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Research paper

Comparison of hybridoma screening methods for the efficient detection of high-affinity hapten-specific monoclonal antibodies

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Abstract

This study compares diverse microplate-based hybridoma screening methods for the generation of hapten-(aflatoxin-) specific monoclonal antibodies (MAbs). Standard indirect enzyme-linked immunosorbent assay (ELISA) screenings (with immobilization of hapten–protein conjugate and use of enzyme-labeled anti-mouse IgG as tracer) were compared with direct ELISAs (with antibody immobilization and use of a hapten-enzyme conjugate as tracer). Although direct ELISA is rarely used for routine hybridoma screenings, it showed considerable advantages compared to the indirect assays. Standard indirect ELISA screening can lead to a considerable number of false positives (up to about 50% false positives of all 373 supernatants tested) if the antibody concentrations in the supernatants are too high. Direct ELISAs gave useful screening results for the different supernatant dilutions chosen. At most 3 false positives were detected out of 373 supernatants. However, the sensitivity of the direct ELISA screening is generally lower compared to indirect ELISA, and individual high-affinity MAbs might be classified as false negative. Therefore, a modified indirect ELISA screening was also developed. It includes pre-incubation of the supernatants in anti-mouse IgG-coated microplates which are then transferred into the (indirect) hapten conjugate-coated microplates. This screening method leads to excellent results with good overall selectivity and sensitivity. It can also be conveniently combined with the direct ELISA screening. Using these improved screening methods, aflatoxin-specific MAbs could be generated with IC_{50} values down to 3 ng/l (aflatoxin concentration).

Keywords: Monoclonal antibody; Hapten-specific; Hybridoma screening; ELISA; Aflatoxin; Mycotoxin

Abbreviations: AF, aflatoxin; BSA, bovine serum albumin; CMO, carboxymethoxyloxime; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; HAT, hypoxanthine/aminopterin/thymidine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HT, hypoxanthine/thymidine; IC_{50}, concentration of analyte that causes a 50% decrease of the maximum response; LC, liquid chromatography; MAb, monoclonal antibody; MALDI, matrix-assisted laser desorption/ionization; MCD, mean coupling density; MS, mass spectrometry; NHS, *N*-hydroxysuccinimide; PBS, phosphate buffered saline; POD, horseradish peroxidase; RPMI, Roswell Park Memorial Institute; TG, bovine thyroglobulin; TMB, tetramethylbenzidine; TOF, time-of-flight.

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1. Introduction

Monoclonal antibodies (MAbs) are useful biological tools for various analytical applications, e.g. in clinical chemistry, food analysis, and environmental monitoring. In addition, antibodies are increasingly used as human therapeutics. Immunization of animals, mainly mice, in combination with hybridoma technology is still the most common method for the generation of MAbs. Regardless of the intended application, the selection of high-affinity MAbs is often preferred. An efficient hybridoma screening procedure is a crucial step that usually has to be accomplished within about one day (Burrin and Newman, 1991). Thus, the ideal screening method should be fast, reliable, and easy to accomplish, especially if there is little or no equipment available in the laboratory for carrying out automated immunoassays. It should clearly detect high-affinity MAbs with a minimum of both false positives and false negatives. In addition, useful screening results must be obtained relatively independent of the MAb concentration in the supernatants, because optimization of the ELISA parameters (such as supernatant dilution and coating conjugate dilution) prior to the screening is usually much too time-consuming or even impossible, especially as the screening often only involves a single measurement per MAb.

The microplate-based antigen-immobilized ELISA (indirect ELISA) is the de facto standard screening method for the detection of hapten-specific antibodies (Grol and Schulze, 1990), although other methods have been reported, e.g. BIAcore screening (Canziani et al., 2004), flow-based immunoassay (Sasaki et al., 2005), and time-resolved fluorescence assay (Daigo et al., 2006). It usually includes immobilization of a hapten–protein conjugate on the microplate surface, the addition of (diluted) hybridoma culture supernatant and use of a (enzyme-) labeled secondary (anti-mouse IgG) antibody. For the generation of anti-hapten MAbs, simultaneous non-competitive/competitive indirect ELISAs can be performed (Abad and Montoya, 1994; Mercader and Montoya, 1999; Moreno et al., 2001; Manclús et al., 2004). Yet an alternative immunoassay, although rarely used, is known for the screening of hapten-specific MAbs (henceforth referred to as direct ELISA) (Cho et al., 2005; Schetters, 1993; Hack et al., 1987). It includes immobilization of a capture (anti-mouse IgG-) antibody, addition of supernatant, and use of a hapten-enzyme conjugate. Qualitative differences between these two screening methods have been suggested (Kane and Banks, 2000). We have had diverse experiences with the two immunoassays in our laboratories (Matschulat et al., 2005; Mangler et al., 1994; Winklmair et al., 1997; Weller, 1992), which encouraged us to carry out a systematic comparative study.

For this work, aflatoxin-specific MAbs were produced. Aflatoxins, a sub-group of mycotoxins, are low molecular weight secondary metabolic products of moulds (*Aspergillus flavus* and *Aspergillus parasiticus*) that can contaminate various food matrices. Due to their extreme carcinogenicity these toxins are of great concern. Strict maximum permissible limits exist in most countries worldwide (Food and Agriculture Organization, 2004). High-affinity aflatoxin-specific MAbs are therefore useful tools for analytical food chemistry (Eaton and Groopman, 1994).

2. Materials and methods

2.1. Safety note

Aflatoxins are highly carcinogenic and should be handled with extreme care. Aflatoxin-contaminated material should be decontaminated with an aqueous solution of sodium hypochlorite (5%).

2.2. Materials, reagents, and equipment

Flat-bottom, transparent 96-well polystyrene microplates were obtained from Greiner (Frickenhausen, Germany). Sephadex G-25 columns were purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). An IsoStrip mouse monoclonal antibody isotyping kit was purchased from Roche (Mannheim, Germany). Anti-mouse IgG (whole antiserum, produced in goat) was purchased from Sigma (St. Louis, MO). Monoclonal aflatoxin-specific reference antibodies were obtained from LCTech (Dorfen, Germany). Solid horseradish peroxidase (POD, EIA grade) was obtained from Roche. POD-labeled anti-mouse IgG antibody (H+L, produced in horse, affinity purified) was obtained from Axxora (Lörrach, Germany). Bovine serum albumin (BSA, fraction V, ~99%) and casein were obtained from Sigma. Thyroglobulin (TG) from bovine thyroid glands was obtained from Fluka (Buchs, Switzerland). Aflatoxins B1, B2, G1, and G2 were obtained from Sigma. Aflatoxin standard stock solutions in acetonitrile (0.1 mg/ml) were prepared by a validated method (Nesheim et al., 1999). 3,3′,5,5′-tetramethylbenzidine (TMB, ≥99%), carboxymethoxylamine hemihydrochloride (~98%), *N*,*N*′-dicyclohexylcarbodiimide (DCC, ≥99%), *N*-hydroxysuccinimide (NHS, ≥97%), dioxane (≥99.5%, H_2O ≤0.01%), acetonitrile (HPLC grade), H_2O_2 (35%), Tween 20, and dimethyl sulfoxide (DMSO, ≥99.5%) were obtained from Sigma. All