Development of enzyme-linked immunosorbent assay (ELISA) for T-2 toxin using monoclonal antibodies

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モノクローナル抗体を用いた T-2 トキシンの酵素免疫測定法の開発

Summary

Two monoclonal antibodies (7D4, 6E9) reactive with T-2 toxin (T-2) were prepared. The antibody 7D4 was also reactive with T-2 hemisuccinate (T-2 HS) and acetyl T-2 toxin, but less reactive with HT-2, 3'-OH-T-2, and 3'-OH-HT-2 toxins. By using the antibody 7D4 in the indirect competitive ELISA method, the least detectable limit for T-2 was about 25 pg per assay.

T-2 toxin (T-2) is one of the well known toxic metabolites of *Fusarium*, and exhibits a potent inhibition of protein synthesis in mammalian cells^{1,2}. At present, a contamination of this toxin in foods and feeds is a serious problem in health. We prepared monoclonal antibodies (mAbs) for T-2, useful for ELISA, to develop a more specific and sensitive assay method.

Materials and Methods

The method proposed by Chu et al.³ was modified for the preparation of T-2 HS-bovine serum albumin (T-2 HS-BSA), T-2 HS-ovalbumin (T-2 HS-OVA), and T-2 HS-kyhole limpet hemocyanin (T-2 HS-KLH).

Spleen cells of BALB/c mouse immunized with

T-2 HS-BSA were fused with SP 2/0 myeloma cells using 50% polyethylene glycol 4000. Hybridoma cells secreting antibodies against T-2, which were cloned by repeating the limiting dilution method, were injected intraperitoneally into the mice. Then mAb produced were purified from the ascites.

The activity of mAb against T-2 was detected with ELISA by using T-2 HS-OVA and T-2 HS-KLH as antigen. The cross reactivity of mAb for T-2 analogs was analyzed by the indirect competitive ELISA method.

Results and Discussion

We prepared two stable hybridoma clones (7D4, 6E9) producing mAb for T-2. As shown in Figs. 1 and 2, the mAbs 7D4 and 6E9 exhibited a potent reactivity to T-2, T-2 HS and acetyl T-2, and the mAb 7D4 showed a weak reactivity to T-2 metabolites such as HT-2 and 3'-OH-T-2 toxins. The least detectable limits of T-2 with mAbs 7D4 and 6E9 in the indirect competitive ELISA method were 25 pg and 25 ng per assay, respectively. The sensitivity of the present mAb 7D4 is much higher than the mAb reported

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Fig. 1. The specificity of the mAb 7D4 in the indirect competitive ELISA for T-2 analogs

Fig. 2. The specificity of the mAb 6E9 in the indirect competitive ELISA for T-2 analogs

elsewhere⁴⁾.

As shown in Table 1, the cross reactivities of the mAb 6E9 to T-2 HS and acetyl T-2 were

Table 1. Cross reactivity of monoclonal antibodies

T-2 analogs	monoclonal antibodies	
	7D4	6E9
T-2 HS	0.035*(7.5)**	0.031(465)
Acetyl T-2	<0.025 (>10)	0.532(272)
T-2 toxin	0.260 (1.0)	144 (1.0)
3'-OH-T-2 toxin	16.5 (0.016)	>250(<0.58)
HT-2 toxin	19.7 (0.015)	>250(<0.58)
3'-OH-HT-2 toxin	>250 (<0.0001)	>250(<0.58)
Isovaleric acid	>250 (<0.0001)	>250(<0.58)

* Amount (ng) per assay yielding 50% displacement.

** Numbers in parentheses indicate relative ratio to T-2.

465 and 272 times higher than T-2, whereas those of the mAb 7D4 were 7.5 and 10 times, respectively. These results indicate that the mAb 7D4 shows high sensitivity and specificity for T-2.

ELISA method with the present mAb 7D4 may be simple, rapid, sensitive, and excellent for the detection and quantitation of T-2 in foods, feeds, and biological fluids.

Acknowledgement

We are grateful to Dr. T. Yoshizawa (Kagawa University, Japan) for a kind gift of 3'-OH-T-2 and 3'-OH-HT-2 toxins.

References

- 1) Ueno, Y., Hosoya, M., Morita, Y., Ueno, I., Tatsuno, T.: J. Biochem., 64, 479 (1968).
- 2) Ueno, Y., Nakajima, M., Sakai, K., Ishii, K., Sato, N., Shimada, N.: J. Biochem., 74, 285 (1973).
- 3) Chu, F. S., Grossman, S., Wei, Ru-Dong, Mirocha, C. J.: Appl. Environ. Microbiol., 37, 104 (1979).
- 4) Hunter, J. R., K. W., Brimfield, A. A., Miller, M., Finkelman, F. D., Chu, S. F.: Appl. Environ. Microbiol., 49, 168 (1985).