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COMMUNICATIONS

Monoclonal Antibodies Detect Antigenic Diversity in *Theileria parva* Parasites[1]

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Theileria parva parasites cause cattle diseases that are of considerable economic importance in East Africa. The syndromes include East Coast fever and Corridor disease, whose causative agents are called *Theileria parva parva* and *Theileria parva lawrencei*, respectively. There is some debate whether these are distinct species or strains of the same parasite (1). Vaccination against East Coast fever seems feasible, since cattle that recover from this disease are often immune to reinfection. A major difficulty presently limiting the success of vaccination is that although animals injected with infectious material can be protected against the same preparation, they do not withstand heterologous challenge (2). Thus it is necessary to be able to define this heterogeneity. Attempts to distinguish between *T. parva parva* and *T. parva lawrencei* by using recovered cattle serum have failed (3, 4). The technique of cell fusion can be used to produce antibody preparation of unique specificity even when the immunogen is a complex mixture of antigens (5). Using such antibodies, we report here that antigenic differences between *T. parva* strains can be distinguished.

Theileria parasites in cattle have three morphologically distinct stages: macroschizonts, microshizonts present in lymphoid cells, and piroplasms in erythrocytes. Lymphoblastoid cells infected with macroschizonts may be grown *in vitro* for prolonged periods. BALB/c mice were injected i.p. three times at weekly intervals with 2×10^7 viable cells of a cloned bovine lymphoblastoid line infected with *T. parva parva* (Muguga) (cell line code IR.TPM.1, clone D.7). Three or 4 days before fusion, pairs of mice were boosted i.v. with 2×10^6 viable cells from the same cell line. Immune spleen cells were fused to NS1 myeloma cells by using polyethylene-glycol 1550 and hybrids grown as previously described (5). Suitable hybrids were detected by using an indirect immunofluorescence assay. Briefly, cytocentrifuge preparations of cells from line IR.TPM.1 were fixed in acetone, culture supernatants from the hybrid cells were used as the first layer, and a rabbit anti-mouse immunoglobulin (polyvalent) labeled with tetramethylrhodamine isothiocyanate (protein concentration 50 μg/ml) was the second layer. Many culture supernatants bound to intracellular vesicles and cells from such cultures were cloned twice in soft agarose (6). Seven clones of hybrid cells, which arose from separate wells in the original plates, have been used in this study. These monoclonal antibody preparations were taken from cultures that had been seeded at 2×10^5 cells/ml and allowed to grow to 1 to 2×10^6 cells/ml. A single batch of supernatant was used for each antibody; this was divided in small samples and frozen at −20°C.

It was found by using the indirect immunofluorescence assay that the seven monoclonal antibodies bound to intracellular vesicles in the line used for immunization (Table I). None of these antibodies bound to normal peripheral blood lymphocytes from the bovid that the line was derived from; neither did they bind to a bovine lymphosarcoma line (BL-3). They appear, therefore, to bind to *Theileria* parasite or parasite-induced antigens. The staining pattern of these monoclonal antibodies on peripheral blood lymphocytes from a bovid infected with *T. parva parva* (Muguga) was also examined. All seven antibodies showed the typical intracellular vesicle staining, and the number of cells showing positive fluorescence was approximately equal to the number of cells containing macroschizonts detected by Giemsa staining. Erythrocytes infected with the piroplasms stage of the parasite used for immunization did not show immunofluorescence with any of these monoclonal antibodies. The antigens detected by these antibodies, therefore, seem to be restricted, in the mammalian host, to the lymphoblastoid stages.

The binding of these antibodies to *T. parva parva* and *T. parva lawrencei* macroschizonts that originated from different geographic areas was examined. Cells from different macroschizont-infected cell lines and line IR.TPM.1 were fixed, and binding was assessed by indirect immunofluorescence. All cell preparations were examined by combined fluorescence and phase microscopy, and staining of intracellular macroschizonts was assessed. First, supernatants were titrated on cells from line IR.TPM.1, and optimal dilutions were noted. Samples from the same batch of supernatants were then used on the other cell lines at several dilutions identical and in excess of the optimal one for line IR.TPM.1. Slides were read blind; positive control slides of IR.TPM.1 cells and negative control slides from each line receiving NS1 myeloma supernatants as first layer of the assay were each included in each assay. The intensity of staining was graded between 0 and 4 on an arbitrary scale, and the most intense reading is given in Table I.

The macroschizonts in six cell lines were stained by all the monoclonal antibodies. Macroschizonts in one line were stained by only five out of the seven antibodies, and those from two other cell lines were stained by only four (Table I). Macroschizonts in these cell lines also varied in the intensity of staining.

These studies demonstrate that there are antigenic differ-

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TABLE I

Fluorescence intensity obtained with monoclonal antibodies on T. parva macroschizonts from several lymphoblastoid cell lines

Monoclonal Antibody Secreting Cell Lines	*T. parva*-Infected Lymphoblastoid Cell Line[a]								
	IR.TPM.1 (parva, Muguga[b])	E174 (parva, Muguga)	I.R. TPK.1 (parva, Kiambu)	M421 (parva, Mbita)	C379 (parva, Aitong)	IR.TPL.2 (lawrencei, Manyara)	IR.TPL.1 (lawrencei, Serengeti)	K364 (lawrencei, Laikipia)	KB5 (lawrencei, Laikipia)
BT1/11.2.1	2	2	3	2	3	1	3	4	2
BT1/29.6.4	4	4	4	0	1	3	2	0	0
BT1/30.4.10	3	2	3	0	1	1	2	0	0
BT1/34.10.6	4	3	4	4	4	3	3	0	4
BT5/2.2.5	4	4	4	4	4	1	3	4	2
BT5/29.7.9	4	4	4	4	4	1	4	4	1
BT5/35.3.5	4	3	3	4	3	2	3	3	0

[a] All *T. parva* infected lines are from bovid (*Bos taurus*) except for line KB5 which is from a buffalo (*Syncerus caffer*).

[b] The strain of parasite with which these lines are infected are from the various areas of East Africa indicated under the line code.

The assay was repeated 2 to 6 times for each cell line and more than 20 times for the line IR.TPM.1. The variation in fluorescence intensity between repeat assays was minimal.

ences in the macroschizont stage of various strains of *T. parva*. When an antibody gave positive staining with a particular *T. parva*-infected lymphoblastoid line, most macroschizonts in that line were stained. Furthermore, the monoclonal antibodies bound to practically all macroschizonts examined from the peripheral blood cells of a bovid infected with *T. parva*. These observations suggest that it is unlikely that the antibodies recognize antigens that are expressed at a particular developmental stage of the macroschizont. We consider, therefore, that they detect antigenic diversity in the *T. parva* species. It has been debated whether genetic recombination or somatic mutation could be responsible for antigenic diversity in this species (7). By using monoclonal antibodies as markers it may be possible to demonstrate genetic recombination in *T. parva* in an analogous manner to that used by Carter (8) with isoenzyme markers in malaria.

Antigenic differences were detected between K364 and KB5. The cell line KB5 was established from an infected buffalo; several months later parasites from the same buffalo were transferred to a bovine host via the tick vector, and the line K364 was established from the infected bovid. Thus, the antigenic diversity demonstrated here might also reflect antigenic variation within a strain. This interpretation is supported by the work of Young *et al.* (9), who reported that *T. parva lawrencei* parasites isolated from the same buffalo over several months were not necessarily cross-protective.

The significance of the antigenic differences detected by these monoclonal antibodies in terms of vaccination remains to be demonstrated. Some vaccine studies have been conducted with the *Theileria* strains used here: cattle vaccinated with *T. parva lawrencei* (Laikipia, KB5) were inconsistently protected against challenge with *T. parva lawrencei* (Serengeti) (2). It is possible that this is due to KB5 lacking one or more of the antigens recognized by the antibodies BT1/29.6.4, BT1/30.4.10, or BT5/35.3.5. The use of these antibodies, or ones raised subsequently, to type the antigens of *T. parva* strains would provide a rational approach to select suitable vaccine strains. The importance of such antigenic markers for rapid diagnostic and screening tests, especially for the appearance of new types, is self-evident.

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REFERENCES

1. Uilenberg, G. 1976. Tick-borne livestock diseases and their vectors. 2. Epizootiology of tick-borne diseases. World Animal Rev. 17:8.
2. Radley, D. E., A. S. Young, C. G. D. Brown, M. J. Burridge, M P. Cunningham, F. L. Musisi, and R. E. Purnell. 1975. East Coast Fever. 2. Cross-immunity trials with a Kenya strain of *Theileria lawrencei*. Vet. Parasitol. 1:43.
3. Burridge, M. J., C. D. Kimber, and A. S. Young. 1973. Use of the indirect fluorescent antibody technique in serologic studies of *Theileria lawrencei* infections in cattle. Am. J. Vet. Res. 34:897.
4. Lawrence, J. A. 1977. The serological relationship between *Theileria parva* (Muguga) and *Theileria lawrencei* from Rhodesia. Vet. Rec. 100:470.
5. Galfre, G., S. C. Howe, C. Milstein, G. W. Butcher, and J. C. Howard. 1977. Antibodies to major histocompatibility antigens produced by hybrid cell lines. Nature 266:550.
6. Cotton, R. G. H., D. S. Secher, and C. Milstein. 1973. Somatic mutation and the origin of antibody diversity. Clonal variability of the immunoglobulin produced by MOPC 21 cells in culture. Eur. J. Immunol. 3: 135.
7. Levine, N. D. 1971. Taxonomy of the piroplasms. Trans. Am. Microsc. Soc. 90:2.
8. Carter, R. 1978. Studies on enzyme variation in the murine malaria parasites *Plasmodium berghei, P. yoellii, P. vinckei* and *P. chabaudi* by starch gel electrophoresis. Parasitology 76:241.
9. Young, A. S., C. G. D. Brown, M. P. Cunningham, and D. E. Radley. 1978. Evaluation of methods of immunizing cattle against *Theileria lawrencei*. *In* Tick-borne Diseases and Their Vectors. Edited by J. K. H .Wilde. University of Edinburgh. P. 293.