Infection and transformation of dendritic cells from bovine afferent lymph by *Theileria annulata*

S. A. STEPHENS* *and* C. J. HOWARD

Institute for Animal Health, Compton, Newbury, Berkshire RG20 7NN, UK

SUMMARY

Following incubation with sporozoites of the protozoan parasite *Theileria annulata*, dendritic cells (DC), extracted from bovine afferent lymph, became infected and transformed into large, rounded, continuously proliferating cell lines. Phenotypic analysis of the transformed cells by immunostaining and flow cytometry revealed that they expressed MHC class I and II antigens, the myeloid marker MyD1 (SIRPα) and the bovine WC6 (workshop cluster 6) molecule. Transformed DC cell lines differed from those produced from infection of macrophages and B cells in that some lines expressed CD21 and a proportion of cells continued to express the antigen stained by the mAb CC81, a marker which defines a subpopulation of DC in afferent lymph. Both of the main populations of DC that have been identified in bovine afferent lymph appeared to be equally permissive for infection and transformation with *T. annulata*. These findings raise the possibility that the transformed proliferating cells characteristic of *in vivo* infections could be derived from DC as well as macrophages. This could have consequences for understanding the pathogenesis of the disease and for developing methods to manipulate immune responses to eliminate the parasite.

Key words: *Theileria annulata*, dendritic cells, afferent lymph, transformation.

INTRODUCTION

Theileria annulata is a tick-transmitted protozoan parasite that is the cause of tropical theileriosis in cattle. The disease is characterized by the infection and transformation of mononuclear cells which then multiply and disseminate throughout the lympho-haemopoetic system of the infected animal. Previous *in vitro* studies have shown that *T. annulata* sporozoites preferentially invade cells of the monocyte/macrophage lineage (identified by their expression of CD14 or the myeloid marker recognized by the mAb IL-A24 (MyD1, the bovine homologue of SIRPα (Brooke, Parsons & Howard, 1998)) but they can also infect B cells (Glass *et al.* 1989; Spooner *et al.* 1989). More recent work has shown that the parasite can also specifically infect CD5+ macrophages and B1 B cells (Moreau *et al.* 1999). Following infection all of these cell types become transformed; they do not require antigenic stimulation or exogenous growth factors and can be maintained indefinitely as continuously proliferating cell lines (Dobbelaere *et al.* 1988). The presence of the parasite in the cell cytoplasm appears to modulate a number of signal transduction pathways which promote cell survival and proliferation (Dobbelaere & Heussler, 1999), thus leading to uncontrolled replication. Transformation also leads to de-differentiation of infected cells, including the loss of

expression of lineage-specific cell surface markers and functions (Sager *et al.* 1997; Sager, Davis & Jungi, 1999). This can make it difficult, or even impossible, to identify the cell type from which transformed cell lines are derived.

The primary cell type that becomes infected and transformed *in vivo* has been proposed to be monocyte/macrophages, based on the expression of MHC class II antigens, the myeloid antigens CD11b and MyD1 and the lack of expression of T or B cell-specific markers by transformed cells (Campbell & Spooner, 1999; Forsyth *et al.* 1997; Howard *et al.* 1993). However, recent further analysis of cell lines derived from *in vivo* infections has also provided evidence that some of the transformed cells present in infected cattle are derived from B cells (Moreau *et al.* 1999). This result, combined with an earlier finding of CD3 expression on a single *in vivo* infected cell line (Howard *et al.* 1993), could indicate that a wider range of host cell types may be infected *in vivo* than has previously been suggested from the results of *in vitro* studies. The host cell type infected could have significant consequences for the pathogenesis of *T. annulata* infection.

It has been suggested that MHC class II expression, although not a specific receptor, is necessary for *T. annulata* infection of cells (Glass *et al.* 1989). In cattle cells of both the monocyte/macrophage lineage and B cells express MHC class II molecules. Another cell type that also expresses MHC class II is the dendritic cell (DC) but there appear, however, to be no previous reports of

* Corresponding author. Tel: 00 44 (0) 1635 578411. Fax: 00 44 (0) 1635 577263. E-mail: sue.stephens@bbsrc.ac.uk

infection of DC by *T. annulata*. The possibility that DC might be a prime target for the parasite is particularly interesting since these cells are known to be central to the initiation of immune responses, being the only antigen presenting cell that can initiate immune responses in naïve animals (Steinman, 1991; Banchereau & Steinman, 1998). DC present at the body surfaces form a network of cells that come into contact with invading microbes prior to migrating through the afferent lymphatics to the draining lymph node where they present processed antigen to T cells (Steinman, 1991; Steinman, Pack & Inaba, 1997). They are, therefore, present both at the likely site of infection with the sporozoites and in the draining lymph node, which has been suggested to be the site of transformation of target cells by the parasite (Campbell & Spooner, 1999). Afferent lymph draining from peripheral tissues into lymph nodes contains a population of DC called Afferent Lymph Veiled Cells (ALVC). In cattle ALVC can be collected by cannulation of efferent lymphatic ducts following ablation of the prescapular lymph node (Emery, MacHugh & Ellis, 1987). This technique allows the collection of *ex vivo* DC draining from the skin in a normal physiological state unaltered by culture. These cells, therefore, represent a stage of DC development and maturation which potentially could be infected by *T. annulata* sporozoites during a natural infection by the tick vector. In addition to showing high intensity expression of MHC class II antigen a proportion of ALVC also express the myeloid marker MyD1 (Howard *et al.* 1997; McKeever *et al.* 1991) and therefore they could represent a potential origin of MyD1+ transformed cells previously identified in *in vivo* infections.

The main objective of this study was firstly to determine whether it was possible to infect and transform DC with *T. annulata* and secondly to investigate whether both MyD1+ and MyD1− ALVC were equally permissive for infection. These two populations of ALVC have previously been shown to differ in their ability to stimulate T cell responses and differential infection might therefore affect the pathogenesis of the disease following *T. annulata* infection (Howard *et al.* 1997).

MATERIALS AND METHODS

Monoclonal antibodies and flow cytometry

A range of murine mAb that recognize cattle leukocyte antigens were used for cell staining and sorting, the majority of which have been described within the International Ruminant Leukocyte Antigen Workshops (Howard & Naessens, 1993; Howard *et al.* 1991; Naessens & Hopkins, 1996) and used in previous studies of ALVC (McKeever *et al.* 1991;

Howard *et al.* 1997). Either fluorescein isothiocyanate-labelled goat anti-mouse Ig (GAMIg-FITC) or a combination of the isotype specific GAMIgG1-FITC and phycoerythrin-labelled GAMIgG2a mAb (Southern Biotechnology Associates, AL, USA) were used as secondary reagents for immunofluorescent staining. The cells were then analysed by flow cytometry on a FACScalibur (Becton Dickenson, Mountain View, CA, USA) as has been previously described (Shapiro, 1988; Howard *et al.* 1997).

Isolation of dendritic cells, monocytes and B cells

ALVC were collected, essentially as previously described (Emery *et al.* 1987), by cannulation of the efferent duct of the prescapular lymph node following previous ablation of the node. The lymph nodes were removed from the calves between 8 and 16 weeks of age under local anaesthetic. After allowing a minimum of 6 weeks for the afferent and efferent lymphatics to anastomose the efferent ducts were cannulated under general anaesthetic and afferent lymph (AL) collected for between 1 and 8 days. ALVC were distinguished from the other cells in afferent lymph using flow cytometry on the basis of their high forward scatter (FSC) and high level expression of a 210 kDa antigen currently termed WC6 (Howard & Naessens, 1993; Howard *et al.* 1997). Previous analysis has shown that all ALVC have high intensity expression of MHC class II but can be divided into 2 major populations based on the expression of other surface antigens (Howard *et al.* 1997; McKeever *et al.* 1991). The major population expresses the myeloid antigen MyD1 (SIRPα) (Brooke *et al.* 1998), has low intensity or no expression of the integrin CD11a and is not recognized by the mAb CC81. Conversely the minor population shows high intensity expression of CD11a and the antigen recognized by the mAb CC81 (CC81Ag) but does not express MyD1. In this study the 2 major populations of ALVC were sorted, essentially as has been previously described (Howard *et al.* 1997), on a FACStar-plus (Becton Dickenson) on the basis of their expression of the antigen recognized by the mAb CC81.

Monocytes and B cells were also isolated from fresh blood samples. Briefly, peripheral blood mononuclear cells (PBMC) were separated from heparinized blood by the use of a density gradient (Histopaque 1083, Sigma-Alrich, Dorset, UK). Cells were stained with mAb for CD14 (mAb CCG33) to isolate monocytes or surface immunoglobulin expression (Ig light chain, mAb IL-A59) to isolate B cells, then sorted by the use of anti-murine-IgG magnetic beads and MinMACS™ magnetic columns (Miltenyi Biotech GmbH, Bergisch Gladbach, Germany). The purity of the cell populations sorted was confirmed by flow cytometry. The minimum purity of samples used for infection was 97% for B

Table 1. Infection and transformation of different cell types by *Theileria annulata*

(The table shows the number of days post-infection that clusters of large, round, proliferating, transformed cells were first visible microscopically. N.T., No transformed cells seen up to 21 days post-infection. Target cells were isolated by staining with mAb for WC6 (ALVC), CD14 (monocytes) or surface immunoglobulin M expression (B cells) and sorting by flow cytometry or magnetic beads. Cells were all incubated with 0·1 tick-equivalent of *T. annulata* sporozoites.)

Cell type	No. of target cells per well			
	5×10^4	10^4	10^3	10^2
ALVC	8	10	14	NT
Monocytes	10	12	14	NT
B cells	10	12	14	NT

cells and macrophages and $> 99\%$ for the FACS-sorted ALVC.

Infection of cells with sporozoites

Cells were infected with *T. annulata* sporozoites of Hissar stock obtained from ground up tick supernatants which had been stored frozen in liquid nitrogen (kindly supplied by C. G. D. Brown, Centre for Tropical Veterinary Medicine, Royal (Dick) School of Veterinary Studies, University of Edinburgh). After rapid thawing, sporozoites were resuspended at 1 tick-equivalent per ml (TE/ml) in RPMI 1640 with Glutamax and 25 mM HEPES medium (Gibco BRL) containing 10% heat-inactivated foetal calf serum, 50 μg/ml gentamycin and 50 μM 2-mercaptoethanol. Cell infections were carried out in 96-well plates. Between 10^2 and 5×10^4 target cells and 0·1 TE of *T. annulata* sporozoites were added to triplicate wells. In wells containing $< 5 \times 10^4$ target cells an appropriate number of γ-irradiated PBMC (3000 rads) were added per well as filler cells to maintain a constant ratio of host cells to sporozoites. Plates were incubated at 37 °C, in 5% CO_2 for several days until proliferation of transformed *T. annulata* infected cells could be seen. Transformed cells were very large, rounded and easily distinguishable from uninfected cells. Culture and passage of the transformed cells was continued in the same media. Phenotypic characterization of transformed cells by immunostaining and flow cytometry was carried out at several time-points following infection. In total 10 different cell lines derived from ALVC infected with *T. annulata* were stained for phenotypic analysis. The intensity of expression of some antigens (mean fluorescence intensity, MFI) was calculated from the geometric mean (Gm) of the histogram for stained cells minus the Gm for the same cell line stained with isotype-matched control mAb.

Infection and transformation of dendritic cells by T. annulata

A summary of the results of infection of the different types of sorted cells is given in Table 1. The production of transformed, proliferating cells was seen with all 3 of the different cell types in wells containing 10^3 or more target cells. Transformed cells were seen earliest in the wells containing the most target cells. ALVC appeared to be readily infected with *T. annulata*, transformed cells being visible in wells containing 5×10^4 ALVC from as early as 8 days post-infection.

In a further experiment cells representing the 2 major populations of ALVC were sorted on a FACStar-plus and 10^4 target cells per well incubated with 0·1 TE of sporozoites. Proliferating clones of transformed cells were seen following infection of both of the subpopulations of ALVC from 10 days post-infection.

Expression of selected leukocyte differentiation antigens by transformed cells

Phenotypic analysis of the transformed cells was initially carried out to compare the surface marker expression on cell lines derived from all ALVC with that seen on transformed monocytes and B cells. Fig. 1 shows the results of staining cell lines at 21 days post-infection (2nd passage) for a range of typical myeloid, B cell and ALVC antigens. Expression of the WC6 antigen was maintained on both transformed ALVC and B cells at a similar intensity to that seen on the uninfected cells. Interestingly though, monocytes (which are normally WC6 negative) also seemed to start expressing this antigen once they had been transformed by *T. annulata* infection. The myeloid antigen MyD1 (a SIRPα molecule) was also widely expressed on all of the cell lines characterized including those derived from B cells, which do not express this antigen in the uninfected state. There did, however, appear to be a slightly lower mean intensity of expression of MyD1 on the B-cell derived lines compared to the monocyte and ALVC-derived lines. The MFIs for MyD1 expression were as follows; monocyte lines 246·1 ± 112·2, ALVC lines 195·0 ± 71·7 and B cell lines 120·7 ± 30·2 (not significant, Student's *t*-test). There was very little expression of either CC81Ag or CD5 on any of the cell lines examined. A small percentage of cells in ALVC-derived lines appeared to express a very low level of CC81Ag but no expression at all was seen in either monocyte or B cell-derived cell lines. CD21 expression was quite variable between the lines with some ALVC and B cell-derived lines having up to 40% of transformed cells expressing this antigen; however, in other lines there was
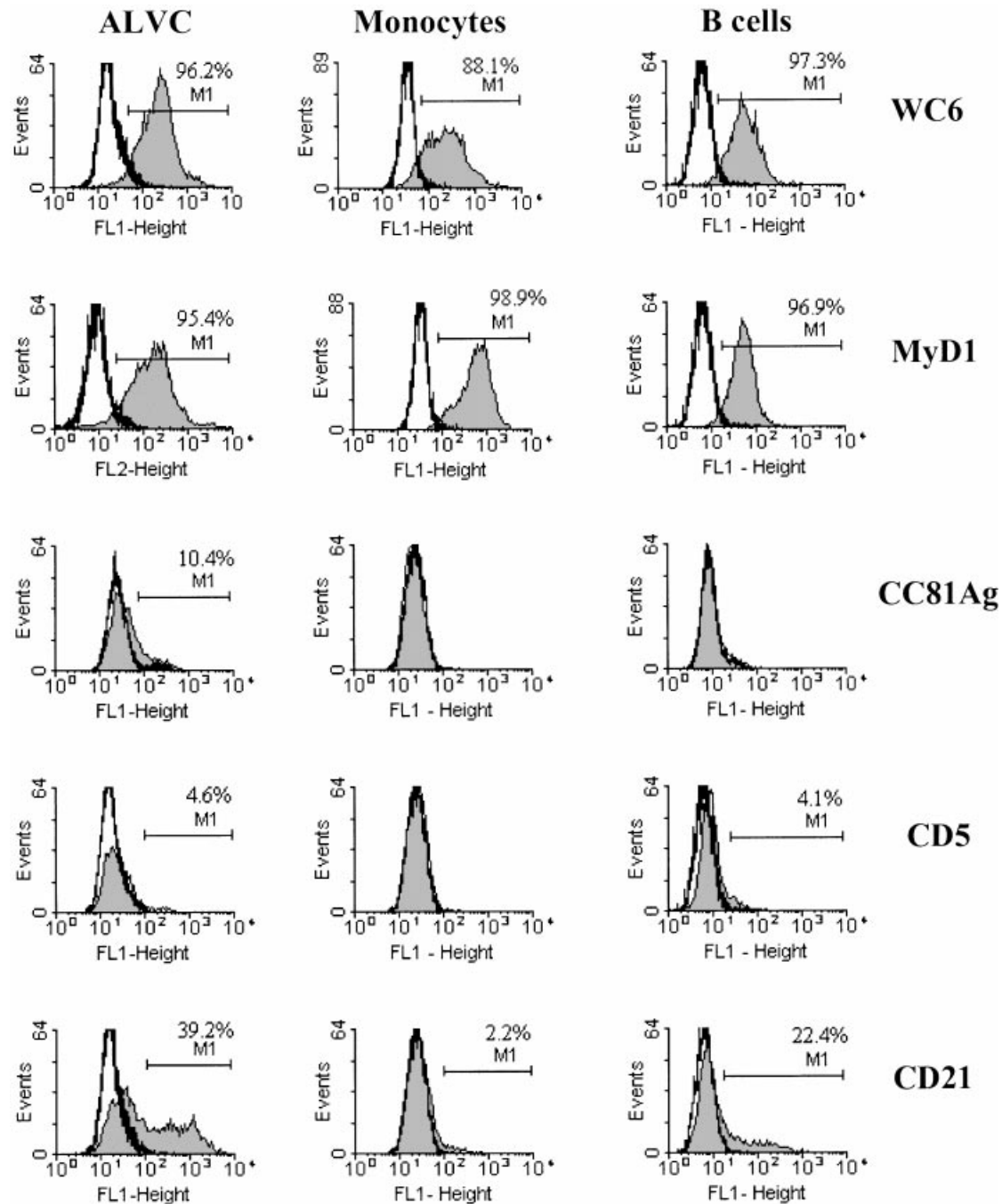
Fig. 1. Comparative expression of typical myeloid and B cell surface antigens by *Theileria annulata*-infected cell lines. Histograms show staining of cell lines produced from sorted ALVC, monocytes and B cells. Cell lines were stained at 21 days after infection (2nd passage) for expression of WC6 (mAb IL-A114 or ILA-53), MyD1 (mAb IL-A24), the antigen stained by mAb CC81, CD5 (mAb CC29) and CD21 (mAb CC21). MAb were detected with FITC-labelled goat anti-mouse IgG1 mAb or PE-labelled goat anti-mouse IgG2a. Open histograms show negative control cells from the same line stained with isotype-matched avian mAb.

virtually no expression. A small number ($< 5\%$) of cells in some monocyte-derived lines also showed low level CD21 expression but the majority were negative. There was no evidence of expression of either CD1b (mAb CC14) or surface immunoglobulin (sIg) (mAb IL-A59) on any of the ALVC-derived cell lines examined (results not shown).

Comparison of MHC expression transformed cells

Fig. 2 shows typical results for the expression of MHC classes I and II on infected ALVC, monocyte and B cell derived lines at 19–25 days post-infection (p.i.) (2nd passage). The expression of MHC class II by infected ALVC cell lines appeared to be much
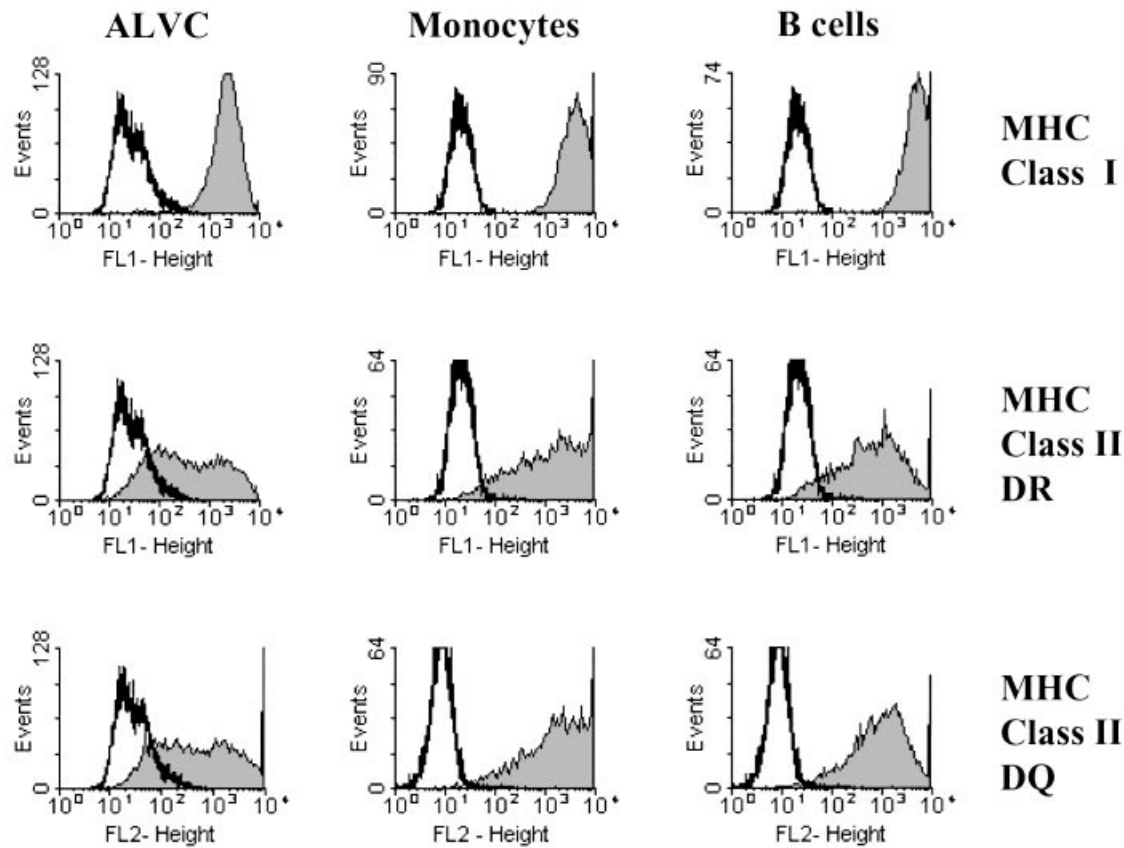
Fig. 2. Comparative expression of MHC class I and class II on *Theileria annulata*-infected cell lines derived from ALVC, monocytes or B cells respectively. AL was stained with mAb IL-A53 (WC6) and phycoerythrin (PE) labelled anti-murine mAb. ALVC (high FSC, WC6+) were FACS sorted and infected with *T. annulata*. Monocytes and B cells for infection were isolated from peripheral blood by the use of mAb CCG33 (CD14) or IL-A59 (sIg) and anti-murine magnetic beads. Cell lines were stained at 19–25 days post-infection (2nd passage) for expression of MHC class I (mAb IL-A88), MHC class II DR (mAb CC108) and DQ (mAb CC158) detected with FITC or PE conjugated goat anti-mouse IgG. Open histograms show isotype-matched negative controls – cells from the same lines stained with avian mAb AV20 (IgG1) or AV37 (IgG2a).

more variable than that seen and previously reported for monocytes and B cells (Howard *et al*. 1993; Glass *et al*. 1989; Spooner *et al*. 1989). Whilst most of the cell lines examined did show expression of MHC class II at the time of the second passage (19 days p.i.) the intensity of expression was noticeably lower on many of the infected cells than on uninfected ALVC or on infected monocyte or B cell lines. The sample shown in Fig. 2 shows a typical staining profile for infected ALVC lines where there appears to be a bimodal distribution of MHC class II expression. Although some transformed ALVC continue to express MHC class II at high intensity, a significant proportion of infected cells seem to have down-regulated expression of this antigen. In some of the samples stained up to 70% of transformed ALVC showed low or no expression of MHC class II at this time-point. Further staining of ALVC lines at different time-points produced even more variable results. Some lines appeared to show down-regulation of MHC class II expression up to 60 days p.i.

whereas other lines showed no consistent pattern of expression over time.

Comparison of ALVC subsets after transformation with sporozoites

The infection of the 2 separate populations of ALVC showed that it is possible to infect and transform both CC81Ag+ and CC81Ag− ALVC with *T. annulata*. The transformed cell lines derived from both of these populations were remarkably similar in phenotype for all of the lines examined (Fig. 3). As expected, all of the lines expressed the WC6 antigen but more surprisingly the majority of cells in all lines also expressed MyD1, suggesting that expression of this antigen is upregulated following infection and transformation of the CC81Ag+ ALVC (which do not express MyD1 in the uninfected state (Howard *et al*. 1997)). There was no significant expression of CD5 by either population following transformation but some residual expression of CC81Ag seemed to
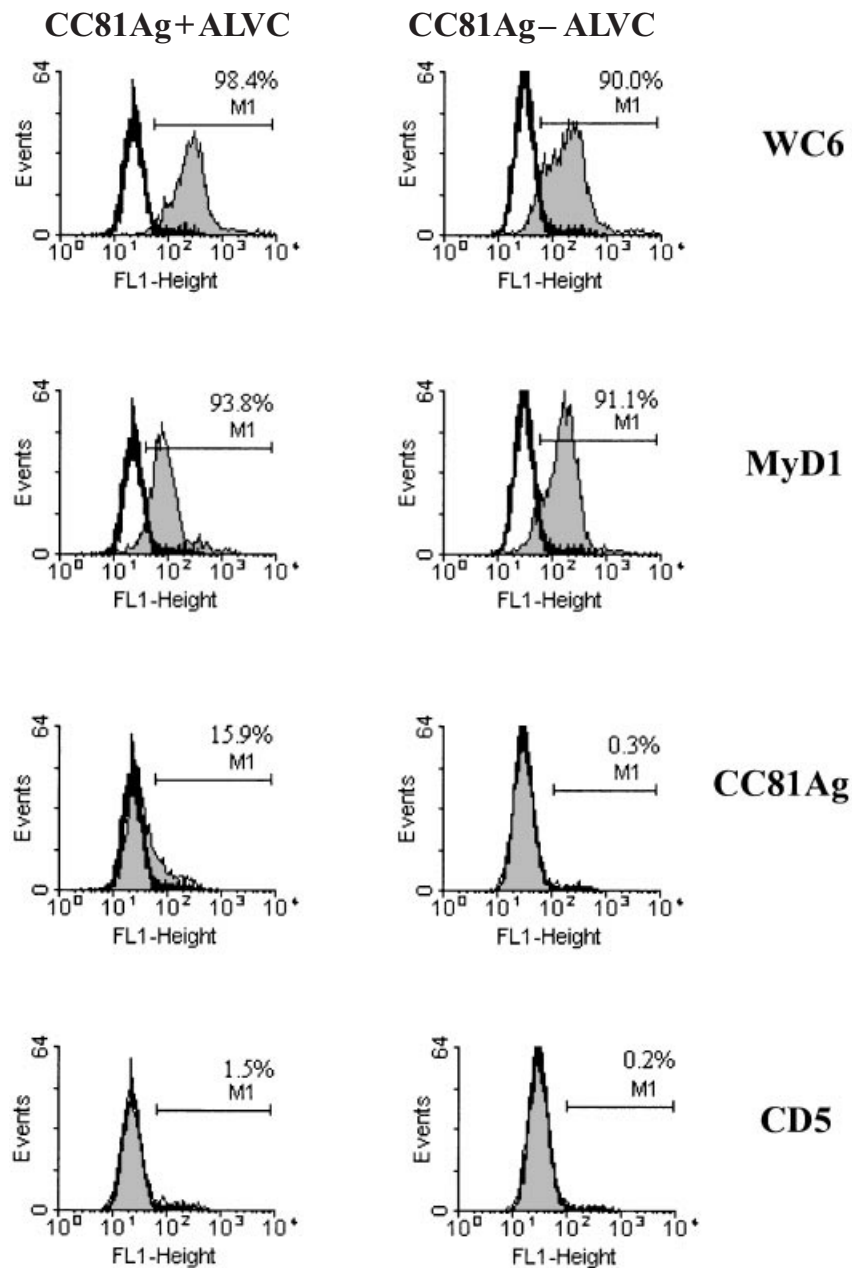
Fig. 3. Comparative expression of surface antigens by CC81Ag+ and CC81Ag− ALVC-derived cell lines. AL was stained with mAb CC81 (CC81Ag) and IL-A53 (WC6). ALVC (WC6+) were FACS sorted into CC81Ag+ and CC81Ag− populations and infected with *Theileria annulata*. Infected cell lines were stained at 19 days (2nd passage) for expression of WC6 (mAb IL-A114), MyD1 (mAb IL-A24), CC81 antigen and CD5 (mAb CC29) detected with FITC conjugated goat anti-mouse IgG. Open histograms show negative controls – cells from the same lines stained with avian mAb AV20.

remain on a minority of cells in lines derived from CC81Ag+ ALVC. There was no evidence of any CC81Ag expression on CC81Ag− ALVC derived cell lines.

DISCUSSION

The results of these experiments provide the first evidence that bovine dendritic cells can become infected with, and transformed by, the parasite *T. annulata*.

Establishing with certainty the identity of cells which have become infected and transformed by *T. annulata* remains problematic due to the changes in expression of lineage-specific markers by transformed cells. However, the results from this study strongly support the conclusion that transformed cell lines were generated from ALVC rather than from contaminating monocytes or B cells in the samples. Firstly all the ALVC samples were sorted by flow cytometry and were > 99 % pure populations. Secondly, the CC81Ag+ population of ALVC

could easily be identified and separated from afferent lymph because of the exclusive high intensity expression of this antigen. In dilution studies using different starting numbers of each of the cell types it was noticed that the length of time until transformed and proliferating cells were visible microscopically was inversely proportional to the number of sorted 'target' cells present (excluding irradiated filler cells in the wells). Wells containing 5×10^4 sorted ALVC initially showed signs of proliferation as early as 8 days post-infection which was 2 days earlier than the results for similar numbers of either monocytes and B cells. Proliferation in wells containing 10^3 initial cells was not visible until at least 14 days post-infection for any of the cell types. Cell infection and transformation also failed to establish in wells containing 10^2 or less sorted cells. These results support the conclusion that the cell lines resulted from infection of the predominant cell type in the well rather than a minority of contaminating cells. The fact that transformation and proliferation was seen earliest in the wells containing ALVC could suggest that they are even more susceptible to infection than either monocytes or B cells. This clearly has implications when considering the most likely cell types to be transformed in natural *in vivo* infections.

The phenotypic analysis of *T. annulata*-infected cell lines also provides further evidence to support the conclusion that it is specifically the ALVC, not a minority of contaminating cells, which have become transformed in this study. Although expression of some lineage-specific markers appeared to be lost following infection and transformation the cell lines derived from ALVC did seem to show some characteristics which differed from both monocyte and B cell derived lines. In particular the apparent maintenance of low-level CC81Ag expression on a minority of cells at the time of the 2nd passage was only seen on ALVC-derived cell lines, more specifically this was only seen on CC81Ag+ ALVC-derived cell lines. Whilst ALVC-derived cell lines were similar to B cell-derived lines in that they showed variable expression of CD21, they did not express sIg, which has been reported in one study as a characteristic of transformed B cell clones (Sager, Bertoni & Jungi, 1998).

The pattern of expression of MHC class II on cell lines derived from infected ALVC also appears to be slightly different and more variable compared to that seen for both monocyte and B cell-derived lines, where expression was uniformly of high intensity. The expression of MHC class II varied considerably between different ALVC cell lines and time-points after infection and it was not possible to identify a specific pattern of expression by ALVC. This could have important consequences *in vivo* since MHC class II expression or absence on infected cells could affect their ability to present antigens. The reason for the variable expression is not clear; however, it could be related either to the stage of maturity of the ALVC at the time of infection or possibly the growth phase of the proliferating cells at the time of staining.

The expression of MyD1 by all of the infected lines was not unexpected since transformed B cells have been previously reported to upregulate expression of a similar myeloid marker stained by the mAb DH59B (Sager *et al.* 1999). It was interesting to note in this study that CC81Ag+ ALVC, which do not normally express MyD1, were also able to upregulate expression of this antigen following transformation. In this respect they seem to resemble infected B cells. Another observation from this study was that transformed B cell and CC81Ag+ ALVC appeared to show a lower mean intensity of expression of MyD1 than transformed monocytes or CC81Ag− ALVC. This loss of lineage markers and upregulation of markers considered to identify monocyte/macrophages emphasizes the difficulty in determining cell of origin from phenotypic studies of transformed cells.

The lack of previous reports of the infection of dendritic cells by *T. annulata* could be due to a number of factors. Firstly, the majority of phenotypic studies have been carried out in *in vitro* culture systems. Although bovine dendritic cells have been successfully produced by the culture of monocytes with appropriate cytokines (Werling *et al.* 1999) it is not known how these monocyte-derived DC relate to those found *in vivo*. It is possible that these DC are at a different maturation stage and have different functional properties compared to ALVC. A previous study with subsets of monocyte/macrophages has suggested that there may be differences in susceptibility to *T. annulata* infection depending on the maturation state of the cell, with mature monocytes reported to be more readily infected than immature ones (Campbell *et al.* 1994). A similar situation may occur with DC. The use of *in vitro* systems where PBMC are the target cells for infection might also be expected to favour the production of monocyte-derived transformed cell lines due to the much greater numbers of monocytes present in peripheral blood compared to DC precursors. Conversely, given the high frequency of DC present in the skin and lymph nodes where natural exposure to sporozoites would occur, transformed cells recovered from *in vivo* infections might be more likely to be derived from DC.

Although there have been no previous studies showing infection of DC with *T. annulata*, the related parasite, *T. parva*, has been reported to infect, but not transform, DC (Wells, Awino & McKeever, 1999). It is possible that this difference in ability of these two parasites to transform DC may be related to differences in the type of cell they target, since *T. parva* predominantly infects T cells whereas the main target for *T. annulata* is generally

considered to be cells of monocytic lineage (Glass *et al.* 1989).

The use of DC collected *ex vivo* from a superficial lymphatic vessel and unaltered by culture provides evidence that this type of infection could occur under natural physiological conditions. In view of the readiness with which the DC were infected and transformed by the sporozoites and the previous suggestion that, after subcutaneous inoculation, infected cells are probably generated from infection of lymph node cells (Campbell & Spooner, 1999) it seems highly likely that a primary target cell for the parasite in the lymph node may be the resident or recently immigrated dendritic cell as well as the resident macrophage. Indeed it is DC, not macrophages, that are the predominant APC in the T cell paracortex of the lymph node.

Previous studies have identified *T. annulata*-infected transformed cells as 'myeloid' based on their expression of MHC class II, CD11b and the MyD1 antigen (Glass & Spooner, 1990; Howard *et al.* 1993; Campbell & Spooner, 1999; Forsyth *et al.* 1997). The results from this study suggest that infected DC might be expected to express a similar phenotype and therefore would be impossible to distinguish from transformed macrophages on this basis.

Studies on the mechanisms of immune evasion by *T. annulata* have provided evidence that transformation of cells following infection leads to production of a number of cytokines which have been suggested to subvert the immune response by production of ineffective T cell responses (Campbell *et al.* 1997; Campbell & Spooner, 1999; Brown *et al.* 1995). Although antigen presentation by transformed cells leads to widespread T cell activation it has been suggested that the CD8+ T cells produced are not capable of cytotoxic activity, possibly due to a lack of CD2 expression affecting their ability to adhere to target cells (Campbell & Spooner, 1999; Campbell *et al.* 1997). Whilst the suggestion that transformed cells may be derived from DC rather than macrophages *in vivo* does not change this current understanding of the possible pathogenesis of the disease it does raise the question of the validity of *in vitro* systems where infection of purified CD14+ monocytes is used as a model. Previous work with the related parasite *T. parva* has shown differences in pathogenicity depending on the cell type infected by the parasite (Morrison, MacHugh & Lalor, 1996). In this case it is possible that there may be differences in cytokine production between transformed macrophages and DC which could lead to differences in the immune response depending on the primary cell type infected. Furthermore, there is now evidence to suggest that the 2 populations of ALVC tend to have different profiles of cytokine production (Stephens *et al.* submitted for publication) in the uninfected state. If these differences

were maintained following infection and transformation this might also lead to different immune responses depending on which cell type is initially transformed. It is clear that further work needs to be done to investigate cytokine production by the different populations of DC following transformation. Finally, the possibility that transformed cells *in vivo* may be derived from DC could affect the approaches which are required to manipulate immune responses to eliminate the parasite.

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