kDa	394.2 kDa 69 kDa	– – – –

* Dedicated to Prof. Dr. J. Eckert (Zürich) on the occasion of his 60th birthday

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Fig. 1. Trophozoites of *Pneumocystis carinii* in a bronchoalveolar lavage detected by a direct immunofluorescence antibody test using the *anti-Toxoplasma* monoclonal antibody *5Bl0jFITC*

specific reaction of the mAb with IgGs attached to the surface of the parasites (a phenomenon described by Blumenfeld et al. 1990 for *P. carinii* and by Budzko et al. 1989 for *T. gondii)* can be excluded. A prominent and, in humans, strongly antigenic protein of *P. carinii* with a molecular weight of about 69 kDa has been described by several authors (Kovacs et al. 1989; Linke et al. 1989).

Considering these findings, we came to the conclusion that there must be an identical epitope in *T. gondii* and in *P. carinii* that is located on both of the parasites' surfaces. Although the systematic position of *P. carinii* is still being debated, there are some strong indications of its fungal nature (Walker et al. 1990; Walzer and Cushion 1989; Watanabe et al. 1989). It is presently unclear whether our finding indicates a phylogenetic relationship between *T. gondii* and *P. carinii,* or represents the consequence of analogous antigen evolution due to selection pressure by the host, or simply reflects a curious, accidental epitopic coincidence.

Acknowledgements. The authors gratefully acknowledge the excellent technical assistance of Ms, I. Blöschl. This study was supported in part by grant P7151-MED from the Austrian Fonds zur Förderung der wissenschaftlichen Forschung.

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