# Monoclonal Antibodies Register

# Monoclonal Antibodies against Type-A Staphylococcal Enterotoxin

B. HOLEČKOVÁ, J. GONDOL', V. KALINÁČOVÁ, M. FOTTA

Department of Bacteriology and Hybridoma Technology, Research Institute of Veterinary Medicine, Košice, Slovakia

## Background

Type A staphylococcal enterotoxin (SEA) is a single-chain, heat-stable protein with a molecular weight of 27.8 kDa and isoelectric point (pI) of 7.3 (Su and Wong, 1997), which is produced by enterotoxigenic strains of *Staphylococcus aureus*. SEA, along with staphylococcal enterotoxins B and C (SEB and SEC), belong to enterotoxins most frequently occuring in foodstuffs. By its ability to form complexes with molecules of the MHC class II and subsequently to activate a large number of T lymphocytes, it falls into the group of superantigens (Svensson et al., 1997). Due to the proliferation of a large proportion of T lymphocytes, an excessive quantity of lymphokines is released that are likely to contribute to the development of enterotoxin-induced disease – staphylococcal enterotoxicosis (Marrack and Kappler, 1990). Relatively little is known of antigenic determinants of staphylococcal enterotoxins and how these may relate to the structure and function of the toxins (Wood, et al., 1997). To detect staphylococcal enterotoxins in foodstuffs, immunochemical methods (RIA, ELISA) are mostly employed using specific polyclonal and monoclonal antibodies. Monoclonal antibodies against SEA were prepared to be used in the development of the ELISA method.

## Description of the Antibodies

### *Production*

Hybridoma cultures producing monoclonal antibodies against SEA were obtained after immunization of Balb/c mice with purified SEA (Kienle, 1988) and fusion of mice spleen cells with SP2/0-Ag 14 myeloma cells in the presence of polyethylene glycol (PEG 3000). The radioimmunoassay (RIA) was a screening method with $^{125}$I-tracer prepared by the chloramine T method (Greenwood and Hunter, 1963). Limiting-dilution cloning and recloning (Campbell, 1984) was used to prepare hybridoma cell lines. Ascitic fluids were obtained by i.p. injection of hybridomas to Balb/c mice. Affinity constants of ascites were determined by processing experimental data of calibration relationship by means of Scatchard's equation

$$B/F = K (q - [B])$$

where B denotes bound fraction of labelled antigen, F is free fraction of labelled antigen, K is the affinity constant, q is the binding capacity, and [B] is the concentration of nonlabelled binding antigen – standard. The subclass of antibodies was determined by mouse monoclonal antibody isotyping kit (Sigma Israel Chemicals Ltd., Kiryat Weizmann, Rehovot, Israel). The reaction of monoclonal antibodies with crude and purified SEA was detected by Western blot (Harlow and Lane, 1988). The ELISA additivity test (Friquet et al., 1983; Peters and Baumgarten, 1997) was used for epitope analysis. The additivity indices,

$$A.I. = [(2A_{1+2}/A_1 + A_2) - 1] \cdot 100,$$

were calculated for determination of possible competition of antibodies ($A_1$, $A_2$, $A_{1+2}$ are absorptions of antibody alone and together, respectively). Additivity (A : A.I. = 50 – 100%) and non-additivity (NA : A.I. = 0 – 50%) of pairs of monoclonal antibodies were evaluated.

### *Specificity*

Two hybridoma cell lines (SEA-E12, SEA-G11) producing antibodies against SEA were obtained after fusion, screening the produced antibodies by RIA, cloning and recloning. The specificity of monoclonal antibodies to SEA was confirmed by immunoblotting. Antibodies reacted with a 30 kDa protein band of culture supernatant fluids (crude SEA) from SEA-producing *Staphylococcus aureus* strains (FRI 722) corresponding to about a 30 kDa band of purified SEA (Fig. 1). Cross-reactivity of the examined antibodies with staphylococcal enterotoxins B, C and D (SEB, SEC, SED) was not proved by immunoblotting (data not shown).

### *Properties*

The monoclonal antibodies against SEA can bind crude and purified *Staphylococcus aureus* enterotoxin A under denaturing and non-denaturing conditions of SDS-PAGE.

Received June 19, 1998. Accepted December 21, 1999.

Corresponding author: Beáta Holečková, Department of Bacteriology and Hybridoma Technology, Research Institute of Veterinary Medicine, Hlinkova 1/A, 040 01 Košice, Slovakia.

Abbreviations: PEG – polyethylene glycol, SEA (B, C, D) – staphylococcal enterotoxins A (B, C, D).

The properties of the antibodies (IgG) are shown in Table 1. The values of affinity constants indicate the high affinity of antibodies to SEA antigen and a possibility of using them for the development of an immunochemical method (indirect double sandwich ELISA with monoclonal and polyclonal antibodies). The additivity index A.I. = 1% for screening pairs of supernatants signify non-additivity (NA) of tested supernatants: the prepared antibodies probably recognize the same epitope of the SEA antigen and therefore they are not convenient to be selected as combinations for a double-monoclonal sandwich ELISA.

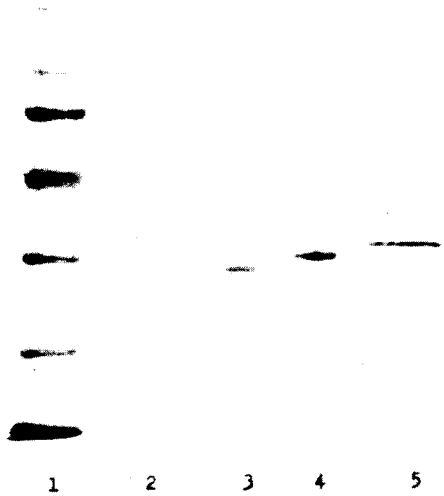


*Fig.1.* SDS-PAGE and Western blot. SDS-PAGE: line 1 – specific molecular weight markers (from top to bottom: 94 kDa, 67 kDa, 43 kDa, 30 kDa, 20.1 kDa, 14.4 kDa ); line 2 – Coomasie brilliant blue staining of culture supernatant fluid from SEA-producing *Staphylococcus aureus* strain FRI 722, line 3 – purified antigen SEA. Western blot: line 4 – immunoreactivity of purified antigen SEA with monoclonal antibody SEA-G11; line 5 – immunoreactivity of crude antigen SEA with monoclonal antibody SEA-G11.

*Table 1. The properties of mouse monoclonal antibodies against staphylococcal enterotoxin A*

| Mouse monoclonal antibody | Subclass of antibody | Affinity constant l/mol | Results of epitope analysis |
|---|---|---|---|
| SEA-E12 | IgG 1 | $2{,}647.10^9$ | NA[1] |
| SEA-G11 | IgG 1 | $1{,}452.10^9$ | NA |

[1]NA – non-additivity (competitive inhibition) of antibodies as determined by ELISA

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