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L-selectin expression differentiates T cells isolated from different lymphoid tissues in cattle but does not correlate with memory

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SUMMARY

L-selectin (LECAM-1, LAM-1) was expressed by a high proportion of $CD4^+$ and $CD8^+$ T cells, as well as almost all of the $\gamma\delta$ T-cell receptor $(TcR)^+$ $(WC1^+)$ T cells, isolated from blood, lymph nodes or tonsils. $CD4^+$ T cells in the lamina propria of the gut villi and $CD8^+$ T cells in the villous epithelium as well as the majority of $WC1^+$ T cells in the gut mucosa were L-$selectin^-$. The proportion of T cells from Peyer's patches that synthesized the molecule was intermediate between the value for blood and gut mucosa. Expression of L-selectin therefore marks T cells in cattle with a distinct tissue distribution that correlates with its function as the peripheral node homing receptor. The proportion of $CD4^+$ and $CD8^+$ T cells in the circulation that were L-$selectin^+$ decreased with age. Unlike CD45R, expression of L-selectin was not related to CD4 T-cell memory as judged by proliferation in transformation assays to soluble antigen. Three-colour immunofluorescent staining demonstrated four subpopulations of CD4 and CD8 T lymphocytes in peripheral blood mononuclear cells (PBMC) that were $CD45R^+$, L-$selectin^+$; $CD45R^+$, L-$selectin^-$; $CD45R^-$, L-$selectin^+$; $CD45R^-$, L-$selectin^-$. $CD4^4$ memory cells were $CD45R^-$ and L-$selectin^+$ or L-$selectin^-$. Taken with earlier studies the reported observations demonstrated that only one of the four phenotypes of the $CD4^+$ T cells in blood is present in the lamina propria of the gut villi and these are $CD45R^-$, L-$selectin^-$. Two of the four phenotypes of $CD8^+$ T cells were present in the gut epithelium; these were $CD45R^+$, L-$selectin^-$ or $CD45R^-$, L-$selectin^-$. Expression of the bovine molecule was not rapidly down-regulated on T cells following activation by exposure to phorbol myristate acetate.

INTRODUCTION

Functionally distinct subpopulations of T lymphocytes can be identified on the basis of expression of surface molecules which participate in specific cellular functions. Thus, expression of molecules such as CD4 and CD8 reflect the capacity of T cells to recognize antigen in the context of class I or class II major histocompatibility complex (MHC) respectively, expressions of certain isoforms of CD45 indicate whether or not cells have experienced antigenic stimulation and expression of homing receptors such as L-selectin (LECAM-1, LAM-1) and VLA-4 reflect the migratory capacity of the cells.[1,2] Definition of the expression of these molecules, coupled with knowledge of their function, can contribute significantly to understanding how such responses are induced *in vivo*.

Abbreviations: IAH, Institute for Animal Health; HEV, high endothelial venules; mAb, monoclonal antibody; OVA, ovalbumin; PBMC, peripheral blood mononuclear cells; PE, phycoerythrin; PMA, phorbol 12-myristate 13-acetate; SA, streptavidin; WC, workshop cluster (bovine).

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There are three major populations of T cells in cattle. In addition to the two populations of $\alpha\beta$ T-cell receptor (TcR) cells which express $CD2^+$ and either $CD4^+$ or $CD8^+$ there is a large population of $\gamma\delta$ TcR T cells that are $CD2^-$, $CD4^-$, $CD8^-$ and can be identified by expression of an antigen with a M_r of 215,000/300,000 called WC1.[3,4] Other numerically minor populations are evident in blood such as $CD8^+$, $\gamma\delta$ T cells and $CD2^+$, $CD8^-$, $CD4^-$, $WC1^-$ cells.[5,6] Recently an isoform of the leucocyte common antigen (CD45) has been shown to define subpopulations within the $CD4^+$ and $CD8^+$ T cells. This molecule was found to be expressed on naive but not memory $CD4^+$ T cells in the peripheral blood of cattle immunized with *Trypanosoma brucei* variable surface glycoprotein antigen.[7] This CD45 isoform is present on a high proportion of CD4 and CD8 T cells in the circulation of young calves and a much smaller proportion of the cells in adults. It is also absent from CD4 T cells in the lamina propria of the gut mucosa indicating that the CD4 T cells in the gut represent a subpopulation of those in the circulation.

In this paper the expression of the peripheral node homing receptor L-selectin, also called LECAM-1 or LAM-1 in man and MEL-14 in mice,[8–10] on $\alpha\beta$ and $\gamma\delta$ T cells isolated from different lymphoid compartments has been established, its relation to memory in CD4 T cells assessed and the implications

of the observations for T-cell migration and function considered.

MATERIALS AND METHODS

Animals and cells

Cattle were conventionally reared *Bos taurus* Friesian or Friesian crossed with Aberdeen Angus held at the Institute for Animal Health (IAH). Immunization was with 2 mg ovalbumin (OVA) subcutaneously in incomplete Freund's adjuvant on three occasions.

Peripheral blood mononuclear cells (PBMC) and suspensions of cells from lymphoid tissues were prepared as described previously[7] from blood taken into heparin or from lymph nodes, tonsils and spleens teased apart in phosphate-buffered saline and separated on Histopaque 1083 (Sigma, Poole, U.K.). Discrete Peyer's patches from the duodenum and gut mucosa taken from areas of the duodenum devoid of Peyer's patches, were prepared as previously recorded except that collagenase was omitted from the enzyme mixture used to digest tissue.[7] Preliminary studies indicated that some batches of collagenase removed L-selectin from cells. Control experiments with collagenase omitted from the reaction mixture showed no effect on expression of the molecule on cells from lymph nodes.

Monoclonal antibody (mAb) and flow cytometry

Monoclonal antibody to bovine CD2 (IAH-CC42), CD4 (IAH-CC8 or IAH-CC30), CD5 (IAH-CC17), CD6 (IAH-CC38), CD8 (IAH-CC63 or IAH-CC58), have been described previously.[4] Monoclonal antibody IAH-CC21 recognizes a 145,000 MW molecule expressed on bovine B cells called WC3,[4,11] mAb IL-A30 reacts with bovine IgM,[12] mAb IL-A24 recognizes an antigen present on bovine monocytes and granulocytes,[13] mAb IAH-CC15 recognizes the 215,000/300,000 MW molecule expressed on $CD4^-$, $CD8^-$ $\gamma\delta$ T cells,[3] called WC1.[4] Monoclonal antibody IAH-CC76 recognizes an isoform of the bovine leucocyte common antigen (CD45R) expressed on naive but not memory $CD4^+$ T cells.[7] Murine mAb IAH-CC32, isotype IgG1, was produced as described previously from the spleen of a mouse that had been inoculated with bovine lymphocytes.[14] This mAb is considered to react with L-selectin on bovine lymphocytes. It stains the same cells in bovine PBMC as mAb LAM1.4 which recognizes human L-selectin but which cross-reacts with bovine cells.[10] Furthermore, mAb LAM1.4 blocks the binding of biotin-coupled mAb CC32 to bovine PBMC and both mAb immunoprecipitate a molecule with the same M_r from surface-iodinated bovine PBMC.

One- and two-colour immunofluorescent staining were analysed with a FACScan (Becton Dickinson, Mountain View, CA) as described previously.[7] For sorting PBMC were stained with fluorescein isothiocyanate (FITC) and biotin-coupled mAb and streptavidin–phycoerythrin (SA–PE) in a similar manner and sorted with a FACStar-plus. Three-colour immunofluorescent staining involved reacting cells with two mAb of different isotypes followed by FITC goat anti-mouse IgG1 and PE goat anti-mouse IgG2a (Southern Biotechnology Associates, Birmingham, AL) incubation with normal mouse serum then biotin-coupled third antibody followed by allophycocyanin–streptavidin (APC–SA; Becton Dickinson). Samples were analysed on a FACStar-plus with excitation wavelengths of 488 nm from an argon-ion laser and 633 nm from a helium-neon laser.

Biochemical characterization

Cell surface proteins of PBMC were labelled with ^{125}I using lactoperoxidase, immunoprecipitated with protein G-Sepharose (Pharmacia, Uppsala, Sweden) and examined under reducing conditions by SDS–PAGE. Precipitated molecules were visualized in dried gels by autoradiography.[14]

Assessment of proliferative responses

PBMC from calves immunized with OVA were stained with FITC-coupled mAb CC30 (CD4) and biotin-coupled CC32 (L-selectin) or CC76 (CD45R) followed by SA–PE and sorted. 2×10^5 sorted cells were cultured with 10^5 autologous PBMC that had been exposed to 20 Gy γ-irradiation from a ^{137}Cs source. OVA was added to appropriate wells in the 96-well microculture plates to give a final concentration of 20 μg/ml. Culture medium was RPMI-1640 medium supplemented with 10% heat-inactivated foetal calf serum (FCS), 2 mM L-glutamine, 25 mM HEPES and 50 μg/ml gentamycin. Cells were cultured for 5 days at 37° in a humidified atmosphere of 5% CO_2 in air. Proliferation was measured by the addition of 1 μCi of [^{3}H]thymidine for the final 18 hr of the culture period.

Effect of phorbol myristate acetate on surface antigen expression

PBMC were cultured in medium with and without various concentrations of phorbol 12-myristate 13-acetate (PMA) with and without ionomycin for periods of 10 min to 1½ hr. Cells were stained with mAb and the effect on intensity of immunofluorescent staining assessed by flow cytometry.

RESULTS

Biochemical characterization of bovine L-selectin

Analysis of immunoprecipitates obtained with mAb CC32 or LAM1.4 from surface labelled PBMC, by SDS–PAGE run under reducing conditions, revealed a molecule of 90,000 MW (Fig. 1). This value is slightly larger than the value of 80,000 MW reported for MEL-14 in mice[9] and the M_r of 74,000 for L-selectin expressed on human lymphocytes.[15]

Expression of L-selectin on subpopulations of leucocytes from blood

Differences in expression of L-selectin by leucocytes in the circulation was assessed by two-colour flow cytometry (Fig. 2). The molecule was demonstrated on subpopulations of T lymphocytes that expressed the CD2, CD4 and CD8 antigens. The $\gamma\delta$ T cells that were $WC1^+$ all expressed L-selectin. A subpopulation of B cells that were $sIgM^+$ and $WC3^+$ was L-$selectin^+$. Monocytes in PBMC that reacted with mAb IL-A24 and granulocytes from lysed blood, identified on the basis of their high granularity, all expressed L-selectin.

The proportion of L-$selectin^+$ T cells decreases with age

The proportions of the CD4 and CD8 subpopulations in PBMC that were L-$selectin^+$ changed markedly with age (Table 1). The majority of $CD4^+$ or $CD8^+$ T cells in the blood of 4–6-day-old

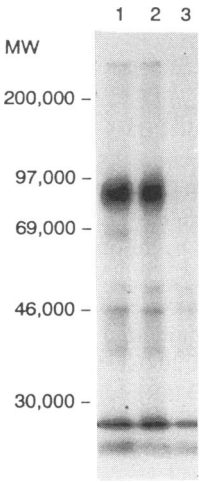

Figure 1. Biochemical analysis of bovine L-selectin recognized by mAb CC32 and LAM-1.4. Immunoprecipitates from ^{125}I surface-labelled PBMC were run on 7·5% polyacrylamide gels under reducing conditions. Immunoprecipitates with mAb CC32 (lane 1), mAb LAM-1.4 (lane 2) and mAb to respiratory syncytial virus (lane 3).

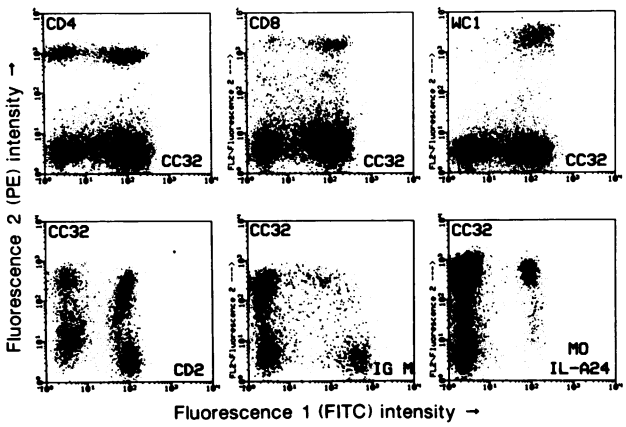

Figure 2. Two-colour immunofluorescent staining with mAb to bovine L-selectin. PBMC were stained with the first mAb followed by FITC goat anti-mouse Ig (FL1, FITC), biotin-conjugated second mAb and streptavidin-PE (FL2, PE). Monoclonal antibodies were—CC32, L-selectin; CC8, CD4; CC63, CD8; CC15, WC1; CC42, CD2; IL-A30, IgM; IL-A24, monocytes and granulocytes in PBMC.

calves were positive, 90% and 83% respectively, whereas only 50% of the CD4$^+$ and 60% of the CD8$^+$ T lymphocytes from adult cows were L-selectin$^+$. The proportions in calves about 6 months of age were intermediate.

The majority of T cells in the gut mucosa do not express L-selectin

In order to investigate whether subpopulations of T cells that were L-selectin$^+$ or L-selectin$^-$ had a distinctive and heterogenous tissue distribution two-colour flow cytometry was used together with mAb specific for the three major populations of bovine T cells to stain cells isolated from different tissues. Significant L-selectin$^+$ and L-selectin$^-$ subpopulations of lymphocytes that expressed the CD4 or CD8 antigens were present in suspensions of cells from the spleen and lymph nodes from adult cattle (Table 2). In marked contrast, < 1% of CD4 or CD8 T cells isolated from the gut mucosa were L-selectin$^+$. The proportion of CD4$^+$ or CD8$^+$ T cells from discrete Peyer's patches that were L-selectin$^+$ was 19%, a value intermediate between that seen for cells from the gut mucosa and lymph nodes. Interestingly, in the tonsil 67% and 80% of the CD4$^+$ and CD8$^+$ T cells were L-selectin$^+$, a higher value than seen in any other tissue examined including blood and most similar to values seen for prescapular lymph nodes, the opposite to findings in the gut. The proportion of WC1$^+$ T cells in many of the suspensions of lymphocytes from adult tissues was low but in contrast to blood the majority of WC1$^+$ cells in spleen and gut mucosa were L-selectin$^-$.

Response of sorted CD4$^+$, L-selectin$^+$ and CD4$^+$, L-selectin$^-$ cells to stimulation *in vitro* with OVA

In order to establish whether expression of L-selectin was associated with memory the proliferative response of PBMC and of sorted cells from PBMC from an animal immunized with OVA were assessed. The results of one experiment in which CD4$^+$, L-selectin$^+$ and CD4$^+$, L-selectin$^-$ cells were compared with autologous irradiated PBMC used as antigen-presenting cells are shown in Table 3. Both the L-selectin$^+$ (CC32$^+$) and the L-selectin$^-$ (CC32$^-$) subpopulations proliferated in response to OVA. In repeated experiments identical results were obtained. As a further control, CD4$^+$, CD45R$^+$ (CC76$^+$) and CD4$^+$, CD45R$^-$ cells sorted from PBMC from the same calf were tested for their response to OVA using the same *in vitro* conditions (Table 4). In these sorted cell populations the responding lymphocytes were entirely within the CD45R$^-$ population confirming previous findings with variable surface glycoprotein from *T. brucei*.[7]

L-selectin expression does not correlate with expression of CD45R

The changes in L-selectin expression associated with age and lack of expression on CD4$^+$ cells in the gut mucosa correlated with previous observations relating to CD45R but proliferative responses failed to confirm any relation. Three-colour immunofluorescent staining was used to examine coincident expression of L-selectin and CD45R on CD4 and CD8 T cells (Table 5). Four subpopulations of CD4$^+$ and CD8$^+$ cells were evident in the circulation of both calves and adult cattle indicating that expression of L-selectin and CD45R were independently variable.

L-selectin expression is retained by T cells exposed to PMA

Down-regulation of L-selectin as a consequence of, or in association with, activation of T cells would profoundly affect the migration of the cells. In a series of experiments PBMC were incubated with PMA concentrations of 3 ng/ml, 10 ng/ml and 50 ng/ml for 10, 60 or 90 min with and without 0·6 μg/ml ionomycin and the effect on expression of the CD4 and CD8 antigens and L-selectin assessed. In all cases similar results were obtained to that shown in Fig. 3. A fall in the intensity of staining with mAb to the CD4 antigen was observed and no change in intensity of

Table 1. Differences in the expression of L-selectin, recognized by mAb CC32, by peripheral blood T cells from cattle of different ages

	Percentage of PBMC with indicated phenotype*					
	L-selectin					
	+	−	+	−	+	−
Animal age	$CD4^+$		$CD8^+$		$WC1^+$	
3–6 days	26±4·8	3±2·2	15±5·7	3±2·1	31±12·9	<1±0
6 months	23±7·7	10±1·3	10±1·9	6±0·6	7±1·6	<1±0
>3 years	12±3·2	14±0·4	15±4·2	8±2·1	4±2·4	<1±0·3

* Percentage ± SD given for groups of three animals

Table 2. Expression of L-selectin, detected with mAb CC32, by $CD4^+$, $CD8^+$ or $WC1^+$ cells in lymphoid tissues of adult cattle

	L-selectin					
	+	−	+	−	+	−
Tissues*	CD4		CD8		WC1	
PsLN	18±11·4	14±8·4	11±5·4	3±5·4	3±1·8	1±0·4
BLN	14±8·4	18±11·4	11±5·4	3±0·8	3±1·8	1±0·4
MLN	9±4·1	32±7·7	8±0·4	10±4·2	1±0·7	2±0·4
Spleen	4±1·4	9±6·8	7±5·1	18±8·3	3±0·8	5±2·6
DPP	6±2·5	25±8·7	3±0·9	13±5·0	1±5·0	1±0·5
Mucosa	1±0	25±18·7	1±0·5	47±16·3	2±0·9	7±1·7
Tonsil	22±8·3	11±2·6	8±4·8	2±0·9	1±0·2	1±0·1

* Tissues: PsLN, prescapular lymph node; BLN, bronchial lymph node; MLN, mesenteric lymph node; DPP, discrete Peyer's patch; mucosa, non-Peyer's patch gut lucosa; $n=3$, mean % ± SD given.

Table 3. Proliferation induced by OVA in sorted $CD4^+$, L-$selectin^+$ and $CD4^+$, L-$selectin^-$ T cells

	C.p.m.* incorporated by indicated cell population				
	PBMC	PBMC†	Stained PBMC not sorted	Sorted $CD4^+$ L-$selectin^+$	Sorted $CD4^+$ L-$selectin^-$
Cells alone	259	209	148	157	175
Cells + OVA	35,550	126	—	403	132
Cells + PBMC†	—	—	—	143	163
Cells + PBMC† + OVA	—	—	53,409	38,191	47,198

* Mean of three wells.
† PBMC exposed to 20 Gy irradiation.

staining with mAb to the CD8 antigen. Intensity of staining with mAb CC32 was marginally less in cells exposed to PMA or PMA and ionomycin than for controls but the reduced intensity was much less than that observed for staining with CD4 mAb. Thus, a district down-regulation of expression of L-selectin as a result of incubation in the presence of PMA with or without additional ionomycin was not evident. PMA and ionomycin induced extensive proliferation of cultures of bovine PMBC; PMA alone induced a small proliferative response.

DISCUSSION

Analysis of the expression of L-selectin has allowed a more precise definition of the phenotypes of T cells in cattle and the

Table 4. Proliferation induced by OVA in sorted CD4$^+$, CD45R$^+$ and CD4$^+$, CD45R$^-$ T cells

	C.p.m.* incorporated by indicated cell population			
	PBMC	PBMC†	Sorted CD4$^+$ CD45R$^+$	Sorted CD4$^+$ CD45R$^-$
Cells alone	263	556	194	252
Cells + OVA	29,334	327	265	268
Cells + PBMC†	—	—	227	248
Cells + PBMC† + OVA	—	—	202	69,651

* Mean of three wells.
† PBMC exposed to 20 Gy irradiation.

Table 5. Three-colour immunofluorescent staining of PBMC

Phenotype	Percentage of CD4$^+$ or CD8$^+$ T cells stained for L-selectin and for CD45R ± SD	
	Calves* ($n=3$)	Adult† ($n=4$)
CD4$^+$		
L-selectin$^+$ CD45R$^+$	85 ± 5·0	23 ± 1·6
L-selectin$^+$ CD45R$^-$	6 ± 1·3	16 ± 3·3
L-selectin$^-$ CD45R$^+$	2 ± 1·3	9 ± 0·8
L-selectin$^-$ CD45R$^-$	7 ± 3·1	52 ± 2·4
CD8$^+$		
L-selectin$^+$ CD45R$^+$	76 ± 2·2	49 ± 9·2
L-selectin$^+$ CD45R$^-$	15 ± 2·4	7 ± 2·2
L-selectin$^-$ CD45R$^+$	4 ± 1·0	27 ± 11·3
L-selectin$^-$ CD45R$^-$	5 ± 1·4	18 ± 4·0

* $n=3$, age 4–6 weeks.
† $n=4$, age > 3 years.
mAb CC32-L-selectin, CC76-CD45R, CC8-CD4, CC63-CD8.

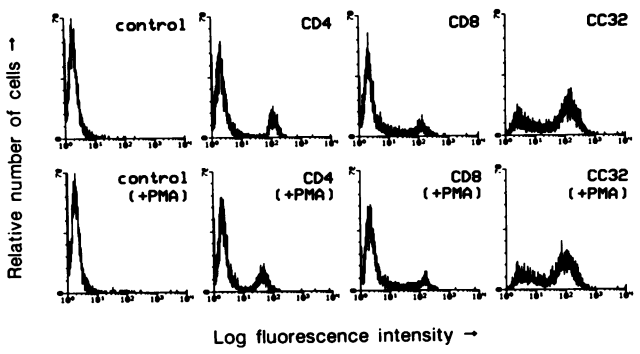

Figure 3. Effect of incubation with PMA on surface staining. PBMC were incubated in medium or in medium containing 50 ng PMA for 90 min and stained with mAb to indicated antigen followed by FITC goat anti-mouse Ig. Control, minus mAb plus FITC anti-mouse Ig.

results obtained have a number of implications concerning their migration. Our observations show that certain of the defined phenotypes of T cells exhibit a very restricted distribution within different tissues. In blood taken from adult cattle four phenotypically distinct subpopulations of cells are evident within the CD4 or CD8 populations of T cells. These are L-selectin$^+$, CD45R$^+$; L-selectin$^+$, CD45R$^-$; L-selectin$^-$, CD45R$^+$; L-selectin$^-$, CD45R$^-$. In the non-Peyer's patch gut mucosa CD4$^+$ T cells are primarily situated in the lamina propria.[16] Taken with earlier findings[7] the observations indicate that the CD4$^+$ cells at this site are all L-selectin$^-$ and CD45R$^-$. The bovine gut CD8$^+$ T cells, which are primarily situated in the epithelium,[16] were also L-selectin$^-$ but previous studies have shown them to be CD45R$^+$ or CD45R$^-$.[7] Although all of the WC1$^+$ $\gamma\delta$ T cells in the circulation were L-selectin$^+$ the majority of these cells in the gut were L-selectin$^-$. The lack of expression of L-selectin essentially on all three of the major bovine T-cell populations in the gut epithelium thus is consistent with its function as the peripheral node homing receptor.

As animals aged the proportion of CD4$^+$ and CD8$^+$ T cells in the circulation that were L-selectin$^-$ increased. Lymphocytes derived from the gut-associated lymphoid tissues may contribute to this increasing proportion of L-selectin$^-$ T cells in the circulation; lymphocytes that have recently emigrated through the thoracic duct from the gut are estimated to contribute 5–10% of the lymphocytes in PBMC.[17] In contrast to the CD4$^+$ and CD8$^+$ T cells the WC1$^+$ $\gamma\delta$ T cells in the circulation remained L-selectin$^+$ as animals aged. The absence of WC1$^+$, L-selectin$^-$ cells in the blood may reflect a failure of the gut population to recirculate extensively from the mucosa. In mice there is evidence for a locally self-renewing population of intra-epithelial $\gamma\delta$ T cells[18] but the relation of these cells to those in cattle is unknown. The WC1$^+$ lymphocytes comprise only about 3% of cells in lymph nodes and are primarily situated in the subcapsular cortex and trabeculae, not the paracortex.[3] Although these cells are a very minor proportion of the resident T cells in nodes, it has been reported in sheep[19] that they represent 12% of PBMC and comprise 10% of lymphocytes in efferent lymph, which must be derived from lymph nodes. If the proportion of WC1$^+$ cells is calculated as a percentage of the total T cells (CD4 + CD8 + WC1) then these $\gamma\delta$ cells comprise 34% of T cells in blood, 36% of T cells in afferent lymph and 16% of T cells in efferent lymph. The smaller proportion of the WC1$^+$ cells in the T-cell populations in efferent lymph compared to blood would appear to reflect a reduced capacity,

compared to CD4 T cells, to recirculate through the node. It has been estimated that 90% of lymphocytes in efferent lymph are derived from the blood[20] and taken together the observations indicate that some recirculation of the WC1$^+$ T cells occurs through the node that involves L-selectin and binding to high endothelial venules (HEV).

The expression of L-selectin on Peyer's patch T cells was intermediate between that of the mucosa and lymph nodes. Monoclonal antibody to mouse L-selectin has been shown to partially block homing of lymphocytes to Peyer's patches[21] indicating a role for this molecule in binding to HEV in the organized lymphoid tissue in the gut wall, although it is clear that the HEV of the Peyer's patches also express molecules specific for homing receptors other than L-selectin.[1,22] Other studies in mice[23] indicated that the number of T cells in the gut mucosa increases after birth upon antigenic stimulation of the Peyer's patches. Differences in L-selectin expression seen on T cells from the bovine Peyer's patch may partially distinguish recent immigrants from the blood and potential emigrants likely to home preferentially to the gut mucosa. Other preliminary studies in cattle (C. J. Howard, unpublished observations) have demonstrated CD8$^+$ T cells in the epithelium of the gut of a mature foetus and neonatal calves but few CD4$^+$ T cells in the lamina propria of the gut villi. The CD4$^+$ T cells found in the lamina propria of the gut postnatally may therefore be derived from the Peyer's patches following local exposure to antigen. A large number of L-selectin$^-$ T cells were evident in the spleen, an observation consistent with the known absence of HEV in this organ and in accord with *in vivo* observations in mice where mAb MEL-14 failed to block lymphocyte homing to the spleen.[17,21] Expression of L-selectin on T cells from the tonsil contrasted markedly with those of the gut epithelium or Peyer's patches. The greater majority of tonsillar T cells were L-selectin$^+$ and thus would be expected to migrate preferentially via the peripheral nodes and not the gut. Thus memory T cells derived from the tonsil should circulate primarily through the peripheral nodes, and vice versa, allowing potentiation of secondary systemic responses.

As noted above, the proportion of L-selectin$^-$ cells within the CD4 and CD8 T-cell populations in the circulation of cattle increased with age. A similar observation was made previously, and confirmed here, for expression of restricted isoforms of CD45.[7] In this case expression of CD45R was restricted to naive T cells, as judged by proliferative responses to soluble antigen, and as animals aged the proportion of T cells in the peripheral circulation that were CD45R$^-$ increased. Furthermore, all of the CD4$^+$ T cells in the gut mucosa were CD45R$^-$, i.e. had the phenotype of memory cells. Evidence has been presented in mice that memory CD4$^+$ T cells are MEL-14$^-$.[24] However, human L-selectin$^+$ and L-selectin$^-$CD4$^+$ memory T cells proliferated in response to soluble antigen although the time–courses varied.[15,25] The T-cell proliferative response to soluble antigen of sorted cells taken from a calf that had been inoculated subcutaneously was entirely within the CD4$^+$, CD45R$^-$ population but equally distributed within the CD4$^+$,L-selectin$^+$ and CD4$^+$, L-selectin$^-$ populations. It is therefore apparent that memory CD4$^+$ cells defined by proliferation assays are CD45R$^-$ and L-selectin$^+$ or L-selectin$^-$. It would be expected that the route of administration of antigen could affect the phenotype of the memory cell. The association of memory with lack of L-selectin expression in mice may be a result of priming splenic T cells following intraperitoneal inoculation in contrast to the intramuscular route usually employed for tetanus toxoid in man. In cattle and man memory T cells may have been generated from both L-selectin$^+$ and L-selectin$^-$ naive precursors derived from cells that had entered the node from blood, following binding to HEV for L-selectin$^+$ cells, or from afferent lymph, for both negative and positive cells. We have no evidence for, or against, the alternative possibility that L-selectin$^+$ cells may become temporarily or permanently negative after stimulation by antigen.

Expression of bovine L-selectin was not rapidly lost after exposure of lymphocytes to PMA unlike the molecule on human cells[26–28] for which extremely rapid down-regulation was elicited in association with the activation of protein kinase C. Long-term down-regulation of L-selectin could markedly alter the recirculation pathway after activation, rapid down-regulation may be involved in de-adhesion of cells after diapedesis or prevention of recirculation after local activation.[28] Only expression of L-selectin by lymphocytes following PMA exposure was examined in cattle. Longer-term activation experiments and an examination of neutrophil expression could reveal the functioning of different mechanisms.

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