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Bovine alloreactive cytotoxic cells generated *in vitro*: target specificity in relation to BoLA phenotype

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Summary. Cytotoxic cells of bovine origin were generated in primary MLC using stimulator cells of BoLA w8/w11 phenotype. Bovine lymphoblasts transformed by the protozoan parasite *Theileria parva parva* acted as target cells in studies of the specificity of cytotoxicity. When responder cells in MLC did not share w8 or w11 with stimulator cells, cytotoxicity was evident with all targets bearing w8 or w11, or both, and was almost entirely restricted to these products of the BoLA-A locus. When responder and stimulator cells shared both w8 and w11, cytotoxicity was also generated. Whether this was specific for the products of other putative Class I loci in cattle, or for the products of a Class II region, remains to be determined. These results suggest that the determinants recognized by appropriately generated bovine alloreactive cytotoxic cells are identical with, or closely related to, determinants characterized by BoLA w8 and w11 defining alloantisera.

Abbreviations: BoLA, bovine lymphocyte antigen; CTL, cytotoxic T lymphocyte; FBS, fetal bovine serum; HLA, human lymphocyte antigen; MHC, major histocompatibility complex; MLC, mixed lymphocyte culture; PBM, peripheral blood mononuclear cells; TCGF, T-cell growth factor(s).

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INTRODUCTION

The BoLA system has hitherto been defined serologically (Amorena & Stone, 1978; Spooner *et al.*, 1978) with alloantisera raised by immunization with tissue or lymphocytes or derived from parous cows. Two international comparison tests have been carried out and workshops subsequent to these have agreed upon 17 specificities (prefix w), two of which are considered to be subgroups of BoLA w6 (Spooner *et al.*, 1979; Anon, 1982). The workshop specificities are believed to represent polymorphic determinants on Class I MHC antigens coded for by alleles at a single locus (BoLA-A locus).

Products of the Class I loci of other species have been shown to be the principal recognition structures for alloimmune CTL (Alter *et al.*, 1973; Eijsvoogel *et al.*, 1973; Nabholz *et al.*, 1974; Thistlethwaite *et al.*, 1984) and to restrict the recognition by cytotoxic cells of 'foreign' antigen on cell surfaces. This has been shown to occur in response to virus-infected target cells of mouse (Zinkernagel & Doherty, 1975) and man (McMichael *et al.*, 1977) and in responses to minor histocompatibility antigens (Goulmy *et al.*, 1977) and hapten-modified targets (Shearer, Rehn & Garbarino, 1975; Dickmeiss, Soeberg & Svejgaard, 1977). The experience of others working with human systems has indicated that the determinants recognized by allo-

antisera, at least in some instances, differ from those recognized by CTL (Biddison *et al.*, 1980). Thus, restriction of influenza-immune CTL was found not to completely correlate with the presence of the HLA-A2 serologically-defined specificity on virus-infected target cells. Subsequent biochemical studies have revealed heterogeneity in the A2 specificity (Krangel, Biddison & Strominger, 1983), the variants being recognizable by both virus-immune and alloimmune CTL, but not by alloantisera. Further, it was suggested on the basis of these results that there are distinct regions on human MHC antigens carrying determinants recognized either by CTL or alloantisera. Such a dichotomy in the recognition of determinants in mice has also been suggested as a result of studies using H-2 mutant strains (Klein, 1978).

The study reported here was undertaken as a prelude to studies of bovine MHC restriction in the recognition of 'foreign' antigen by cytotoxic cells. The specificities of uncloned bovine cytotoxic cell populations generated in MLC were determined and related to the serologically-defined BoLA phenotypes of stimulator and target cells.

MATERIALS AND METHODS

Donor cattle

A total of 23 cattle were used. Animals 191, 470, 816 and 995 were used as sources of responder cells. The serologically defined BoLA-A phenotypes of these cattle were w7/w10, w6/w7, w8/w11 and w8/w11, respectively. They had not been exposed to infection with *T. parva* and were serologically negative for antibodies to *T. parva* macroschizonts. Stimulator cells were derived from animals 811, 817 and 821, all of which were of the w8/w11 phenotype. The remaining cattle served as sources of PBM for the establishment of target cell lines.

Generation of cytotoxic cells

Cytotoxic cells were generated in MLC utilizing isolated PBM. In the case of animal 191, responder PBM were derived from defibrinated venous blood. Other responder and stimulator PBM were derived from venous blood collected into equal volumes of Alsever's solution.

Mononuclear cells were separated from blood by centrifugation on ficoll/diatrizoate sodium, S.G. 1077, (Ficoll-Paque, Pharmacia Fine Chemicals, Uppsala, Sweden) for 30 min at 1050 ***g***. Cells from defibrinated blood were washed once in RPMI-1640 medium (Gibco Ltd, Uxbridge, Middlesex, U.K.). Cells derived from blood collected into Alsever's solution were washed three times in Alsever's solution by repeated suspension and centrifugation (10 min at 170 ***g***). The washed PBM were finally resuspended in RPMI-1640 medium containing 10% FBS (Gibco Ltd), 2 mM L-glutamine, 50 μg/ml gentamycin and 5×10^{-5} M 2-mercaptoethanol. Responder and stimulator cells were resuspended at concentrations of 5×10^6/ml and $2{\cdot}5 \times 10^6$/ml, respectively.

Stimulator cells were exposed to 5000 rads of gamma irradiation from a caesium (^{137}Cs) source prior to use.

MLCs were established in 24-well tissue culture plates (Costar no. 3524) by the addition of 1-ml aliquots of responder and stimulator cell suspensions per well. The cells were incubated for 6 or 7 days at 37° in a humidified atmosphere of 5% CO_2 in air. At the end of the incubation period, the cells were harvested from the wells, pelleted by centrifugation and resuspended at appropriate viable cell concentrations in RPMI-1640 medium supplemented with 10% FBS (assay medium).

Long-term maintenance of cytotoxic cells

Cells harvested from 6-day MLCs were centrifuged over ficoll/diatrizoate sodium at 1050 ***g*** for 30 min. Viable cells taken from the interface were restimulated in 24-well tissue culture plates in 2 ml culture medium containing $2{\cdot}5 \times 10^6$ responders and 5×10^6 stimulator cells. Stimulator PBM were prepared and irradiated as for the initial MLC. Harvesting of responder cells and restimulation were repeated twice at weekly intervals, after which 5×10^5 responder cells/well were restimulated at 7–10-day intervals, with 5×10^6 irradiated cells in 2 ml medium containing 10 or 20% of a culture supernatant containing TCGF.

TCGF was prepared by culturing bovine PBM separated from blood collected into Alsever's solution, at a concentration of 4×10^6/ml in the presence of $2{\cdot}5$ μg/ml concanavalin A (Sigma Chemical Company, St Louis, MO; type IV) and collecting the culture supernatants after 24 hr.

Target cells

Lymphoblastoid cell lines infected with the protozoan parasite *Theileria parva parva* (Muguga) served as target cells. The initiation of parasitized cell lines from PBM and their maintenance were performed as described by Stagg *et al.*, (1981). The schizont stage of

the parasite occurs intracellularly in bovine lymphoid cells, and schizont and cell replication are synchronous (Hulliger *et al.*, 1964). The mechanism responsible for maintaining replication of the host cell for essentially indefinite periods is not known, but it does appear to be dependent on the continued presence of the parasite (Pinder *et al.*, 1981).

Previous studies have demonstrated that infection of bovine leucocytes with *Theileria* parasites does not result in qualitative alterations in the expression of serologically-defined determinants associated with those BoLA workshop (Class I) specificities characterized to date (Spooner & Brown, 1980; Teale *et al.*, 1983). Nevertheless, preliminary experiments were undertaken to compare the suitability, as targets in cell-mediated lympholysis assays, of a panel of *T. parva parva*-infected lymphoblasts and a panel of uninfected lymphoblasts derived from the same donor cattle, maintained in culture with concanavalin A and TCGF. These experiments revealed no qualitative differences in ^{51}Cr release patterns between the two panels of lymphoblasts when tested with various effectors generated *in vitro*. In view of the simple maintenance requirements of the parasitized cell lines, these were used in the studies reported here in preference to uninfected lymphoblasts.

Target cells obtained from cultures in exponential growth phase were pelleted by centrifugation and resuspended at 2×10^7/ml in assay medium. The cells were labelled with ^{51}Cr by the addition of an equal volume of ^{51}Cr sodium chromate (Amersham International, Amersham, Bucks, U.K.; code CJS.4) made to 1 mCi/ml in RPMI-1640 medium, followed by incubation with periodic mixing at 37° for 60 min. The cells were then washed three times in assay medium prior to use.

The assay medium utilized a batch of FBS selected, on the basis of preliminary experiments, to give low spontaneous release of ^{51}Cr.

Cytotoxicity assay

Assays were carried out in duplicate in microculture plates (Costar no. 3596) using 5×10^4 target cells per well and different numbers of effector cells to give effector:target ratios ranging from 160:1 to 5:1. Spontaneous release of ^{51}Cr was measured in triplicate wells containing target cells and medium only. Maximum release of isotope was assessed by the addition of target cells in 50 μl assay medium to 200 μl tap water, in triplicate, followed by two cycles of rapid freezing and slow thawing.

With the exception of cells used for estimation of maximum ^{51}Cr release, cells were incubated for 4 hr in a humidified atmosphere of 5% CO_2 in air. Following resuspension of cells and centrifugation of assay plates (7 min at 160 ***g***), standard aliquots of supernatants were harvested for counting of gamma emmissions in a Beckman Gamma 5500 counter. Percent specific ^{51}Cr release was used as a measure of target cell lysis. This was calculated as:

$$\% \text{ specific } {}^{51}Cr \text{ release} = \frac{\text{test release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100.$$

Cold target inhibition

Unlabelled (cold) target cells were added to effector cell suspensions in microculture plates 10 min before the addition of ^{51}Cr-labelled (hot) target cells. Cold:hot ratios of 10, 5, 2·5 and 1·25:1, were assayed in duplicate at different effector:hot target ratios. In some assays, two cold targets were added in combination. In these cases, each cold target cell was added at half the equivalent level for single cold target inhibition.

Histocompatibility testing (BoLA typing)

Cell donor cattle were assigned BoLA types on the basis of the results of a microlymphocytotoxicity assay, using PBM as described by Teale *et al.* (1983) and a panel of alloantisera prepared in the AFRC Animal Breeding Research Organization, Edinburgh, U.K. The panel included sera designated as defining reagents in two international comparison tests (Spooner *et al.*, 1979; Anon, 1982).

The same panel of typing sera was used for BoLA typing of target cell-line cells. The method was as for PBM except that cells were added to test wells at a concentration of 10^6/ml.

All donors and target cell lines were BoLA-typed prior to use. Lines were typed subsequent to the fourth *in vitro* passage. In all cases, the BoLA-A locus-coded phenotypes of target cell lines corresponded with those of PBM from the donor cattle.

RESULTS

Primary MLC with BoLa mismatched responders and stimulators

The pooled results of cytotoxicity assays utilizing effectors originating from the PBM of animals not

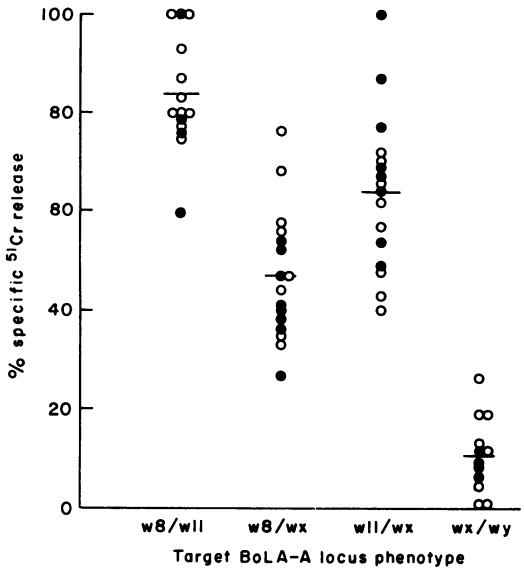

Figure 1. Cytotoxic activity of non-w8/w11 effectors, generated *in vitro* with w8/w11 stimulator cells, related to target BoLA phenotype. Results with four effectors at an effector: target ratio of 80: 1 are shown. Two originated from PBM of animal 191 (●) in coculture with PBM of stimulators 811 and 821, and two originated from PBM of animal 470 (○) in coculture with the same stimulator cells. MLCs were established as described in the Materials and Methods. wx and wy denote third-party (non-w8, non-w11) specificities. Horizontal bars denote mean specific release of ^{51}Cr.

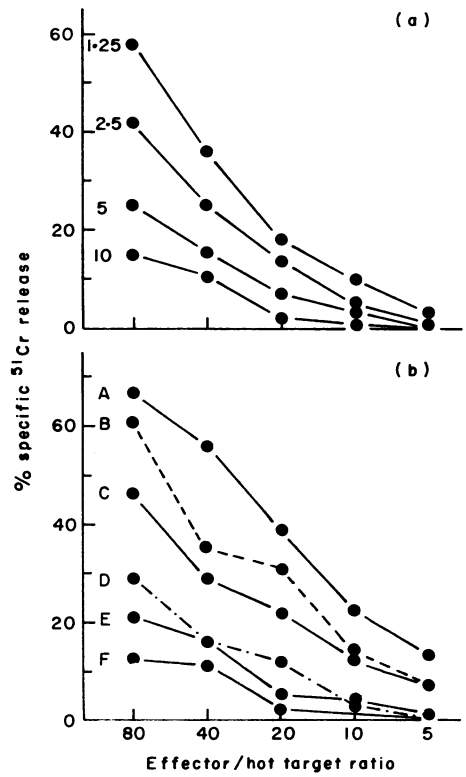

Figure 2. Inhibition of specific ^{51}Cr release from the 821 target (w8/w11) by unlabelled (cold) targets using effector 191 generated in coculture with 821 PBM, at various effector: hot target ratios. (a) Cold target 821 at cold: hot target ratios of 10, 5, 2·5 and 1·25: 1. (b) Cold target inhibition by five different cold targets used at a cold: hot target ratio of 10: 1, 'A' depicts ^{51}Cr release at the various effector: hot target ratios in the absence of cold targets. The five cold target blockers and their BoLA phenotypes were: 'B', 829 (w13/w16); 'C', 836 (w8/w13); 'D', 834 (w6/w11); 'E', 817 (w8/w11); 'F', 821 (w8/w11).

sharing either BoLA-A locus specificity with stimulators of the w8/w11 phenotype, are shown in Fig. 1. Four effector populations were generated in responder PBM from two cattle following stimulation with w8/w11 PBM populations from two different cattle. A panel of A-locus related and unrelated target cells was used to assess the specificity of cytotoxicity. In all combinations, cytotoxic activity was generated which was evident with all w8/w11 targets, and further, when target and stimulator were derived from the same donor, cytotoxicity was not significantly greater than with other w8/w11 targets. Cytotoxicity with non-w8/w11 targets (mismatched) was low or inapparent, whereas intermediate levels of cytotoxicity were observed with all targets possessing only w8 or w11 (half-matched targets).

The effectors differed in their cytotoxic capability with half-matched targets. Both 191 effectors achieved greater cytotoxicity on w11 targets (mean specific ^{51}Cr release at effector to target ratio 80: 1 of 72%) than on w8 targets (mean, 42%). The levels of cytotoxicity on w11 half-matched targets approached those obtained on w8/w11 targets (mean 79%) and, in individual cases, exceeded them. The 470 effectors exhibited similar levels of cytotoxicity on w8 and w11 half-matched targets.

Cold target inhibition

Inhibition of ^{51}Cr release was assayed in order to confirm specificity for the w8 and w11 target entities. Specificity was confirmed utilizing effectors generated in the PBM of animal 191 during stimulation with 821 PBM. Specific release from the ^{51}Cr-labelled 821 target in the presence of unlabelled 821 target cells at

Table 1. Inhibition of ^{51}Cr release from a w8/w11 target (821) by unlabelled targets of various BoLA phenotypes with 191 (w7/w10) effector originating in MLC with 821 stimulator cells

Cold target	BoLA phenotype	% inhibition
821	w8/w11	78
811	w8/w11	55
817	w8/w11	68
828	w8/w11	70
814	w13/w20	20
829	w13/w16	9
818	w6/w8	38
836	w8/w13	31
S807	w11/w13	67
834	w6/w11	57
S807/818*	as above	75
836/834*	as above	78

Effector:hot target ratio, 80:1.
Cold target:hot target ratio, 10:1.
* Cold targets added in combination, each at a 5:1 cold:hot ratio.

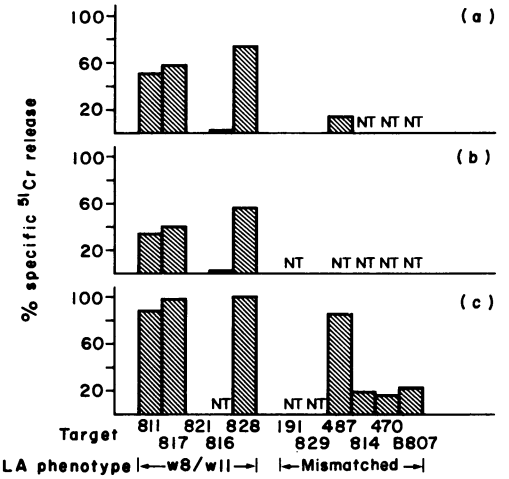

Figure 3. Cytotoxic activity of three effectors of w8/w11 phenotype following *in vitro* stimulation with w8/w11 PBM. (a) effector 995/stimulator 811 (effector:target ratio 80:1); (b) effector 995/stimulator 817 (effector:target ratio 80:1); (c) effector 816/stimulator 811 (effector:target ratio 160:1). NT, not tested.

different cold target:hot target ratios is depicted in Fig. 2a. Figure 2b shows ^{51}Cr release at the 10:1 cold:hot target ratio with 821, matched w8/w11, w8 and w11 half-matched and mismatched cold targets. The percentage inhibition of ^{51}Cr release by cold targets of varying A-locus compatibility with the stimulator at the 10:1 cold:hot target ratio, is given in Table 1.

The specificity for w8 and w11 was clearly confirmed. It is also apparent that the preference for w11 over w8 shown by the 191 effectors in the first series of experiments is reflected in these results. The simultaneous presence of two unlabelled targets, one carrying w8 and the other w11, reduced ^{51}Cr release in each of two such combinations to levels approximating that observed on an irrelevant hot target in the absence of cold inhibitors (data not shown). Moreover, the mean ^{51}Cr release (16% at effector to target ratio of 80:1) with half-matched cold target combination blocking was lower than the mean ^{51}Cr release (22%) achieved with matched w8/w11 cold targets at an equivalent cold:hot ratio.

Primary MLC with A-locus matched stimulator and responder cells

In a third series of experiments, the generation of cytotoxic effector cells in MLCs involving responder and stimulator cells of the same A-locus type, as defined by our panel of alloantisera, was studied and their target specificity assessed. In some of these responder/stimulator combinations, cytotoxic effectors were generated. Their target specificity is shown in Fig. 3.

Effector 995 showed the same pattern of cytotoxicity on a panel of five w8/w11 targets following generation in MLC with 811 or 817 cells. Thus, with both stimulators, 811, 817, and 828 targets were lysed, whereas 816 and 821 targets were not. Of three non-w8/w11 targets tested, only 487 was lysed but the specific ^{51}Cr release was markedly less than with lysed w8/w11 targets.

Effector 816, following stimulation with 811 or 817 (data not shown), showed the same pattern of cytotoxicity on w8/w11 targets as both 995 effectors. Cytotoxicity on the A-locus mismatched target 487 was also obtained and was of similar magnitude to that on positive w8/w11 targets. No significant cytotoxicity was evident with the panel of targets used with the 816 or 995 effectors following stimulation with 821.

Following a separate generation of 816 effectors cocultured with the 811 stimulator, cytotoxicity was detected with a w8 half-matched target and another A-locus mismatched target. The levels of ^{51}Cr release (33% and 32%, respectively) compared with a mean release of 32% with positive w8/w11 targets, and less

than 5% with other targets, at an effector:target ratio of 80:1. The cells originating in this MLC were maintained in culture for several months as described in the Materials and Methods. When assayed for cytotoxicity following 6 weekly restimulations with 811 PBM and a further 4-week period of maintenance in TCGF without restimulation, significant cytotoxicity was detected with a fourth non-w8/w11 target which had not previously been tested and which has no BoLA workshop specificities detectable with our panel. In this case, at an effector:target ratio of 10:1, specific ^{51}Cr release was 29%, which compared with 25% with the 817 w8/w11 target and less than 3% with non-w8/w11 targets.

DISCUSSION

It is clear from these studies that, where responder cells did not carry w8 or w11, target specificity of cytotoxic cells correlated well with the serologically-defined w8/w11 phenotype of stimulator and target cells. The finding that such effectors did not distinguish between different w8/w11 targets, qualitatively or quantitatively, is consistent with the response being predominantly to determinants closely associated with the w8 and w11 specificities. It is unlikely that significant cytotoxicity was restricted to antigens common to all such targets and coded for at other loci in these cases because of the heterogeneity of the stimulator and target cell donor cattle. The group of donors carrying either or both of the target specificities were the progeny of five sires and a larger number of dams.

Further evidence that cytotoxicity was predominantly, and possibly entirely, directed against w8- and w11-associated determinants in these instances was obtained in the cold target inhibition assays. The simultaneous presence of w8 and w11 on different cold targets in two combinations in such assays abrogated specific cytotoxicity. In these cases, the stimulator cell in the generation of cytotoxicity derived neither haplotype from the same sire as any of the inhibitors.

It is possible, should other loci exist in cattle which code for target determinants in cell-mediated lympholysis, that responses to them are dominated by responses to A-locus products when responder cells do not share these with stimulators. The possibility that the response to either or both of the w8 and w11 specificities is polyclonal, with a variety of epitopes being recognized, is the subject of continuing studies.

It is clear, however, that it is possible to generate bovine cytotoxic cells in primary MLC when both responder and stimulator cells carry w8 and w11. It would seem that in our panel of w8/w11 cattle, 811, 817 and 828 can be distinguished from 816, 821 and 995 on the basis of susceptibility to lysis in such situations. The relationship of donor cattle, which has already been discussed, strengthens the view that, in the cases studied, cytotoxicity was directed against epitopes on other allelic products than w8 and w11. In addition, such effectors were demonstrated to have the ability to lyse four non-w8/w11 targets of varying A-locus phenotype.

It is unlikely that these results can be explained by target heterogeneity with respect to susceptibility to natural killer cell activity. The levels of lysis which could be achieved in the 4-hr assay were considerable. Moreover, the different responder PBM consistently gave rise to cytotoxic effector cells during stimulation with PBM of some cattle and not with others. Finally, in this respect, the target cell lines which were lysed in this series of experiments have been used to assay specificity of CTL on other occasions following generation in MLC using non-w8/w11 stimulator cells and A-locus mismatched responders. Lysis in these situations has not been observed.

The nature of the proposed second locus coding for determinants recognized by primary *in vitro*-generated cytotoxic cells is not yet clear. Evidence for a second serologically-defined BoLA Class I locus has been reported by Stear, Newman & Nicholas (1982). Although the typing antisera used were not operationally monospecific, these workers reported two specificities which did not cocap with defining sera and which were inherited together. Our own serological observations, while not conclusive, strongly support the existence of such a locus, and attempts to confirm this are in progress.

A further consideration is that, in situations where responder and stimulator are matched for A-locus products, cytotoxic cells may be generated against Class II MHC antigens. Molecules of Class II type have been identified by biochemical characterization on bovine cells (Hoang-Xuan *et al.*, 1982). Serological studies also suggest the existence of a Class II genetic region in cattle (Newman, Adams & Brandon, 1982). In addition, there is evidence of expression of Class II products on *Theileria parva parva*-infected lymphoblasts (Black *et al.*, 1981). Studies in mice have shown that it is possible to develop cytotoxicity directed against Class II antigens in primary MLC (Wagner *et al.*, 1974). The possibility that the cytotoxicity gener-

ated in the studies reported here, when responder and stimulator cells were identical with respect to A-locus products, was directed against the products of the Class II region of the bovine MHC cannot, therefore, be excluded.

It is clear from these studies, however, that serologically-defined BoLA-A locus products have the capacity to function as target elements for primary *in vitro*-generated bovine cytotoxic cells. Further, it would appear that the epitopes recognized on BoLA w8 and w11 by alloantisera and cytotoxic cells, if not identical, are closely associated on the same molecules.

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REFERENCES

ALTER B.J., SCHENDEL D.J., BACH M.L., BACH F.H., KLEIN J. & STIMPFLING J.H. (1973) Cell mediated lympholysis. Importance of serologically defined H-2 regions. *J. exp. Med.* **137,** 303.

AMORENA B. & STONE W.H. (1978) Serologically defined (SD) locus in cattle. *Science*, **201,** 159.

ANON. (1982) Proceedings of the second international bovine lymphocyte antigen (BoLA) workshop. *Anim. Blood Groups. biochem. Genet.* **13,** 33.

BIDDISON W.E., WARD F.E., SHEARER G.M. & SHAW S. (1980) The self determinants recognised by human-virus immune T cells can be distinguished from the serologically defined HLA antigens. *J. Immunol.* **124,** 548.

BLACK S.J., JACK R., LALOR P. & NEWSON J. (1981) Analyses of *Theileria*-infected cell surface antigens with monoclonal antibodies. In: *Advances in the Control of Theileriosis* (eds A. D. Irvin, M. P. Cunningham and A. S. Young), p. 327. Martinus Nijhoff Publishers for the Commission of the European Communities, The Hague, The Netherlands.

DICKMEISS E., SOEBERG B. & SVEJGAARD A. (1977) Human cell-mediated cytotoxicity against modified target cells is restricted by HLA. *Nature (Lond.)*, **270,** 526.

EIJSVOOGEL V.P., DU BOIS M.J.G.J., MEINESZ A., BIERHORST-EIJLANDER A., ZEYLEMAKER W.P. & SCHELLEKENS P.T.A. (1973) The specificity and activation mechanism of cell-mediated lympholysis (CML) in man. *Transplant. Proc.* **5,** 1675.

GOULMY E., TERMIJTELEN A., BRADLEY B.A. & VAN ROOD J.J. (1977) Antigen killing by T cells of women is restricted by HLA. *Nature (Lond.)*, **266,** 544.

HOANG-XUAN M., CHARRON D., ZILBER M-T. & LEVY D. (1982) Biochemical characterisation of class II bovine major histocompatibility complex antigens using cross-species reactive antibodies. *Immunogenetics*, **15,** 621.

HULLIGER L., WILDE J.K.M., BROWN C.G.D. & TURNER I (1964) Mode of multiplication of *Theileria* in cultures of bovine lymphocytic cells. *Nature (Lond.)*, **203,** 728.

KLEIN J. (1978) Genetics of cell-mediated lymphocytotoxicity in the mouse. *Springer Seminars in Immunopathol.* **1,** 31.

KRANGEL M.S., BIDDISON W.E. & STROMINGER J.L. (1983) Comparative structural analysis of HLA-A2 antigens distinguishable by cytotoxic T lymphocytes. II. Variant DK1: evidence for a discrete CTL recognition region. *J. Immunol.* **130,** 1856.

MCMICHAEL A.J., TING A., ZWEERINK H.J. & ASKONAS B.A. (1977) HLA restriction of cell-mediated lysis of influenza virus-infected human cells. *Nature (Lond.)*, **270,** 524.

NABHOLZ M., VIVES J., YOUNG H.M., MEO T., MIGGIANO V., RIJNBEEK A. & SHREFFLER D.C. (1974) Cell-mediated cell lysis *in vitro*: genetic control of killer cell production and target specificities in the mouse. *Eur. J. Immunol.* **4,** 378.

NEWMAN M.J., ADAMS T.E. & BRANDON M.R. (1982) Serological and genetic identification of a bovine B lymphocyte alloantigen system. *Anim. Blood Groups biochem. Genet.* **13,** 123.

PINDER M., KAR S., WITHEY K.S., LUNDIN L.B. & ROELANTS G.E. (1981) Proliferation and lymphocyte stimulatory capacity of *Theileria*-infected lymphoblastoid cells before and after the elimination of intracellular parasites. *Immunology*, **44,** 51.

SHEARER G.M., REHN T.G. & GARBARINO C.A. (1975) Cell-mediated lympholysis of trinitrophenyl-modified autologous lymphocytes. Effector cell specificity to modifed cell surface components controlled by the H-2K and H-2D serological regions of the murine major histocompatibility complex. *J. exp. Med.* **141,** 1348,

SPOONER R.L. & BROWN C.G.D. (1980) Bovine lymphocyte antigens (BoLA) of bovine lymphocytes and derived lymphoblastoid cell lines transformed by *Theileria parva* and *Theileria annulata*. *Parasite Immunol.* **2,** 163.

SPOONER R.L., LEVEZIEL H., GROSCLAUD F., OLIVER R.A. & VAIMAN M. (1978) Evidence for a possible major histocompatibility complex (BLA) in cattle. *J. Immunogenet.* **5,** 335.

SPOONER R.L. *et al.* (1979) Analysis of alloantisera against bovine lymphocytes. Joint report of the 1st international bovine lymphocyte antigen (BoLA) workshop. *Anim. Blood Groups biochem. Genet.* **10,** 63.

STAGG D.A., DOLAN T.T., LEITCH B.L. & YOUNG A.S. (1981) The initial stages of infection of cattle cells with *Theileria parva* sporozoites *in vitro*. *Parasitology*, **83,** 191.

STEAR M.J., NEWMAN M.J. & NICHOLAS F.W. (1982) Two closely linked loci and one apparently independent locus code for bovine lymphocyte antigens. *Tissue Antigens*, **20,** 289.

TEALE A.J., KEMP S.J., YOUNG F. & SPOONER R.L. (1983) Selection by major histocompatibility type (BoLA), of

lymphoid cells derived from a bovine chimaera and transformed by *Theileria* parasites. *Parasite Immunol.* **5,** 329.

THISTLETHWAITE J.R., AUCHINCLOSS M., PESCOVITZ M.D. & SACHS D.M. (1984) Immunologic characterization of MHC recombinant swine: role of class I and II antigens in *in vitro* immune responses. *J. Immunogenet.* **11,** 9.

WAGNER H., GOTZE D., PTSCHELINZEW W. & ROLLINGHOFF M. (1975) Induction of cytotoxic T lymphocytes against I-region-coded determinants: *in vitro* evidence for a third histocompatibility locus in the mouse. *J. exp. Med.* **142,** 1477.

ZINKERNAGEL R.M. & DOHERTY P.C. (1975) H-2 compatibility requirement for T cell mediated lysis of target cells infected with lymphocytic choriomeningitis virus. Different cytotoxic T cell specificities are associated with structures coded for in H-2K or H-2D. *J. exp. Med.* **141,** 1427.