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

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Summary

Two monoclonal antibodies (7 D 4, 6 E 9) reactive with T-2 toxin (T-2) were prepared. The antibody 7 D 4 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 7 D 4 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 cells1). 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.2) 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 mAbs produced were purified from the ascites.

Results and Discussion

We prepared two stable hybridoma clones (7 D 4, 6 E 9) producing mAb for T-2. As shown in Figs. 1 and 2, the mAbs 7 D 4 and 6 E 9 exhibited a potent reactivity to T-2, T-2 HS and acetyl T-2, and the mAb 7 D 4 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 7 D 4 and 6 E 9 in the indirect competitive ELISA method were 25 pg and 25 ng per assay, respectively. The sensitivity of the present mAb 7 D 4 is much higher than the mAb reported
As shown in Table 1, the cross reactivities of the mAb 6E9 to T-2 HS and acetyl T-2 were 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.

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References