皮膚型ウシ白血病細胞に対するモノクローナル抗体の作出

誌名	Japanese journal of veterinary science
ISSN	00215295
著者	西, 英機 小沼, 操 和田, 正秀
巻/号	49巻5号
掲載ページ	p. 909-912
発行年月	1987年10月

Monoclonal Antibodies against Bovine Skin Leukosis Cells

Hideki NISHI, Misao ONUMA, Masahide WADA, Rikio KIRISAWA, and Yoshimi KAWAKAMI
Department of Veterinary Microbiology, Rakuno Gakuen University, Ebetsu 069, Japan
(Received 25 March 1987/Accepted 30 May 1987)

Jpn. J. Vet. Sci. 49(5): 909–912, 1987

KEY WORDS: monoclonal antibody, skin leukosis, tumor antigen.

Enzootic bovine leukosis (EBL) is common and caused by bovine leukemia virus (BLV), while sporadic bovine leukosis (SBL) is subdivided into calf (CLS), skin (SLS) and thymic (TLS) forms of bovine lymphosarcoma.

Previously we obtained monoclonal antibodies against tumor-associated antigen (TAA) expressed on EBL tumor cells [1]. Since the antibody, c143 showed highly specific for EBL tumor cells, it has been used for diagnosing EBL [4] and for analysis of the biological nature of the TAA. Recently we succeeded in the establishment of a lymphoid cell line, SBLC-1, from a tumor of SLS [5]. To know the nature of the TAA expressed on SBL tumor cells and its use as a diagnostic tool, we examined the production of monoclonal antibodies against SBLC-1 cells.

SBLC-1 cells showed T cell character, tumorigenicity in athymic nude mice and contained no bovine leukemia provirus [5]. About 3-month-old BALB/c mice were immunized subcutaneously with 1×10^8 viable SBLC-1 cells emulsified with the same volume of Freund's complete adjuvant, followed by injection intraperitoneally with the same number of cells 2 weeks later. The mice were given injections intraperitoneally with the same number of the cells after 2 weeks and then sacrificed 2 days after the last injection. The spleen cells were fused with P3×63Ag 8.653 cells in the presence of 50% Polyethylen Glycol 1,000. Presence of antibodies against SBLC-1 cells was examined by a complement-dependent antibody cytotoxicity (CDAC) test as previously described [1, 6]. Hybridomas that produced antibodies reactive to normal bovine peripheral blood lymphocytes (PBL) were eliminated. Hybridomas producing antibodies reactive to SBLC-1 cells were cloned 3 times in soft agar culture. Hybridomas were propagated in tissue culture or in the peritoneal cavity of the pristan-primed mice. Reactivities of the antibodies against various cells were determined by a CDAC test and/or by an indirect fluorescent antibody (IFA) test. To know the specificity of the antibodies, an adsorption test was performed using culture fluid as previously described [1].

SBLC-1 cell line was used to examine the effect of the antibodies on cell growth. SBLC-1 cells (1×10^5 cells/well) were suspended in 1 m*l* of Dulbecco's minimal essential medium, containing 10% fetal calf serum plus either 50 μl of ascitic fluid of monoclonal antibodies D8E3 or D4A9, and plated in a 24-well plate in triplicate wells. The cells were incubated at 37°C for 72 hrs. After incubation, the number of live cells was determined by the trypan blue dye exclusion test. For control, culture fluid from P3×63Ag8.653 or 50 μl of ascitic fluid from a monoclonal antibody against *Theileria sergenti* [2] was used in place of D8E3 and D4A9.

To identify the molecule that reacted with each monoclonal antibodies, Western blotting was performed. Tumor cells (10^7 cells) were mixed with 200 μl of lysate buffer (0.2% Triton X100, 2mM Phenylmethylsulfonyl fluoride, 0.02M Tris-HCl pH8.2) in an ice bath for 1 hr to make tumor cells lysate. The lysate was electrophoresed in 10% polyacrylamide slab gel in the presence of SDS and transferred to nitrocellulose sheets as previously described [7]. Transferred proteins were detected either by staining with Coomassie blue or immunologic reaction. For immunological detection, nitrocellulose sheets containing the transferred proteins which were reacted with monoclonal antibodies were washed and then reacted with a peroxidase-conjugated anti-mouse IgG $(ab')_2$ antibody (Zymed Lab, Inc, San Francisco, CA).

Hybridomas which reacted to SBLC-1 cells but not to normal bovine PBL were selected by a CDAC test. A total of 10 hybridomas producing antibodies against SBLC-1 cells were identified, and cloned in soft agar. The antibody titers of culture fluids from each of the 10 hybridomas were 1:4 to 1:8 by the CDAC test against SBLC-1 cells. All the antibodies reacted to SBLC-1 cells and the ranges of cytotoxic indices (CIs) were 68.9 to 85.5 but not to normal bovine PBL, FLK

Table 1. Reactivity of monoclonal antibodies against SBLC-1 by CDAC test

	Mean CI±SD of					
	D8E3	D4A9	5A12	D8D1	D4B3	5A11
Normal bovine lymphocytes(n=25)	3.0±6.3	4.1±5.6	2.9±5.5	4.9±7.6	2.4±4.4	3.3±5.5
EBL(n=9)	5.6±3.3	4.4±3.6	5.9±5.9	5.2±3.8	5.3±2.5	3.7±5.4
FLK	1.3	0	3.6	2.2	0	0
SBLC-1	72.3	84.3	73.2	68.9	77.8	69.4
Bovine fetal thymus (n=3)	16.9±3.3	16.1±8.1	56.6±8.0	49.3±8.2	32.4±9.2	46.7±4.5

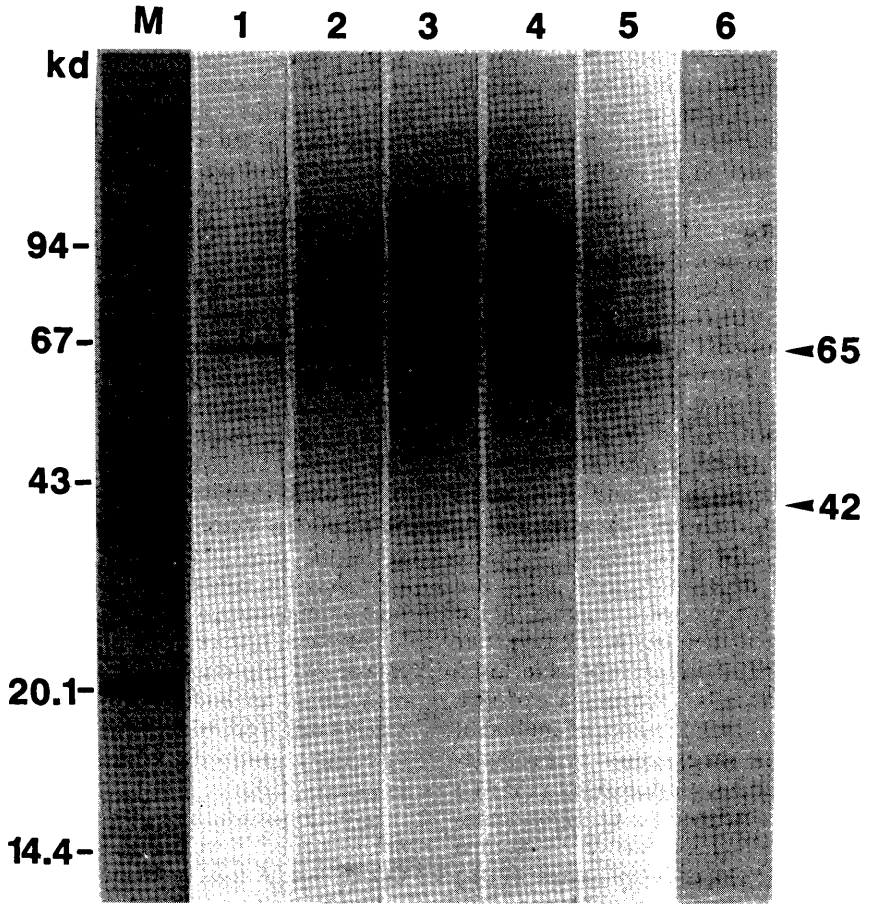

Fig. 1. Western blot analysis of SBLC-1 lysate with monoclonal antibodies. Lane M: Molecular weight marker, stained with Coomassie brilliant blue. Lanes 1 to 5: Monoclonal antibodies against SBLC-1 cells. Lane 1 to 5 are D4A9, 5B4, D4B11, D4B3 and 5A12, respectively. Lane 6: Monoclonal antibody c143.

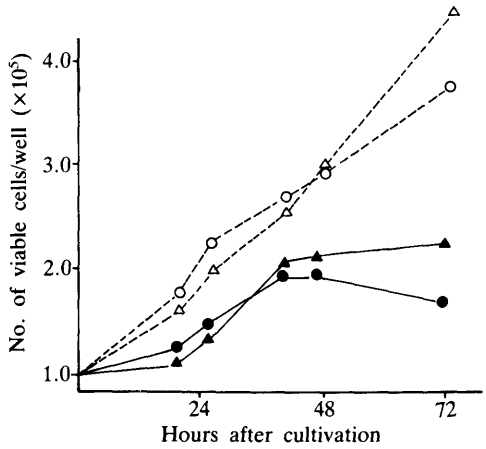

Fig. 2. Effect of monoclonal antibodies on the growth of SBLC-1 cells. ●, medium containing D4A9; ▲, medium containing D8E3; ○, medium containing monoclonal antibodies against *Theileria sergenti*; △, medium containing culture fluid from P3×63Ag8.653.

cells which is persistently infected with BLV, nor EBL tumor cells (Table 1). Furthermore, 4 (D8D1, D4B3, 5A11, 5A12) out of the 10 hybridomas reacted to bovine fetal thymus cells and the ranges of CIs of these 4 clones were 32.4±9.2 to 56.8±8.0. Similar reactivity of c143 to fetal thymus, especially thymic medulla was observed by immunohistological staining [3]. Adsorption tests were done using a clone 5A12 which reacted with bovine fetal thymus cells as well as SBLC-1 cells, to further confirm its specificity. After adsorption, its residual cytotoxicity against SBLC-1 was examined. Adsorption of 5A12 with SBLC-1 or bovine fetal thymus cells abolished its reactivity with SBLC-1 cells, however, adsorption of 5A12 with normal bovine PBL did not diminish its reactivity. These results indicated that monoclonal antibodies against SBLC-1 obtained may recognize the new antigen (s), tumor-associated antigen expressed on SBLC-1 cells. Although c143 reacted with SBLC-1 cells as well as EBL tumor cells [5], 5A12 did not react with EBL tumor cells suggesting that there are common and distinct TAAs expressed on EBL and SLS tumor cells.

Western blotting was performed to determine polypeptides that reacted with each monoclonal antibody. Five monoclonal antibodies (D4A9, 5B4, D4B11, D4B3 and 5A12) tested recognized a polypeptide with molecular weight (M. W.) of 65,000 (65 k) against SBLC-1 lysate, whereas c143 recognized a polypeptide with M. W. of 42 k (Fig. 1). c143 reacted with EBL and SBLC-1 tumor cells, and recognized polypeptides with M. W. of 74 k and 42 k, respectively indicating that polypeptides recognized by c143 in different tumor cell populations are different.

To know the biological activity of the monoclonal antibodies to SBLC-1 cells, SBLC-1 cells were cultured in a medium containing D8E3 or D4A9. The number of live cells was reduced in cultures containing either D8E3 or D4A9 as compared with cultures containing either P3×63Ag8.653 or ascitic fluid from a clone of *Theileria sergenti* (Fig. 2). Since both D8E3 and D4A9 showed no cytotoxicity to the SBLC-1 cells in the absence of rabbit complement, this result suggests the inhibitory effect of D8E3 and D4A9 for *in vitro* growth of SBLC-1 cells. The similar inhibitory effect of c143 against EBL tumor cells was also observed [1]. Although, however the exact mechanism of this inhibitory effect is not clear, there is a possibility that the TAA is a kind of receptor for growth factor thus the antibody against TAA may cause the inhibition of cell growth. The nature of the TAAs expressed on bovine lymphosarcoma cells and comparison of TAA on EBL and SBL tumor cells are under progress.

ACKNOWLEDGMENT. This work was supported in part by grant (61480085) from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1. Aida, Y., Onuma, M., Ogawa, Y., Mikami, T., and Izawa, H. 1985. *Cancer Res.* 45: 1174–1180.
2. Kobayashi, N., Onuma, M., Kirisawa, R., Ohgitani, T., Takahashi, K., Sasaki, N., and Kawakami, Y. 1987. *Jpn. J. Vet. Sci.* 49. 697–702.
3. Okada, K., Sakaguchi, I., Numakunai, S., Aida, Y., Onuma, M., and Ohshima, K. 1987. *Jpn. J. Vet. Sci.* 49: 373–377.
4. Onuma, M., Aida, Y., Okada, K., Ohshima, K., Kawakami, Y., and Izawa, H. 1985. *Jpn. J. Cancer Res.* (Gann) 76: 959–966.
5. Onuma, M., Nishi, H., Okada, H. M., Kirisawa, R., Chihaya, Y., and Kawakami, Y. 1987. *Jpn. J. Vet. Sci.* 49: 137–140.
6. Onuma, M., Suneya, M., Yamamoto, S., Mikami,

T., and Izawa, H. 1982. *J. Comp. Pathol.* 92: 559–565.

7. Towbin, H., Staehelin, T., and Gordon, J. 1979. *Proc. Natl. Acad. Sci. U. S. A.* 76: 4350–4354.

要　　約

皮膚型ウシ白血病細胞に対するモノクローナル抗体の作出（短報）：西　英機・小沼　操・和田正秀・桐沢力雄・川上善三（酪農学園大学家畜微生物学教室）——皮膚型ウシ白血病細胞株（SBLC—1）に対するモノクローナル抗体を作出した。得られた10クローンはいずれも正常ウシ末梢血リンパ球とは反応せず，SBLC—1細胞とのみ反応した。検査したクローンのうち4クローンはウシ胎仔胸腺細胞とも反応し，4クローンはウェスタン・ブロット法で65,000のポリペプタイドを認識した。2クローンは培養液中に加えることによりSBLC—1細胞の増殖を抑制した。