Detection of *Aspergillus* Galactomannan: Comparison of an Enzyme-Linked Immunoassay and a Europium-Linked Time-Resolved Fluoroimmunoassay

A. PAUGAM,1* J. SARFATI, 2 R. ROMIEU,3 M. VIGUIER,3 J. DUPOUY-CAMET,1 AND J. P. LATGE2

Laboratoire de Mycologie1 and INSERM 445,3 Hôpital Cochin, and Laboratoire des Aspergillus, Institut Pasteur,2 Paris, France

Received 4 May 1998/Returned for modification 8 June 1998/Accepted 7 July 1998

With a view to improving the sensitivity of serological detection of *Aspergillus* galactomannan (GM), a europium-linked time-resolved fluoroimmunoassay was developed. This method was compared to an enzyme-linked immunosorbent assay using a peroxidase-conjugated detector antibody. No increase in the sensitivity of the detection of GM standards was seen with the europium-based fluoroimmunoassay.

Detection of *Aspergillus* antigens is the most promising approach to serological diagnosis of invasive aspergillosis (4, 8). Research has focused on the detection of the galactomannan antigen (GM), a major cell wall component and secreted molecule of *Aspergillus* species (1, 6). GM is composed of linear α-(1-2) α-(1-6)-linked mananns with β-(1-5) galactofuranose containing side chains (6). A rat antialgalactofuran immunoglobulin M (IgM) monoclonal antibody (MAb) named EB-A2 (14) has been used as a captor and detector in a direct double-sandwich enzyme-linked immunosorbent assay (ELISA) (commercially available as Platelia *Aspergillus*; Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France) (15). This is currently the most sensitive technique for GM detection (13), meaning that it gives the earliest serological diagnosis of invasive aspergillosis (9). Recently, ultrasensitive bioanalytical assays with time-resolved fluorescence detection have been developed (DELFIA [dissociation enhancement lanthanide fluorescent immunoassay]; Wallac, Turku, Finland), based on lanthanide fluorescence (europium, samarium, or terbium). Immunoassays using time-resolved fluorometry have been proposed as a more sensitive alternative to classical ELISAs (2). To improve GM detection, we developed a time-resolved fluorimunoassay in which EB-A2 was conjugated with europium instead of peroxidase.

The sandwich immunoassay was carried out as described by Stynen et al. (13). Briefly, GM was isolated from a culture of *Aspergillus fumigatus* (6). The captor MAb EB-A2 was kindly provided by M. Tabourret (Sanofi Diagnostics Pasteur, Steenverde, France) and was produced and purified as previously described (14). The detector was peroxidase-conjugated EB-A2 (Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France) or europium-conjugated EB-A2 (Wallac labelling service, Turku, Finland). These two conjugates have been stored for at least 3 months at 4°C without any loss of activity. Polystyrene microtiter plates (Nunc; Becton Dickinson) were coated with least 3 months at 4°C without any loss of activity. Polystyrene microtiter plates (Nunc; Becton Dickinson) were coated with 100 µl of EB-A2 at 1 µg per ml at +4°C in 0.1 M carbonate buffer (pH 9.4). After overnight incubation at room temperature, the solution was removed by aspiration and the wells were washed with washing solution and postcoated with 200 µl of 0.05% phosphate-buffered saline (PBS)-Tween and 1% bovine serum albumin at 37°C for 1 h. After a wash with PBS-Tween, 50-µl volumes of twofold serial dilutions of GM in distilled water (1.25 to 0.1 ng per ml) were added and plates were incubated for 1 h at 37°C. After washing with PBS-Tween, 50 µl of peroxidase-conjugated EB-A2 or europium-conjugated EB-A2 at 1 µg/ml was added and the mixture was incubated for 1 h at 37°C.

In the ELISA method, after extensive washings, optical density was read at 490 nm after 30 min of incubation in the dark at room temperature with 100 µl of revelation buffer containing O-phenylenediamine. In the DELFIA method, after extensive washings, Eu³⁺ was released from the chelate within a few minutes by the low pH (≤ 3) of the DELFIA enhancement solution (Wallac). After plates had been agitated for 5 min on a plate shaker, fluorescence was measured with a time-resolved fluorometer (DELFIA Research Fluorometer; Wallac).

The results are shown in Fig. 1. The sensitivity of the sandwich peroxidase-conjugated assay was consistent with that found in previous studies (0.5 to 1 ng/ml) (13). The background fluorescence seen in this time-resolved fluorimetry procedure was high (blank with no antigen, about 85,000 cps). The nonspecific binding of the europium-bound conjugate to the first antibody as well as the high labelling yield for the IgM MAb (22.7 europium molecules per EB-A2 IgM molecule versus about 8 europium molecules per IgG molecule [7]) may be responsible for the high background fluorescence seen in the time-resolved fluorimetry procedure.

No major improvement in sensitivity was obtained with the fluorescence time-resolved immunoassay. The two methods differed only by the substance to which the detector antibody was conjugated (peroxidase or europium). Time-resolved fluorescence immunoassays have been used successfully in virology, hormonology, antitoxin, and cytokine research, etc., and have proved to be 6 to 40 times more sensitive than standard ELISA methods (3, 5, 10, 11); in another application, antihuman immunodeficiency virus antibodies were detected 16 days earlier than by standard ELISA (12). To our knowledge, this is the first time that a time-resolved fluorescence immunoassay has been used to detect a polysaccharide (GM) rather than a polypeptide and the first time that europium-labelled IgM has been used instead of IgG. The chemical nature of the antigen and the IgM detector antibody may explain this lack of increase in sensitivity.

---

* Corresponding author. Mailing address: Laboratoire de Mycologie, Hôpital Cochin, 75014 Paris, France. Phone: 1 42 34 14 97. Fax: 1 42 34 14 96. E-mail: andre.paugam@cch.ap-hop-paris.fr.
This work was supported by Assistance Publique-Hôpitaux de Paris (CRC grant 950228).
We thank David Young for checking the English.

REFERENCES


FIG. 1. Detection of GM by using sandwich europium-linked and peroxidase-linked assays.