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Immune responses of cattle to *Theileria parva* (East Coast fever): specificity of cytotoxic cells generated *in vivo* and *in vitro*

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Summary. Examination of the specificity of cytotoxicity generated *in vitro* and *in vivo* against infected bovine lymphoblasts revealed that cytotoxic T lymphocytes (CTL) obtained from cattle immune to *Theileria parva* recognized parasite-induced alterations associated with major histocompatibility complex (MHC) antigens on the membrane of infected autologous cells. By comparison, cytotoxicity generated *in vitro* in an autologous Theilerial-lymphocyte culture (AuTLC) contained both CTL and activity akin to that of natural-killer (NK) cells. The addition to the AuTLC of 2 inhibitors of glycosylation, tunicamycin (Tun) and 2-desoxy-D-glucose (2-DOG) abolished both the proliferative response and the generation of cytotoxicity. While the addition of Tun or 2-DOG in conventional cell-mediated lympholysis (CML) assays did not modify the effector function of cytotoxic cells, pretreatment of target cells with either compound prevented lysis by CTL, but not by NK cells. Although parasite-induced antigens have not been purified from infected bovine lymphoblasts, the present study indicated that these are likely to be glycoprotein or carbohydrate in character, and that their recognition on autologous cells is a consistent feature of CTL from immune cattle.

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INTRODUCTION

During infection of cattle with the protozoan agent *Theileria parva*, the schizogenous form of the parasite (the macroschizont) resides within the cytoplasm of transformed bovine lymphocytes and can divide synchronously with the host cell (Hulliger, Wilde, Brown & Turner, 1964). Infected lymphoblasts from clinical cases (Malmquist, Nyindo & Brown, 1970) or normal bovine peripheral blood leucocytes (PBL) which have been transformed by incubation with sporozoites of *T. parva* can be cultivated continuously *in vitro* as parasitized cells (Brown, Stagg, Purnell, Kanhai & Payne, 1973). These initiate proliferative responses of mixed lymphocyte (MLR) type in normal autologous PBL (Pearson, Dolan, Stagg & Lundin, 1979; Emery & Morrison, 1980), from which genetically-restricted and non-specific cytotoxicity has emerged (Pearson *et al.*, 1979; Eugui & Emery, 1981). Recently Pearson, Hewett, Roelants, Stagg & Dolan (1982) confirmed in blocking studies, components of both types of cytotoxicity from *in vitro* MLRs. In contrast, only genetically-restricted cytotoxic T lymphocytes (CTL) appear among PBL of animals convalescent from, or immune to infection with, *Theileria parva* (Emery, Eugui, Nelson & Tenywa, 1981a; Emery, Tenywa & Jack, 1981c; Eugui & Emery, 1981). Such cytotoxic responses appear to provide a major mechanism of resistance against reinfection with the homologous isolate of the parasite (Emery, Morrison, Nelson & Murray, 1981b).

The genetic restriction of bovine CTL generated *in vivo* suggests recognition of parasite-induced alterations to antigens determined by or associated with the major histocompatibility complex (MHC). While the nature of the antigenic changes for *T. parva* are unknown, membrane-associated viral glycoproteins have been implicated as targets for murine CTL during infections with ectromelia, lymphocytic choriomeningitis (Jackson, Ada, Hapel & Dunlop, 1976) and herpes simplex viruses (Lawman, Courtney, Eberle, Schaffer, O'Hara & Rouse, 1980). The present study compared the specificities of CTL derived *in vivo* and *in vitro* against *T. parva*, with particular emphasis being directed towards analysis of the nature of the antigenic changes induced by *T. parva* on membranes of infected lymphocytes and their role in the initiation and effection of cell-mediated immunity. The results indicate that the majority of cytotoxic activity generated *in vitro* is genetically-restricted and that glycosylation of membrane determinants is intimately involved in the immunogenicity of autologous parasitized lymphoblasts.

MATERIALS AND METHODS

Cattle

Hereford steers aged 4–9 months and weighing approximately 100–150 kg were housed in insect-proof isolation units and fed concentrate rations. All cattle were screened monthly as negative for the presence of antibodies to *Theileria, Trypanosoma, Babesia* and *Anaplasma* species.

Infection and Immunization

For lethal infections, 1·0 ml of *T. parva* (Muguga), ILRAD stabilate IRPOI 001/v was inoculated subcutaneously in the neck. The origin of this stabilate has been described previously (Morrison, Buscher, Murray, Emery, Masake, Cook & Wells, 1981). For immunization, a lethal dose of stabilate was inoculated concurrently with a single intramuscular dose of long-acting tetracycline (Terramycin-LA, Pfizer, Sandwich, Kent, U.K., batch 803-53021-1) at a dose rate of 20 mg/kg body weight as recommended by Radley, Brown, Cunningham, Kimber, Musisi, Purnell, Stagg & Young (1975).

Separation of PBL from bovine blood

Peripheral blood leucocytes were isolated from blood containing 10 i.u./ml heparin (Novo Industrie A/S, Copenhagen, Denmark) or from difibrinated bovine blood by density centrifugation (Emery & McCullagh, 1980a). Leucocytes harvested from the Ficoll-paque (Pharmacia, Uppsala, Sweden) were washed twice in RPMI 1640 culture medium (Flow Labs, England) supplemented with 10% (v/v) heat-inactivated foetal bovine serum (FBS, Flow Labs, England, batch 29041104), 10^{-4} M 2-mercaptoethanol (Sigma Chemical Co., St. Louis, Mo. U.S.A.), 10 mM HEPES buffer, 100 i.u./ml penicillin, 100 μg/ml streptomycin and 20 μg/ml glutamine. For some studies PBL were cryopreserved and later thawed and reconstituted according to the methods of Holden, Oldham, Ortaldo & Herberman (1976).

Preparation of Lymph Node Cells (LNC)

Lymph nodes were surgically removed from cattle & suspensions of LNC prepared in RPMI 1640 culture medium containing 0·7% w/v ethylenediamine tetracetic acid (EDTA) as described previously (Emery & Morrison, 1980).

Transformed lymphocytes used for assays

Parasitized lymphoblastoid cells. Normal PBL from eight calves were transformed *in vitro* after cultivation with sporozoites of *T. parva* (Muguga) following the method described by Brown *et al.* (1973). Parasitized cells used for cultures and cytotoxicity assays were prepared from the exponential phase of cell growth (1 day after passage) of 100–200 day old cultures and were greater than 90% parasitized by macroschizonts of *T. parva*. Macroschizonts were detected by direct immunofluorescence as previously described (Emery & Morrison, 1980) and the macroschizont index (MSI) was expressed as the percentage of parasitized lymphocytes.

Bovine lymphosarcoma cells. Cultured bovine lymphosarcoma cells (BL-3) were originally established from biopsies of a clinical case of the disease in Kenya and have been maintained *in vitro* for in excess of 3 years. The cells were kindly provided by Dr C. G. D. Brown of the Kenya Agricultural Research Institute. Bovine leucosis virus (BLV) has not been detected in BL-3 cells to date.

Autologous lymphoblasts. Lymphoblasts were harvested from 3-day cultures of PBL which had been incubated at a final concentration of 5×10^6 per ml with 20 μg/ml concanavalin A (Con A-Industrie

Biologique, Clindy, France) (Emery and Morrison, 1980).

Incubation of parasitized leucocytes with various compounds

Three compounds were used to treat either LNC and PBL from infected cattle, or parasitized lymphoblasts from cultures *in vitro*. Tunicamycin (Tun), a gift from Dr R. Hamill, (Eli Lilly and Co., Indianapolis, Ind., U.S.A.), was used at final concentrations of 0·1 and 0·5 μg/ml, and 2-desoxy-D-glucose (2-DOG; Serva, Heidelberg, FDR) was used at a final concentration of 0·1% (w/v). Compound 993C (Wellcome) was used at a final concentration of 0·1 mg/litre as recommended by McHardy (1978). For each treatment, infected cells were incubated at a concentration of 10^6 per ml on feeder layers derived from bovine aortic endothelium and kindly supplied by Mr R.T. Nelson at this Institute. Cells were incubated in complete RPMI tissue culture medium (see above) for either 2 days (Tun and 2-DOG) or 4 days (993C) at 37° in a humidified atmosphere of 5% carbon dioxide in air.

Mixed leucocyte reaction (MLR), autologous (Au) and allogeneic (Al) Theilerial-lymphocyte cultures (TLC)

The proliferative responses which proceed from cultured mixtures of parasitized lymphoblasts (stimulators) and normal autologous or allogeneic PBL (responders) differ from conventional MLRs in that secondary responses *in vitro* are not accelerated and sera active against lymphocyte-defined antigens are not inhibitory (Lalor & Emery, unpublished data). Hence the reaction has been termed an autologous (Au) or allogeneic (Al) Theilerial-lymphocyte culture (TLC). The MLR, AuTLC and AlTLC were conducted as described previously (Emery & McCullagh, 1980b; Emery *et al.*, 1981a). Briefly, to assay proliferative responses, 100 μl of a final concentration of 5×10^6 responder PBL per ml were added to an equal volume of infected LNC or PBL (*in vivo*) or parasitized lymphoblasts (*in vitro*). These stimulator cells were suspended at concentrations of 5×10^6, $2{\cdot}5 \times 10^6$ and 10^6 per ml respectively after having been incubated with mitomycin C, 25 μg/ml (Sigma Chemical Co., St. Louis, Mo., U.S.A.) for 30 min at 37°. The culture was incubated for 4 days at 37° in a humidified atmosphere (Emery & Morrison, 1980) and the stimulation index (SI) was calculated from the formula: SI = (c.p.m. in test wells)/(c.p.m. of PBL in medium).

For the generation of cytotoxic cells in the cultures, 1 ml of stimulator cells containing 5×10^5 parasitized lymphoblastoid cells was added to 1 ml of responder PBL (5×10^6 per ml) in wells of a culture plate (24 × 16 mm, Linbro Scientific, Conn., U.S.A.). The cultures were incubated for 5 days at 37° in a humidified atmosphere of 5% carbon dioxide in air as described previously (Emery *et al.*, 1981a).

Cell-mediated lympholysis (CML) assays

The cytotoxicity assay employed the release of 51-chromium over a 4 hr period in a standard technique (Pearson *et al.*, 1979). The results were expressed as the percentage specific lysis calculated from the formula: %lysis = 100 × (c.p.m. in test − c.p.m. in spontaneous release)/(c.p.m. in total release − c.p.m. in spontaneous release)

The CML competitive inhibition assay was conducted with various unlabelled parasitized and normal leucocytes (blocking targets). For the assay, 100 μl of effector PBL computed at effector:labelled target ratios of 40:1, 20:1, 10:1 and 5:1 were added in duplicate to 100 μl of blocking targets computed at blocking:labelled target ratios of 10:1, 5:1, 2·5:1 and 1·25:1 in all combinations. The mixture was incubated at 37° for 10 min before 50 μl of labelled target cells were added to each well. After a further incubation for 4 hr, 100 μl of supernatant were removed from each well and the % lysis computed as above.

Experimental design

Normal PBL from ten calves were transformed *in vitro* by *T. parva* (Muguga) into parasitized lymphoblastoid cell lines. Four calves were inoculated with a lethal dose of *T. parva* (Muguga) after normal PBL had been separated from defibrinated blood and cryopreserved. Nine days later, frozen PBL were reconstituted and cultured in AuTLC with parasitized autologous lymphoblasts (from the cell line) to generate CTL. After a further 5 days, (day 14 of infection) PBL and LNC were prepared from each infected calf and used as target cells for CTL from the AuTLC. Additionally, the day 14 PBL and LNC, together with fresh parasitized lymphoblasts were incubated for 48 hr with Tun and 2-DOG before being used as stimulator cells in AuTLC and AlTLC with frozen-reconstituted PBL.

The remaining 6 calves were immunized against *T. parva* (Muguga). Cytotoxic PBL harvested from peripheral blood on day 16 were tested for lysis of normal parasitized lymphoblasts and target cells treated with Tun, 2-DOG or 993C. The specificity of the lysis was

investigated in competitive inhibition assays. To compare the specificity of CTL derived *in vivo* and *in vitro*, PBL from naive and immune calves were cultured in an AuTLC with parasitized lymphoblasts (cell line) and the nature of the cytotoxicity investigated on a range of target cells using appropriate blocking cells. The importance of glycosylation of membrane determinants for the induction of proliferative and cytotoxic responses and for the activity of CTL were examined by treatment of stimulator and target cells with either Tun or 2-DOG.

RESULTS

Specificity of CTL generated *in vivo*

Cytotoxic T lymphocytes collected from cattle recovering from and immune to *T. parva* lyse autologous but not allogeneic parasitized lymphoblasts (Eugui & Emery, 1981). These CTL exhibit less than 5% lysis of autologous uninfected lymphoblasts and less than 10% specific lysis of the cultured bovine lymphosarcoma, BL-3 (data not shown). Complete inhibition of the genetically-restricted cytotoxicity in six calves was only achieved by competition with unlabelled autologous infected lymphoblasts (Fig. 1). These results indicated that the majority of CTL *in vivo* recognize parasite-induced alterations to autologous MHC determinants on the lymphocyte membrane. A minor proportion of the lytic activity was directed towards non-MHC restricted antigens on uninfected blast cells as relatively similar inhibition curves resulted from addition of autologous Con A blasts, allogeneic infected lymphoblasts and BL-3. With each of these blocking cells at blocker:target (B:T) ratios of up to 10:1, less than 30% of specific lysis was abrogated (Fig. 1).

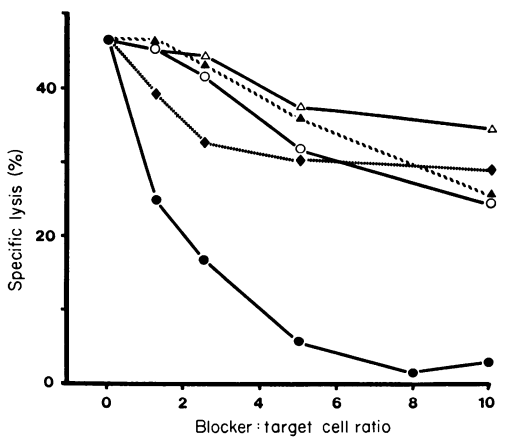

Figure 1. Analysis of the specificity of cytotoxicity for autologous parasitized target cells of CTL from cattle immune to *T. parva*. Results from six calves are expressed as the percentage specific lysis after 4 hr in the presence of competitive inhibition by the following unlabelled (blocking) targets: (●) autologous infected lymphoblasts; (△) autologous infected lymphoblasts treated for 48 hr with tunicamycin (0·1 μg/ml); (○) allogeneic infected lymphoblasts; (♦) autologous Con A blasts; (▲) allogeneic lymphosarcoma, BL-3. The effector PBL:target ratio is 10:1.

Specificity of cytotoxicity generated *in vitro*

In contrast to the findings of Pearson *et al.* (1982), cytotoxicity was elicited in an AuTLC using PBL from either naive or immune cattle as responder cells. No cytotoxicity emerged from cultures of PBL alone. However, the consistent generation of cytotoxicity required the use of defibrinated blood as a source of responder PBL; leucocytes separated from heparinized blood exhibited poor responses after an AuTLC, regardless of whether adherent cells had been removed before culture (data not shown). The reasons for these differences were unclear, but appeared to be associated technically with clumping and adherence of heparinized PBL to the culture vessel. Cytotoxic PBL effectively lysed a range of genetically unrelated target cells, including autologous Con A blasts, autologous

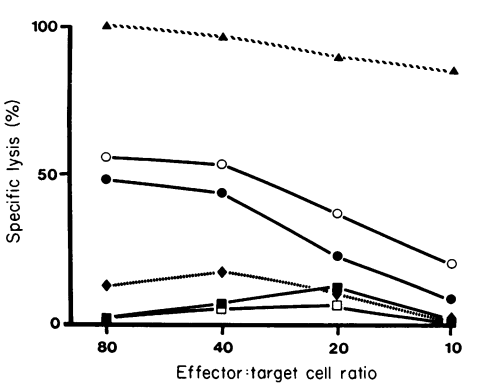

Figure 2. Cytotoxic activity for various target cells of PBL from naive or immune calves after stimulation in an autologous Theilerial-lymphocyte culture (AuTLC). The results are expressed as the percentage specific lysis after 4 hr using the following targets; (●) autologous infected lymphoblasts (6); (○) allogeneic infected lymphoblasts (6); (♦) autologous Con A blasts (3); (▲) BL-3 (6); (■) autologous LNC from infected calves (2) (MSI 12·7%); (□) autologous PBL from infected calves (2) (MSI 7·8%). Numbers of calves in parenthesis. PBL and LNC were prepared from calves 14 days after infection with a lethal dose of *T. parva* (Muguga) as described in 'Materials and Methods'.

and allogeneic parasitized lymphoblasts from infections *in vivo* and *in vitro*, and BL-3 (Fig. 2). Parasitized lymphoblasts *in vitro* which had been incubated with tunicamycin, 2-desoxy-D-glucose or compound 933C were also lysed (Table 2).

Examination by blocking studies of the specificity of lysis by effector cells derived from the AuTLC revealed that both genetically-restricted and natural-killer activity had been generated. The lysis of autologous infected target cells was only blocked efficiently by identical unlabelled targets (Fig. 3a), and closely resembled the activity of CTL *in vivo* (Fig. 1). Lysis of infected allogeneic lymphoblasts was inhibited by competition with specific allogeneic targets; up to 60% of this activity was also blocked by 'cold' autologous and third-party infected cells, whereas BL-3 was less effective (Fig. 3b). Cytotoxicity against BL-3 was prevented by the presence of 'cold' Bl-3 or allogeneic infected cells (90% inhibition), and around 65% lysis was blocked by autologous infected cells at a B:T ratio of 10:1 (Fig. 3c).

Effect of tunicamycin (Tun) and 2-desoxy-D-glucose (2-DOG) on the AuTLC and AlTLC

The effects of either Tun (0·1 or 0·5 μg/ml) or 2-DOG (0·1%) on the proliferative responses elicited by parasitized lymphoblasts in the AuTLC or AlTLC are shown in Table 1. While incubation with either compound did not appreciably affect the viability of the treated cells on feeder layers, it reduced by 30–40% the rate of division of infected lymphoblasts. Each compound when added at the start of the culture period exerted a similar depressive effect on both the AuTLC and AlTLC; there was little difference between the effects of Tun at 0·1 or 0·5 μg/ml in culture. Both Tun and 2-DOG reduced by 75–90% the proliferation arising from cultures containing LNC and PBL from infected cattle, whereas the responses induced by lymphoblasts parasitized *in vitro* were less affected (depressed by 50–70%). Pretreatment of stimulator cells with either compound was largely ineffective unless the respective compound was also included in the TLC. Since proliferative responses returned to 70–85% of those in normal reactions when pretreated stimulator cells were cultured with PBL in normal medium, it was not possible to determine whether the effect of Tun or 2-DOG was entirely restricted to the stimulator cells, or affected the proliferative capacity of PBL as well. However, the presence of Tun or 2-DOG in culture depressed the mitogenic response of PBL to Con A (10 μg/ml) from a stimulation index of 46·5 (SD, 9·6) after 72 hr to 3·8 (SD, 1·8) and 1·2 (SD, 1·4) respectively (data not shown).

Effect of tunicamycin and 2-desoxy-D-glucose on the generation and effector function of CTL

The presence of tunicamycin (0·1 μg/ml) or 2-DOG

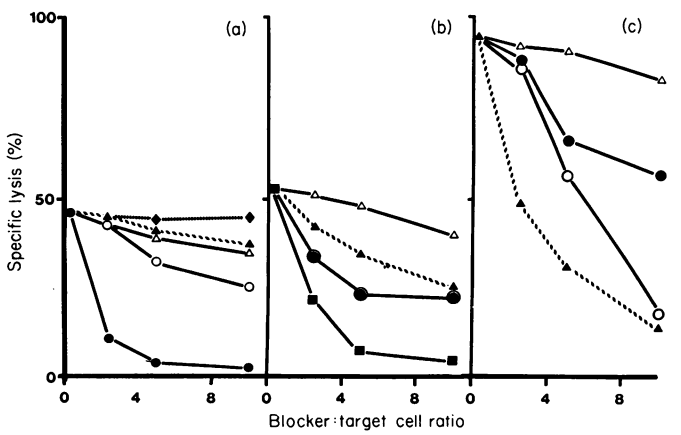

Figure 3. Analysis of the specificity of cytotoxicity of PBL responding in an AuTLC against the following target cells: (a) autologous infected lymphoblasts; (b) allogeneic infected lymphoblasts; (c) BL-3, at an effector:target ratio of 40:1. Competitive inhibition was performed using unlabelled (blocking) targets: (●) autologous infected lymphoblasts; (○) allogeneic infected lymphoblasts; (△) autologous infected lymphoblasts treated with tunicamycin (0·1 μg/ml for 48 hr); (♦) autologous Con A blasts; (■) specific allogeneic infected lymphoblasts (autologous with respect to the labelled target cell); (▲) BL-3. Results are expressed as the percentage specific lysis from four calves.

Table 1. The effect of tunicamycin and 2-desoxy-D-glucose on the proliferative responses of PBL to lymphocytes infected with *T. parva*

Responder PBL	Compound in culture (μg/ml)	Stimulator cell and pretreatment* Cultured infected cells None	Tun	2-DOG	LNC† None	Tun	2-DOG	PBL† None	Tun	2-DOG
Autologous	None	8·8	6·8	7·5	3·8	2·8	3·6	3·2	2·1	3·3
	Tun (0·1)	3·2	3·3	—	0·3	2·1	—	0·8	1·4	—
	(0·5)	2·1	2·4	—	—	—	—	—	—	—
	2-DOG	3·6	—	4·0	0·5	—	0·4	0·9	—	0·7
Allogeneic	None	9·8	6·7	8·2	12·3	19·1	4·3	10·4	11·3	9·9
	Tun (0·1)	3·6	3·0	—	1·3	1·2	—	1·0	1·3	—
	(0·5)	3·8	3·2	—	—	—	—	—	—	—
	2-DOG	3·8	—	3·8	1·1	—	0·6	1·4	—	0·7

* Prior to the initiation of the assay, stimulator cells were incubated on feeder layers for 48 hr in normal medium, medium containing tunicamycin (Tun) (0·1 μg/ml) or 2-desoxy-D-glucose (2-DOG) (0·1% w/v).

† Lymph node cells (LNC) and PBL were harvested from two infected calves 14 days after infection. The percentages of infected LNC were 10·8 and 9·6, and infected PBL were 5·9 and 3·7.

The culture was initiated and incubated for 96 hr as described in Materials and Methods. Results are expressed as the mean stimulation index (SI) from eight calves (cultured infected cells) or the average SI from two calves (LNC and PBL). The autologous PBL responding to infected LNC and PBL had been cryopreserved prior to infection.

Table 2. The influence of tunicamycin (Tun) and 2-desoxy-D-glucose (2-DOG) on the generation and effection of cytotoxicity against autologous lymphoblasts infected with *T. parva*

Generation of CTL	Compound in AuTLC†	Pretreatment (P-) of target cells* and compound in CTL assay P-None None	Tun	2-DOG	P-Tun None	Tun	P-2-DOG None	2-DOG	P-993C None
In vitro	None	71·6	64·0	70·4	82·0	80·6	85·8	86·2	75·6
	Tun	0	0	—	0	0	—	—	—
	2-DOG	0	—	0	—	—	0	0	—
In vivo	n.a.	30·1	29·8	32·4	3·6	2·9	1·8	2·2	8·5

* Target cells (from autologous parasitized cells lines) were incubated for 48 hr on feeder layers in the presence of normal medium, medium containing tunicamycin (0·1 and 0·5 μg/ml) or 2-DOG (0·1%), and the assay for cell-mediated lympholysis (CML) was conducted in the presence or absence of these compounds. Target cells treated with compound 993C were incubated for 96 hr on feeder layers.

† The autologous Theilerial-lymphocyte culture (AuTLC) was initiated as described in 'Materials and Methods' using cell line lymphoblasts as stimulator cells.

The results from the 4 hr assay are expressed as the mean percentage specific release of ^{51}chromium from six calves. (n.a.—not applicable).

(0·1%) in the AuTLC completely inhibited the generation of CTL (Table 2). However, the addition of either compound to conventional CML assays did not affect the ability of CTL generated *in vitro* or *in vivo* to recognize and destroy autologous infected target cells. When target cells were prepared from infected lymphoblasts which had been incubated with Tun or 2-DOG, autologous CTL from immune cattle failed to exhibit cytotoxicity regardless of whether Tun or 2-DOG were included in the 4 hr assay (Table 2). A

similar effect was obtained with compound 993C (Table 2). Furthermore, autologous infected lymphoblasts pretreated with Tun were 50–60% less effective than their untreated counterparts in blocking the CML of PBL from immune cattle (Fig. 1); the inhibition curve was similar to that obtained when unlabelled allogeneic infected cells were used for blocking. In contrast, cytotoxic cells generated *in vitro* were capable of lysing normal and pretreated target cells with efficiencies of around 70% and 85% respectively (Table 2). However, blocking studies revealed that Tun-treated lymphoblasts did not competitively inhibit the lysis of autologous or allogeneic infected target cells by cytotoxic cells generated in the AuTLC (Fig. 3a, b). In addition, treated autologous infected cells did not affect the lysis of BL-3 by similar cells (Fig. 3c).

DISCUSSION

Examination of the specificity of cytolysis by cytotoxic T lymphocytes (CTL) obtained from cattle immune to *Theileria parva* revealed that the majority of activity was directed towards parasite-induced alterations associated with MHC antigens on the membrane of infected autologous cells. By comparison, cytotoxicity generated *in vitro* in an autologous Theilerial-lymphocyte culture (AuTLC) contained both CTL and activity akin to that of natural-killer (NK) cells. The addition to the AuTLC of 2 inhibitors of glycosylation, tunicamycin (Tun) and 2-desoxy-D-glucose (2-DOG) abolished both the proliferative response and the generation of cytotoxicity. While the addition of Tun or 2-DOG in conventional CML assays did not modify the effector function of cytotoxic cells pretreatment of autologous infected lymphoblasts with either compound prevented lysis by CTL, but not by NK cells.

Genetically-restricted CTL obtained from cattle convalescent from and immune to infection with *T. parva* have been presumed to recognize altered membrane determinants associated with MHC (Eugui & Emery, 1981; Emery *et al.*, 1981a). This premise is further supported by the results of blocking assays in the present study and by the failure of CTL *in vivo* to recognize or destroy lymphoblasts pretreated with inhibitors of glycosylation. Parasitized lymphoblasts treated with Tun or 2-DOG probably no longer express sufficient relevant antigens to act as target cells for recognition and lysis by bovine CTL, suggesting that the target antigen(s) may be glycoproteins or carbohydrates. It is apparent that NK cells either effect lysis through receptors which do not require glycosylation for their expression or recognize lower concentrations of membrane-bound antigens than CTL. Examinations of infected cells for those target antigens which are important for CML have implicated viral glycoproteins for herpes simplex (Lawman *et al.*, 1980), matrix proteins, glycoproteins and haemagglutinins for influenza (Brachiale, 1977; Ennis, Martin & Verbonitz, 1977) and glycoproteins for ectromelia (Jackson *et al.*, 1976). However, extensive investigations of lymphoblasts infected with *T. parva* have failed to detect parasite-specific antigens in complement and antibody-dependent cellular cytotoxicity (ADCC) assays using serum from naive, immunized or immune cattle (Creemers, 1982) and solid-phase radioimmunoassays (Emery, unpublished data). By comparison with the present study, this is probably due to the extremely low titres of relevant antibodies in the serum of immune cattle, and biochemical analyses on the membranes of parasitized lymphoblasts are continuing in attempts to identify changes induced by *T. parva*.

The induction of both CTL and NK cell activity in the AuTLC is analogous to observations with the human MLR (Bolhuis & Schellekens, 1981) and studies with Epstein–Barr virus (EBV) (Vaillet, Svedmyr, Stemitz & Klein, 1978; Sugamura & Hinuma, 1980). With EBV and *T. parva*, blocking could discriminate between CTL and NK activity (Sugamura & Hinuma, 1980; Pearson *et al.*, 1982; this study). Although CTL derived from cattle immune to *T. parva* are genetically-restricted, NK-like activity has been demonstrated in PBL from highly parasitotic cattle before either death or recovery (Emery *et al.*, 1981a). Whether non-specific cytotoxicity reflects the initial events in the induction of immunity, or influences the outcome of the disease both remain to be determined, as does its relationship to NK activity derived *in vitro* from the AuTLC.

The precise nature of the effects of Tun and 2-DOG to depress proliferation in the AuTLC and AlTLC could not be defined, since their effects were not restricted to either stimulator or responder cells. By comparison, both compounds have been shown to inhibit glycosylation of viral glycoproteins in cells infected with influenza, ectromelia, herpes viruses and vesicular stomatitis viruses, (Courtney, Steiner & Benyesh-Melnick, 1973; Jackson *et al.*, 1976; Leavitt, Schlesinger & Kornfeld, 1977; Lawman *et al.*, 1980;

Black, Vitetta, Forman, Kang, May & Uhr, 1981). Given that the persistence of membrane-bound H-2 antigens are apparently unaffected by Tun (Lawman *et al.*, 1980) and therefore should not severely affect allogeneic responses (AlTLC), it is probable that the compounds suppressed principally the division of responder PBL. Since Tun and 2-DOG abolished the stimulation of PBL by mitogens, then it was not surprising that the generation of CTL *in vitro*, a process requiring cell division (Cantor & Jandinski, 1974), was also inhibited. Although mitosis was not required for the production of NK cells after the third day of an MLR (Callewaert, Lightbody, Kaplan, Joroszewski, Peterson & Rosenberg, 1978) interference with proliferative or inductive events prior to this stage may have been responsible for the absence of NK activity after AuLTCs containing Tun or 2-DOG.

The induction of protective immunity against *T. parva* necessitates the use of viable parasitic material to establish infection in host lymphocytes (Cunningham, 1977; Emery *et al.*, 1981b). The results of the present study suggest that parasite-altered antigens isolated from infected cells could be tried as an alternative approach to immunoprophylaxis similar to those attempted with influenza (McMichael, Gotch, Cullen, Askonas & Webster, 1980) and Marek's disease (Payne, Powell, Renni & Ross, 1978). Given that CTL provide a major mechanism of resistance against reinfection of cattle with *T. parva*, induction of CTL with isolated antigens in unprimed animals has been reported using liposomes containing H-2 alloantigens (Hale, 1980) and glycoproteins of Sendai virus (McGee, Hale & Panetti, 1980). Generation of CTL against *T. parva* has also been achieved with membrane preparations of autologous infected lymphoblasts (Emery & Jack, in preparation), thereby providing a practical incentive for this avenue of investigation.

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