Bovine Monoclonal Antibody Specific to *Theileria sergenti*

Kazuhiro YOSHIHARA, Yoshio NAKAMURA, Kozo FUJISAKI, and Takashi ONODERA

Third Research Division, National Institute of Animal Health, 3-1-1 Kannondai, Tsukuba 305, Japan

(Received 12 April 1990/Accepted 10 August 1990)

Jpn. J. Vet. Sci. 52(6): 1333-1335, 1990

KEY WORDS: bovine monoclonal antibody, hetero-hybridoma, *Theileria sergenti*.

Theileria sergenti, a causative agent of Japanese bovine theileriosis, induces remittent fever and anemia in cattle. This disease is still thought to be one of the most important epizootic in grazing cattle, in spite of massive efforts of its eradication and control. Since the defensive mechanisms against theileriosis are not known precisely, there are very few reports to develop the vaccine [8]. The premunition using live parasite in erythrocytes before grazing is thought to be more effective at present [2]. However blood inoculation may transmit infectious diseases such as bovine leukemia. Therefore antigenic analysis using monoclonal antibodies against *T. sergenti* is under way to develop a new-type vaccine.

A few reports indicated the antigenic characteristics of *T. sergenti*. Western blot analysis using convalescent serum showed that four different protozoan polypeptides with molecular weights of 23,000 29,000 32,000 and 64,000 are responsible for the induction of immunity [6]. Murine monoclonal antibodies are reactive to two different protozoan polypeptides with

molecular weights of 23,000 and 32,000 [3].

Many researchers have developed bovine monoclonal antibodies to various antigens such as the K99 pilus antigen of *Escherichia coli* [1], *Brucella abortus* lypopolysaccharide [5], and bovine leukemia virus glycoprotein GP51 [9].

In the present study, we attempted to obtain bovine x murine heterohybridomas secreting bovine monoclonal antibodies to *T. sergenti* and described the characteristics of bovine monoclonal antibody. It is important to conduct a more precise antigenic analysis by using specific antibodies derived from a natural host of *T. sergenti*, to develop a useful vaccine.

A Holstein calf, four months of age, was immunized intramuscularly with 2.5 ml of an equal mixture of freezing and thawing *T. sergenti* (Ikeda strain) and Freund's complete adjuvant. Additional similar injection was given 48 days later. On day 50 after immunization, the calf was splenectomized and then infected with *T. sergenti* by infected ticks two weeks after splenectomy. The calf showed persistent parasitemia, the intraerythrocytic stage of piroplasmiasis. On day 85, the axillary lymph node was excised and lymphocytes were separated by using Ficoll-hypaque.

Fig. 1. Activation of lymphocytes. Active growth of lymphocytes are observed around the colony of bovine-mouse heterohybridoma. $\times 40$.

Fig. 2. Immunoelectrophoresis patterns obtained with clone C5 hybridoma supernate or bovine serum by using sheep antibody to bovine IgM.

A total of 1×10^8 bovine lymphocytes and 1×10^7 mouse myeloma cells (P3U1) were fused by using 1 ml of 50% polyethylenglycol 4000 (Merck, Rahway, New Jersey, U.S.A.) as described by Köhler and Milstein [4]. Hybrid cells were selected by HAT medium. In six out of 192 wells, the active growth of lymphocytes was observed around the colonies (Fig. 1). Hybridomas in these six wells were cultured in serum free medium (ASF 104, Ajinomoto Tokyo, Japan). Using the supernates, an indirect ELISA for *T. sergenti* was performed for screening as described previously [7]. One of the 6 wells showed positive reaction to *T. sergenti* antigen, and the hybridomas in this well were cloned by limiting dilution. The clone with high optical density in screening, clone C5, was selected for propagation.

Clone C5 was cultured in ASF 104 at a concentration of 1×10^6 /ml for 4 days. The supernate was subjected to ultrafiltration to get a 10-fold concentrated supernate.

Immunoelectrophoresis, Western blot analysis and indirect fluorescent antibody (IFA) test were performed with this concentrated supernate. The immunoglobulin class of monoclonal antibody was determined by immunoelectrophoresis. Immunoelectrophoresis revealed a single precipitin arc when developed with sheep anti-bovine IgM (Bethyl Laboratories, Montgomery, Texas, U.S.A. 1:100 dilution) (Fig. 2). No precipitin arc was produced with anti-bovine IgG₁ and IgG₂.

For the Western blot analysis, *T. sergenti* antigen was electrophoresed on a 5–20% of gradient polyacrylamide gel (Daiichi Pure Chemicals, Tokyo, Japan) in the presence of SDS and then transferred to nitrocellulose membrane. The transferred proteins reacted to the concen-

Fig. 3. Western blot analysis of *T. sergenti* antigen with clone C5 hybridoma supernate and positive serum.

Fig. 4. Indirect immunofluorescent antibody test of acetone-fixed erythrocytes infected with *T. sergenti*.

trated clone C5 supernate, *T. sergenti* positive bovine serum or culture supernate of myeloma cells. Specific binding of antibodies to the transferred proteins was detected by peroxidase conjugated anti-bovine IgM (1:100 dilution). As a result, three bands with molecular weights of 20,000 26,000 and 39,000 were detected using concentrated clone C5 supernate (Fig. 3). No reaction was detected with the culture supernate of myeloma cells. These three proteins might have similar epitope. Moreover, hybridoma supernate reacted specifically to *T. sergenti* in the cytoplasm of bovine erythrocytes by IFA test (Fig. 4).

In this study we have established the clone of hybridoma which produces bovine monoclonal antibody reactive to *T. sergenti* antigen. This monoclonal antibody (MAb C5) will be useful to analyze antigenic property necessary for vaccine development. We are exploring the possibility to develop subunit vaccine or anti-idiotypic vaccine by using MAb C5.

REFERENCES

1. Anderson, D. V., Tucker, E. M., Powell, J. R., and Porter, P. 1987. *Vet. Immunol. Immunopathol.* 15: 223-237.
2. Ishihara, T. 1968. *Jpn. Agr. Res. Quart.* 3: 23-31.
3. Kobayashi, N., Onuma, M., Kirisawa, R., Ohgitani, T., Takahashi, K., Sasaki, N., and Kawakami, Y. 1987. *Jpn. J. Vet. Sci.* 49: 697-702.
4. Köhler, G. and Milstein, C. 1975. *Nature (Lond.)* 256: 495-497.
5. Nielsen, K. H. and Henning, M. D. 1989. *Vet. Immunol. Immunopathol.* 21: 363-372.
6. Ohgitani, T., Okabe, T., and Sasaki, N. 1987. *Jpn. J. Vet. Sci.* 49: 531-534.
7. Shimizu, S., Suzuki, K., Nakamura, K., Kadota, K., Fujisaki, K., Ito, S., and Minami, T. 1988. *Res. Vet. Sci.* 45: 206-212.
8. Shirakata, S., Onuma, M., Kirisawa, R., Takahashi, K., and Kawakami, Y. 1989. *Jpn. J. Vet. Sci.* 51: 831-833.
9. Tanaka, M., Okabe, T., and Sasaki, N. 1988. *Jpn. J. Vet. Sci.* 50: 1136-1138.

要 約

Theileria sergenti に対する牛型モノクローナル抗体の作製(短報): 吉原一浩・中村義男・藤崎幸蔵・小野寺節(農林水産省家畜衛生試験場)——*T. sergenti* (池田株) 実験感染牛の腋窩リンパ節よりリンパ球を分離しマウスミエローマ細胞(P3U1)と細胞融合を行い, *T. sergenti* に対する牛型モノクローナル抗体を産生するヘテロハイブリドーマを一株作製した。抗体のサブクラスは, IgM で, 分子量39,000, 26,000, 20,000の原虫蛋白を認識していた。また, 間接蛍光抗体法により, 赤血球内の原虫に特異的な蛍光が観察された。