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BOVINE MONOCLONAL ANTIBODIES TO THE F5 (K99) PILUS ANTIGEN OF E. COLI, PRODUCED BY MURINE/BOVINE HYBRIDOMAS

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ABSTRACT

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Lymph node cells from calves immunized with purified pilus antigen of K99[+] enterotoxigenic E. coli (ETEC) were fused with mouse myeloma (NSO) cells, and with non-Ig producing mouse/calf hybridomas or with a bovine Ig-producing mouse/calf/calf secondary hybridoma. Lines secreting bovine monoclonal IgG$_1$ specific for K99 pilus antigen in an ELISA were obtained in each case. The two lines derived from xenohybridoma fusion partners have been secreting anti-K99 bovine monoclonal antibody for over one year in continual passage. None of the antibodies cross-reacted with other pilus types including K88, CFAI, CFAII, 987P or CP; they all inhibited agglutination of horse RBC (which have a K99 receptor) in the presence of K99 antigen; they showed positive fluorescence in an indirect binding assay on K99[+] ETEC and inhibited K99[+] ETEC adhesion to piglet enterocytes. These antibodies have potential prophylactic and therapeutic use in control and treatment of diarrhoea.

1 INTRODUCTION

One of the causative agents of neonatal diarrhoea in calves and other farm animals is non-invasive enterotoxigenic E. coli which have the K99 (now called F5) pilus antigen (K99[+] ETEC) (Smith and Linggood 1972). For disease to develop, gut colonization by bacterial adherence to enterocytes is thought to be essential. Preventive and control measures to date including treatment with antibiotics (Kistwaria, Misra and Choudhuri, 1982) and maternal vaccination programmes (Acres, 1985) have met with varying success. Another treatment is to dose calves with antibodies to K99 pilus antigen, and a murine monoclonal anti-K99 antibody has been used for this purpose (Sherman, Acres, Sadowski, Springer, Bray, Raybould and Muscoplat, 1983).

As an alternative approach, we have investigated the possibility of making bovine monoclonal antibodies to the K99 pilus antigen on the principle that a homologous antibody might ultimately be more effective than a heterologous antibody. In addition, since there are a number of situations in veterinary

pathology where the use of a bovine rather than a murine monoclonal antibody would be advantageous (e.g. in studies of host/parasite relationships or where repeated administration of antibody is required) the present investigation was used as a basis to explore the feasibility of making bovine monoclonal antibodies in general.

Since no suitable bovine myeloma lines are yet available, attempts to produce bovine monoclonal immunoglobulins are at present confined to the use of interspecific hybrids made by fusing mouse myeloma cells with lymphocytes from immunized cattle. This approach has met with some success (Srikumaran, Guidry and Goldsby, 1983, 1984; Tucker, Dain, Clarke and Donker, 1984; Raybould, Crouch, McDougall and Watts 1985). However, one problem with such interspecific hybrids (xenohybridomas) is that like other somatic cell hybrids (Weiss and Green, 1967; Pontecorvo, 1971) they lose the non-murine chromosomes as they divide so that even if at first they secrete immunoglobulins, they usually soon lose the ability to do so. Preliminary experiments at Babraham, indicated that improved stability could be achieved using murine/bovine xenohybridomas (primary xenohybridomas) themselves as fusion partners. First, these had to be grown in the presence of 8-azaguanine to select for amin-opterin sensitivity. They were then used as fusion partners with lymphocytes from immunized calves (Tucker et al 1984; Anderson, Clarke, Stein and Tucker, 1986). The resulting secondary xenohybridomas were further selected and used as fusion partners to produce tertiary xenohybridomas.

The present paper reports the production of bovine monoclonal antibodies to the K99 pilus antigen of non-invasive enterotoxigenic E. coli (K99$^+$ ETEC) using the above fusion and 're-fusion' techniques.

2   MATERIALS AND METHODS

2.1 K99 pilus antigen

Pili production was stimulated by growing E.coli (O9,K(A)?, K99) overnight in Minca medium according to the method of Guinée, Jansen and Agterberg (1976). Their presence was subsequently confirmed by electron microscopy. The pilus antigen was prepared by the method of Altmann, Pyliotis and Mukkur (1982). Briefly, the E.coli were heated at 60°C for 45 min, the suspension blended and the bacteria spun down. The pilus protein was precipitated from the supernatant at pH 4.5 using acetic acid and the precipitate taken up in phosphate buffered saline (PBS), pH 7.2. As the K99 and F41 pilus antigens are frequently co-expressed on bovine K99$^+$ ETEC, the purity of the K99 pilus preparation was confirmed by the fact that 1) on SDS-PAGE a single band of

apparent MW 16.5 K was obtained whereas the F41 antigen has a MW of 29.5 K (De Graaf and Roorda, 1982), 2) the K99[+] ETEC did not agglutinate in a specific slide agglutination test with F41 antisera and 3) the K99[+] ETEC did not haemagglutinate guinea pig red cells in the presence of mannose (De Graaf and Roorda, 1982).

2.2 Immunization

One 18 month old steer (calf 1) was injected on the rump with 14.0 ml (7.0 ml each side) of an emulsion of 4.0 ml Freund's complete adjuvant plus 4.0 ml of a saline suspension of K99 pilus antigen containing 0.27 mg/ml of protein and 6.0 ml of "Span 80" (Sorbitan monooleate, Koch-Light Laboratories Ltd., Colnbrook, Bucks, U.K.). Six weeks later, to stimulate the popliteal lymph node 2.5 ml of a 1:1 mixture of Freund's incomplete adjuvant and a saline suspension of K99 pilus antigen were injected low down on the leg below the node. At the same time, 2.5 ml of the mixture was injected into one rump.

A second 9 month old steer (calf 2) was injected as above with a total of 7.0 ml of a mixture composed of 2.0 ml Freund's complete adjuvant, 2.0 ml of a saline suspension of K99 pilus antigen and 3.0 ml of Span 80. Seven weeks later, 1.0 ml of a 1:1 mixture of Freund's incomplete adjuvant and a saline suspension of K99 pilus antigen was injected below the popliteal lymph node and 1.0 ml into the rump.

The popliteal lymph node was excised from calf 1 on day 3 and from calf 2 on day 4 following the second injection.

2.3 Cell lines

Fusion partners used were mouse myeloma NSO cells (subline of NS1/1Ag4.1), two non-Ig secreting mouse/calf xenohybridomas (53B3 and 54B3) and a bovine IgG1-secreting xenohybridoma (94A1) derived from a 'refusion' of 53B3 (Tucker et al., 1984).

2.4 Cell culture and fusions

The popliteal lymph nodes were chopped in culture medium using a small scalpel. Large lumps of tissue were allowed to settle and lymphocytes in suspension pipetted off and centrifuged and resuspended in culture medium. Lymphocytes were fused with myeloma or xenohybridoma cells at a ratio of 4:1 respectively using 50% (w/v) polyethylene glycol 1500 in RPMI (BDH Chemicals Ltd., Poole, U.K.) by a modification of the method described by McMasters and Williams (1979). Cells were cultured in 24-well plates (Linbro) and seeded at 1×10^6 cells/ml (2.0 ml per well) in a selective medium composed of RPMI 1640 with 10^{-4} M hypoxanthine, 4×10^{-7} M aminopterin, 1.6×10^{-3}M thymidine (Flow Laboratories, Irvine, Scotland) plus 10% horse serum (HS) as described previously (Tucker et al 1984). Control wells of unfused fusion partners and

lymphocytes were set up in each case. Culture supernatants were removed at 14 days post fusion and replaced with fresh medium.

2.5 Cloning

Cells were cloned on soft agar according to the method described by Köhler (1979).

2.6 Antibody detection

A solid phase capture-type enzyme-linked immunosorbent assay (ELISA) was used to detect anti-K99 bovine immunoglobulins (Ig) present in culture supernatants. Flat-bottomed 96-well plates (Dynatech, U.K.) were coated overnight with a 1 in 1000 dilution in bicarbonate buffer (pH 9.6) of rabbit anti-K99 antibody (RAK99) which was used as the capture antibody. This had been absorbed against enterotoxigenic E. coli (ETEC) which, after overnight growth at 18°C, no longer expressed the K99 antigen (Altmann et al., 1982). After washing the plates three times in phosphate buffered saline containing 0.05% Tween 20 (Sigma Chemical Company Ltd., Poole, Dorset), PBST, a 1 in 100 dilution of the K99 antigen preparation in PBST was added for 1 hour at 32°C. The plates were then washed three times in PBST and the culture supernatants were added and incubated for 2 hours at 32°C. After three washes in PBST, a 1 in 5000 dilution in PBST of the conjugate was added and left for another hour.

The conjugate used was alkaline phosphatase-conjugated rabbit antibody specific for bovine heavy and light Ig chains (Jackson Immunoresearch Laboratories, Avondale Pennsylvania). The plates were then washed three times in PBST and developed using p-nitrophenyl phosphate (Sigma) in diethanolamine (Fisons plc, Loughborough, U.K.) buffer pH 9.8 at 1 mg/ml. Optical densities were read with a multi-skan automatic reader at 405 and 519 nm absorbance. Culture supernatants were replaced by PBS and culture medium as negative controls. Serum taken from the immunized calf at slaughter was used as a positive control.

2.7 Haemolysis tests

Supernatants from cell lines derived from fusions using xenohybridoma 94A1 were tested for anti-Forssman activity in a standard haemolytic test against sheep red blood cells as described previously (Tucker et al. 1984).

2.8 Isotype characterization

Immunoglobulins were precipitated from culture supernatants using a 1:1 volume of 50% saturated ammonium sulphate by the method of Jonak (1980). The precipitate was collected by centrigation at 48000 x g for 30 min, dialyzed against 0.2 M NaCl and finally concentrated to approximately 25 times the original culture supernatant volume on a macrosolute concentrator (Amicon, Gloucestershire, U.K.). Culture medium was included as a negative control in

all tests. The Ig class was determined by immunodiffusion using antisera specific for bovine IgG_1, IgG_2 and IgM. Alternatively, Ig molecules were internally labelled by growing 2×10^6 cells in 1.0 ml of lysine-free medium (Gibco Ltd. Middlesex, U.K.) for 16 - 20 hr in the presence of 5 µCi ^{14}C-lysine monohydrochloride (Radiochemical Centre, Amersham, U.K.). The culture supernatant proteins were then separated by SDS-PAGE electrophoresis, followed by autoradiography.

2.9 Specificity tests

ELISA

The pilus antigens K88, CFAI, CFAII, 987P and CP (reviewed Parry and Rooke, 1984) were tested in capture-type assays against culture supernatants. Plates were coated with capture antibodies of the appropriate specificity in each instance.

2.10 Antibody binding to $K99^+$ ETEC

1:1 volumes of culture supernatant and a saline suspension of $K99^+$ ETEC were incubated at room temperature (RT°C) for 30 min. The mixture was spun for 10 min at 3000 g to pellet the $K99^+$ ETEC. The pellet was resuspended and washed 3 times in PBS. An equal volume of FITC-conjugated anti-bovine Ig (Wellcome Research Laboratories, Beckenham, Kent) was added at a dilution of 1 in 40 in PBS for 30 min at RT°C. The solution was again spun for 10 min at 3000 g and the pellet resuspended and washed 3 times with PBS. Samples were then viewed for fluorescence under U.V. light. Controls in which culture medium or PBS replaced culture supernatant were also included.

2.11 Inhibition of $K99^+$ ETEC adhesion to piglet enterocytes

Enterocytes from 12-17 day old piglets, prepared according to the method of Weiser (1973), were stored in liquid nitrogen until use. Adhesion assays were carried out broadly according to the method of Evans, Wrigglesworth, Burdett and Pover, (1971). 1:1 volumes of culture supernatant and $K99^+$ ETEC suspension were incubated in microtitre plates for 10 min at RT°C and then an equal volume of enterocyte suspension was added. The mixture was allowed to incubate for a further 10 min at RT°C. Samples were then viewed by interference microscopy for adhesion of $K99^+$ ETEC to enterocytes. Controls included PBS or complete culture medium as a substitute for culture well supernatants.

2.12 Inhibition of $K99^+$ ETEC agglutination of horse red blood cells (HRBC)

$K99^+$ ETEC cause agglutination of HRBC which have a receptor for the K99 pilus antigen (Gaastra and de Graaf, 1982). A working dilution of the $K99^+$ ETEC suspension was chosen that just gave agglutination of a 3-5% (final concentration) suspension of HRBCs at 4°C. 25 µl of the $K99^+$ ETEC suspension

and 25 µl of culture supernatant or culture medium (negative control) were left together for 30 min at RT°C.   25 µl of a 10% suspension of HRBCs was added and the plates were shaken at 4°C.   Positive or negative haemagglutination was recorded in each case.

2.13 Chromosome analysis

Metaphase spreads of all hybridomas and parental cell lines were made according to established procedures (Moorhead, Nowell, Mellman, Battips and Hungerford 1960).   G-banding was carried out using the method of Lin, Newton and Church (1977).
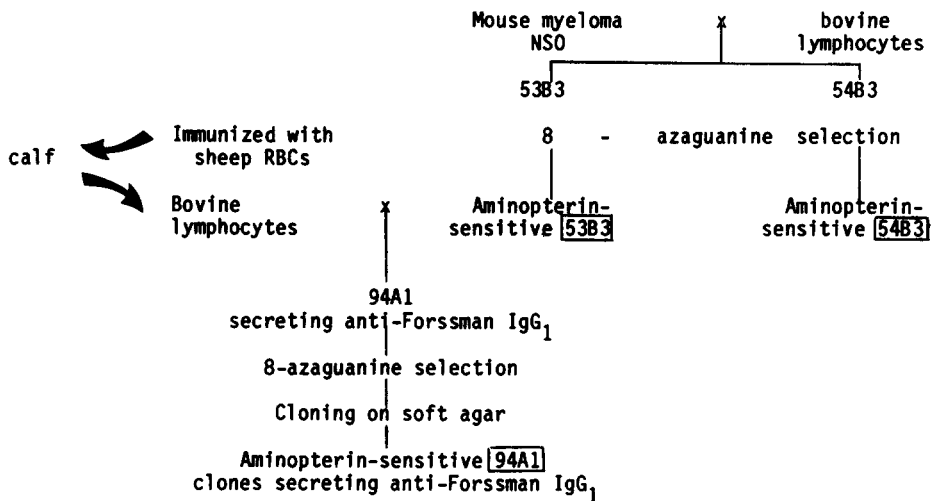
3   RESULTS

Fig. 1.   Derivation of aminopterin-sensitive fusion partners

Fig. 1 summarises the derivation of the aminopterin-sensitive fusion partners used in the present study.   They have been described in detail previously (Tucker et al. 1984).   NSO was the original mouse myeloma line; 53B3 and 54B3 were non-Ig secreting primary xenohybridomas.   94A1 was a cloned secondary xenohybridoma, derived from a 're-fusion' of 53B3 and secreted a bovine IgG_1 of Forssman specificity.

3.1 Fusion efficiency

The above fusion partners were used in six fusions with lymph node cells from two steers which had been immunized with K99 pilus antigen (Table 1).
In all 6 fusions, distinct hybridoma colonies were visible in all culture

plate wells within 7 days after fusion. There was no obvious difference
between the fusions in the mean number of colonies per well; in the case of
54B3, the relatively low number of colonies was probably an underestimate as
many were in suspension thus making counting difficult.

TABLE 1
Fusion details

FUSION PARTNER	Calf 1			Calf 2		
	NSO m N.S	53B3 mxc N.S.	94A1 mxcxc S	53B3 mxc N.S.	54B3 mxc N.S.	94A1 mxcxc S
Total No. of wells screened	144	138	120	288	240	216
Mean No. colonies per well	7	10	7	8	4	8
% No. wells with anti-K99 activity at 21 days after fusion	12	13	22	10	7	17
Lines followed	A			B	C	D and E

m   = mouse
c   = calf
s   = secretor of bovine Ig
N.S. = non-secretor of murine or bovine Ig

3.2 Screening for anti-K99 activity

By 21 days post fusion every supernatant from wells containing fused cells
had an O.D. reading in the K99 ELISA test much higher than that given by RPMI
+ 20% HS alone. Some of this activity could be attributed to residual antibody
production by unfused lymphocytes as judged by the O.D. values obtained for
supernatants from wells with lymphocytes alone. Colonies were therefore picked
out for further study only from those wells that had O.D. values clearly higher
than this 'background' level. Fig. 2 gives an example of O.D. readings
obtained for one culture plate 21 days post fusion. RPMI plus 20% HS gave O.D.
values of 0.05 whereas lymphocyte control wells gave values of approximately
0.35. In order to compare the efficiency of the different fusion partners in
giving rise to positive wells, an arbitrary assessment of the number of
positive wells was made by assuming only those wells with O.D. values 25% above
the mean O.D. value for the plate were positive. By this criterion, only wells
A6, B4 and C2 in Fig. 2 would be considered positive. The values for
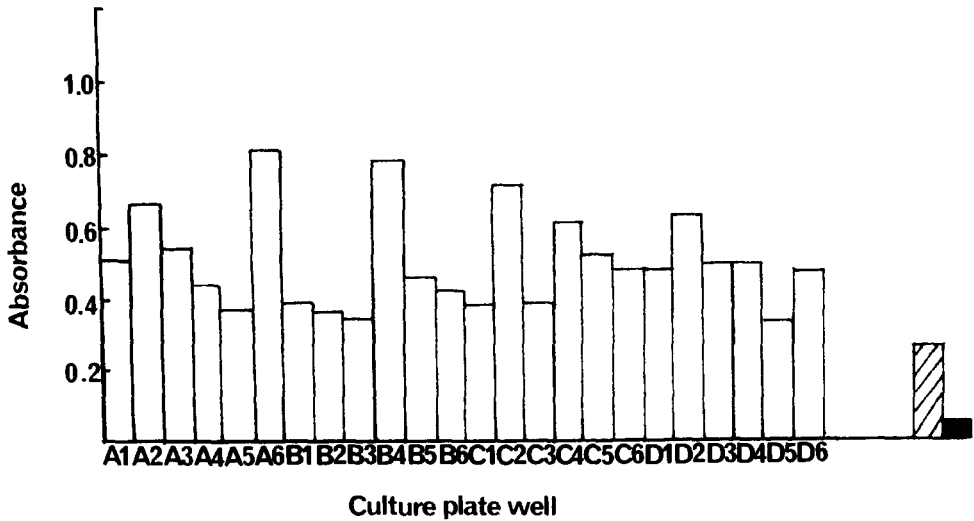percentage number of wells with anti-K99 activity given in Table 1 were

Fig. 2. Bar diagram showing example of range of O.D. readings in a K99 ELISA screening assay from one 24-well culture plate at 21 days post fusion. (RPMI + 20% HS, Black column; Culture supernatant from lymphocyte control well, hatched column).

based on this "rule of thumb" assessment and therefore probably are an underestimate of the actual number of positive wells. Every fusion gave rise to colonies producing anti-K99 antibodies, the highest number of positive wells was obtained when 94A1 was the fusion partner (22%). In the 94A1 fusions, at 21 days, supernatants from all wells showed anti-Forssman activity. Control ELISA tests confirmed, however, that this antibody did not affect the anti-K99 assay.

3.3 Cloning and stability

306 colonies from wells positive for anti-K99 were picked out into separate wells at 4 weeks post fusion. After culturing for two weeks, 35 lines were still positive in the anti-K99 assay; 26 of these lines were derived from fusions which had used 94A1 as the fusion partner, and one of these showed both anti-Forssman and anti-K99 activity. Five lines with the highest O.D. values were selected for cloning. One line (line A, Table 1) was derived from (NSO), one (line B) from 53B3, one (line C) from 54B3 and two came from 94A1. One of the latter (line D) secreted both anti-Forssman and anti-K99, the other (line E) had lost its anti-Forssman activity. Table 2 shows the percentage of positive clones obtained from each line assayed at

approximately 2-3 weeks after cloning.   All clones of line D had retained anti-Forssman activity but none was positive for anti-K99.   On the other hand, all 96 clones isolated from line E secreted anti-K99.

One cloned line from A was selected for further study but lost anti-K99 activity after 7 months in culture.   A cloned line from C and E was cultured for several weeks and then recloned.   50 clones were tested and 77% of C and 98% of E subclones secreted anti-K99 antibody.   One positive subclone each from C and E has maintained secretion of antibody for more than one year in continual passage.

TABLE 2
Cloning efficiency of anti-K99 secreting lines

Line	Derivation	No. of clones assayed	% clones positive for anti-K99	% clones positive for anti-Forssman
A	NSO	30	66	NA
B	53B3	60	20	NA
C	54B3	54	72	NA
D	94A1	60	0	100
E	94A1	96	100	0

NA = not applicable

The ELISA antibody titres of clone A and subclone C and E supernatants (cell density 2×10^5/ml) were assessed periodically.   These were greater than or equal to 512 at 10 weeks post cloning or subcloning.   This titre represented an Ig secretion rate of approximately 5-10 μg/10^6 cells/ml.   After over a year in continual passage the titre of subclones C and E has dropped to 32.

3.4 Chromosome analysis

Metaphase spreads of the xenohybridomas showed the presence of bovine chromosomes. The number of chromosomes in 25 cells from each line was counted and bovine chromosomes distinguished from murine by their clear centromeric regions (Fig. 3).   The number of bovine chromosomes in 53B3, 54B3 and 94A1 agreed with those previously reported (Tucker et al., 1984), 53B3 (mxc) having 7%, 54B3 (mxc) 8% and 94A1 (mxcxc) 14% bovine chromosomes.   Cloned line A (mxc) had 13% bovine chromosomes when counted 2 months after fusion.   A feature of the re-cloned hybridomas from C and E (mxcxc and mxcxcxc) was the high proportion of polyploid (4 n) cells; line C had 15% bovine chromosomes (18.57 ± 4.44 in a total of 124.63 ± 13.13 (4n)) and line E 18-19.5% bovine chromosomes (26.13 ± 4.47 in a total 134.33 ± 7.23 (4n) and 14.33 ± 2.74 in a total of 78.0 ± 8.14 (2n)).
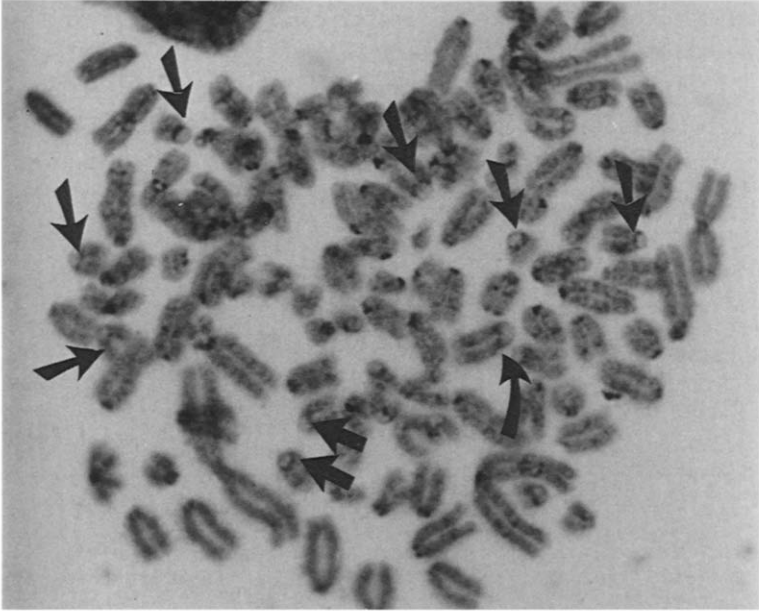
Fig. 3 (x100) G-banded metaphase spread of a tertiary xenohybridoma. 20 bovine chromosomes some of which are indicated (↓), have been identified in this spread in a total of 112 chromosomes. (Calf (2n) = 60; NSO (2n) = 65).

3.5 Antibody characterization and specificity

Concentrated supernatants from lines A, C and E were shown to be of IgG_1 isotype by immunodiffusion tests. The class was confirmed by autoradiography on SDS PAGE gels (Fig.4).

Fusion partners NSO, 53B3 and 54B3 showed no Ig chains, whereas 94A1 had a distinct γ chain but only a faint light chain. Differences in MW were apparent between individual light and heavy chains.

Culture supernatants from A, C and E were tested by ELISA against K88, CFA I, CFA II, 987P and CP pilus antigens. They showed no cross-reactivity.

Culture supernatants from lines A, C and E inhibited the agglutination of horse red cells by K99[+] ETEC. Culture medium alone and supernatants from cultures that had ceased to produce anti-K99 antibody did not inhibit agglutination.

Using an FITC labelled rabbit anti-bovine IgG second antibody, supernatants from lines A, C and E were shown to bind to K99[+] ETEC. Control samples showed no fluorescence.

Supernatants from lines A, C and E inhibited adhesion of K99[+] ETEC to piglet enterocytes. Control samples did not inhibit (Fig. 5).
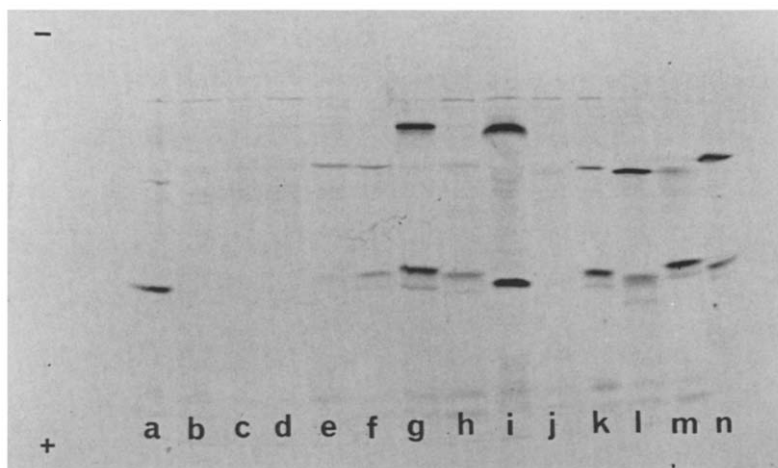
Fig. 4   Autoradiograph of SDS-PAGE of ^{14}C-labelled Ig chains in culture supernatants.
Fusion partners NSO, 53B3 and 54B3 showed no evidence of Ig chain secretion (b-d) whereas 94A1 showed a γ chain and a very weak light chain (e).   The anti-K99 secreting lines A and E show evidence of γ and light chain secretion (k-1).   Controls include mouse monoclonal IgG and M (a & i respectively), bovine monoclonal IgG$_1$ (f,h,n), bovine monoclonal IgG$_2$ (m) and bovine monoclonal IgM (g).   Supernatant from a non-secreting secondary mouse/bovine hybridoma is also included (j).

Fig. 5(a) (x40).   Inhibition of adhesion of K99+ ETEC to piglet enterocytes in the presence of anti-K99 bovine monoclonal antibody.
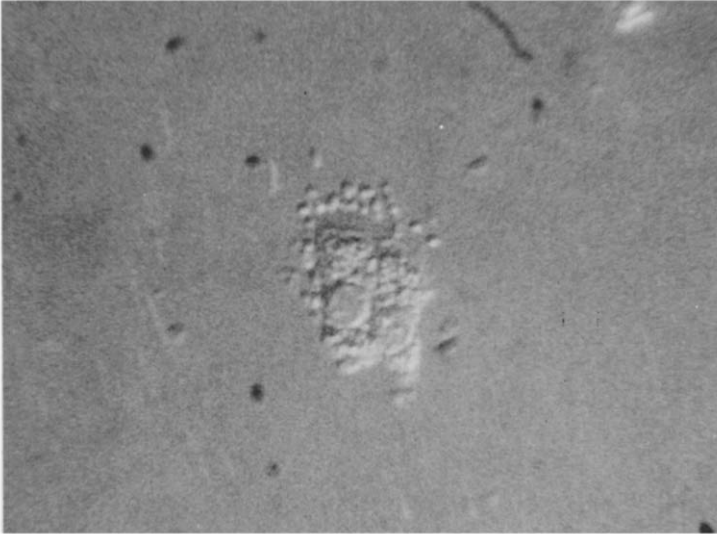
Fig. 5(b) (x40). Adhesion of K99$^+$ ETEC to piglet enterocytes

4   DISCUSSION

Preliminary experiments with murine/ovine hybridomas indicated that the use of such xenohybridomas might offer a feasible approach for the production of ruminant monoclonal antibodies (Tucker, Dain, Wright and Clarke, 1981).

The first reports of the production of monoclonal bovine immunoglobulins by primary xenohybridomas were by Srikumaran et al (1983, 1984). However, these were of unknown specificity.   Raybould et al. 1985 used this technique to produce a bovine monoclonal antibody to bovine enteric coronavirus; this line was stable for at least 3 months.   Tucker et al, 1984 reported the production of a bovine monoclonal antibody to sheep red cells (anti-Forssman) using a xenohybridoma fusion partner. This line has been secreting antibody for over three years in almost continual culture.

The initial reasoning behind the use of xenohybridomas rather than mouse myeloma cells as fusion partners was that they might retain more bovine chromosomes on re-fusion, thereby increasing the chances of retaining the chromosomes relevant for Ig production (Tucker et al 1984).   The present results substantiate this hypothesis in that the use of xenohybridomas as fusion partners resulted in stable lines with an increased proportion of bovine: mouse chromosomes (from 8% to 15% bovine chromosomes in line C and from 14% to 18 and 20% in line E).   The cloned line derived from NSO retained

13% bovine chromosomes and this may be the reason this particular clone was able to maintain antibody secretion for as long as 7 months, before ceasing production. Further chromosomal analysis on more lines will have to be carried out before firm conclusions can be drawn and this will involve identification of individual bovine chromosomes. Earlier results using xenohybridomas indicated that the loci for Ig chain production may be on chromosomes 12 and 22 in the bovine (Tucker et al. 1984); further studies of these lines may help to confirm this assignment.

Reports of the use of mouse x human hybridomas as fusion partners to produce human monoclonal antibodies have had conflicting results (Teng, Lam, Piera and Kaplan 1983; Ostberg and Pusch, 1983; Murphy, Webb, Earle, Russ, Churcher, Tait and D'Aspice, 1986). The latter showed that the failure of primary mouse x human fusions was largely due to a higher death rate of xenohybrids. This was certainly not the case in our studies where primary xenohybridomas often showed just as prolific a growth as mouse x mouse hybridomas. Murphy et al. (1986), also found that secondary xenohybrids continued to survive although they ceased immunoglobulin synthesis. They therefore concluded that this was 'a step in the right direction' towards production of stable lines secreting human monoclonal antibodies. Another factor in assessing stability of the hybridoma lines is the number of times it is necessary to reclone. Raybould et al (1985) cloned only once whereas Srikumaran et al (1983,1984) carried out three early clonings. In our general experience with xenohybridomas it is advantageous to clone as soon as possible after positive colonies have been identified; subsequent further clonings may be necessary, but this will vary from clone to clone.

The subunit composition of the K99 pilus antigen has been the subject of much debate (reviewed Parry and Rooke, 1984). In particular, the question has been raised as to whether or not the haemagglutinin and adhesin on the pilus are on separate sub-units. The fact that the pilus preparation used as the immunogen in the present study gave a single band on SDS-PAGE (approximately MW 16.5 K) and gave rise to monoclonal antibodies that inhibited both adhesion and haemagglutination probably indicates that the molecules on the K99 pilus antigen involved in adhesion and haemagglutination are identical or similar. The monoclonal antibodies should provide useful tools for further studies of the epitopes and receptors involved in the etiology of E. coli infections. Furthermore, the production of homologous monoclonal bovine anti-idiotypic antibodies now becomes possible.

The murine/bovine hybridoma technique described here offers considerable potential for the development of other monoclonal antibodies relevant to the field of veterinary science. To date, using this technique we have produced lines secreting bovine monoclonal antibodies (IgG_1, IgG_2, or IgM) to bovine red cell alloantigens, (Tucker, Métènier and Clarke, 1987) as well as to other bovine pathogens.

5   REFERENCES

Acres, S.D., 1985.   Enterotoxigenic Escherichia coli infections in newborn calves: a review.   J. Dairy Sci. 68: 229-256.

Altmann, K., Pyliotis, N.A. and Mukkur, T.K.S., 1982.   A new method for the extraction and purification of K99 pili from enterotoxigenic Escherichia coli and their characterization. Biochem. J. 20: 505-513.

Anderson, D.V., Clarke, S.W., Stein, J.M. and Tucker, E.M., 1986.   Bovine and ovine monoclonal antibodies to erythrocyte determinants, produced by interspecific (hetero-) myelomas. Biochem. Soc. Trans. 14: 72-73.

De Graaf, F.K. and Roorda, I., 1982.   Production, purification and characterization of the fimbrial adhesive antigen F41 isolated from calf enteropathogenic Escherichia coli strain B41M. Infect. Immun. 36: 751-758.

Evans, E.M., Wrigglesworth, J.M., Burdett, K. and Pover, W.F.R. 1971.   Studies on epithelial cells isolated from guinea pig small intestine.   J. Cell Biol. 51: 452-464.

Gaastra W. and de Graaf, F.K., 1982.   Host-specific fimbrial adhesins of non-invasive enterotoxigenic Escherichia coli strains.   Microbiol. Rev. 46: 129-161.

Guinèe, P.A., Jansen, W.H. and Agterberg, C.M., 1976.   Detection of the K99 antigen by means of agglutination and immunoelectrophoresis in Escherichia coli isolates from calves and its correlation with enterotoxigenicity. Infect. Immun. 13: 1369-1377.

Jonak, Z.L., 1980.   Isolation of monoclonal antibodies from supernatant by $(NH_4)_2$ SO_4 precipitation   In: Monoclonal Antibodies. Hybridomas: A new dimension in Biological Analyses.   Roger H. Kennett, Thomas F. McKearn and Kathleen B. Bechtol (Editors), pp 405-6, Plenum Press, New York.

Kistwaria, K., Misra, S.K. and Choudhuri, P.C., 1982.   A study of the serology, pathogenicity and drug sensitivity of Escherichia coli in a group of diarrhoeic, neonate buffalo calves.   Sri Lanka Vet. J. 30: (2) 14-18.

Köhler, G. (1979).   In Immunological Methods. I.Leftkovitz & B. Pernis (Editors), Academic Press. New York. pp. 397-401.

Lin, C.C., Newton, D.R. and Church, R.B., 1977.   Identification and nomenclature for G-banded chromosomes. Can. Genet. Cytol. 19: 271-282.

McMasters, W.R. and Williams, A.F., 1979.   Identification of Ia glycoproteins in rat thymus and purification from rat spleen. Eur. J. Immunol. 9: 426-433.

Moorhead, P.S., Nowell, P.C., Mellman, W.J., Battips, D.M. and D.A. Hungerford 1960. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp. Cell Res. 20: 613-616.

Murphy, S.M., Webb, G.C., Earle, M.E, Russ, G.R., Churcher, H., Tait, B.D. and d'Apice, A.J.F., 1986. Human monoclonal antibody production by xenohybridomas. Trans. Proc. Vol. XVIII No. 2 (April): 343-346.

Ostberg, L. and Pusch, E., 1983. Human x (Mouse x Human) Hybridomas stably producing human antibodies. Hybridoma 2, no.4: 361-367.

Parry, S.H. and Rooke, D.M., 1984. Adhesins and colonization factors of E.coli In: The Virulence of E.coli. Chapt 4.: 79-153. Soc. Gen. Microbiol.

Pontecorvo, G. (1971). Induction of directional chromosome elimination in somatic cell hybrids. Nature. Lond. 230, 367-369.

Raybould, T.J.G., Crouch, C.F., McDougall, L.F. and Watts, T.C., 1985. Bovine-murine hybridoma that secretes bovine monoclonal antibody of pre-defined specificity. Am. J. Vet. Res. 46: No. 2 426-427.

Sherman, D.M., Acres, S.D., Sadowski, P.L., Springer, J.A., Bray, B., Raybould, T.F.G. and Muscoplat, C.C., 1983. Protection of calves against fatal enteric colibacillosis by orally administered Escherichia coli K99-specific monoclonal antibody. Infect. Immun. 42: No. 2 653-658.

Smith, H.W. and Linggood, M.A., 1972. Further observations on Escherichia coli enterotoxins with particular regard to those produced by atypical piglet strains and by calf and lamb strains: the transmissible nature of these enterotoxins and of a K antigen possessed by calf and lamb strains. J. Med. Micr. 5: 243-250.

Srikumaran, S., Guidry, A.J. and Goldsby, R.A, 1983. Bovine x mouse hybridomas that secrete bovine immunoglobulin G^1. Science 220: 522-523.

Srikumaran, S., Guidry, A.J. and Goldsby, R.A. 1984. Production and characterization of monoclonal bovine immunoglobulins G_1, G_2 and M from bovine x murine hybridomas. Vet. Immunol. Immunopath. 5: 323-342.

Teng, N.N.H., Lam, K.S., Riera, F.C. and Kaplan, H.S. 1983. Construction and testing of mouse-human heteromyelomas for human monoclonal antibody production. Proc. Natl Acad. Sci. U.S.A. 80: 7308-7312.

Tucker, E.M., Dain, A.R., Clarke, S.W. and Donker, R., 1984. Specific bovine monoclonal antibody produced by a re-fused mouse/calf hybridoma. Hybridoma 3: 171-176.

Tucker, E.M., Dain, A.R., Wright, L.J. and Clarke, S.W., 1981. Culture of sheep x mouse hybridoma cells in vitro. Hybridoma 1: (1), 77-86.

Tucker, E.M., Clarke, S.W. and Méténier, L. 1987. The use of murine/bovine xenohybridomas in the production of bovine monoclonal antibodies to bovine blood group determinants. Animal Genetics (in press).

Weiser, M.M. 1973. Intestinal epithelial cell surface membrane glycoprotein synthesis. J. Biol. Chem. 248: 2536-2541.

Weiss, M.C. and Greene, H. (1967) Human-mouse hybrid cell lines containing partial complements of human chromosomes and functioning human genes. Proc. Natl Acad. Sci. U.S.A. 88: 1104-1111.