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Identification of γδT lymphocyte subsets that populate calf ileal mucosa after birth

C.R. Wyatt [a,*], E.J. Brackett [a], L.E. Perryman [b], W.C. Davis [a]

[a] *Department of Veterinary Microbiology and Pathology and Animal Health Research Center, College of Veterinary Medicine, Washington State University, Pullman, WA 99164-7040, USA*

[b] *Department of Microbiology, Pathology, and Parasitology, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough St., Raleigh, NC 27606, USA*

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Abstract

Ileal intraepithelial and lamina propria lymphocytes from newborn, 1.5-week-old, and 3-week-old calves were compared to determine to what extent the mucosa becomes populated after birth. Single and dual fluorescence flow cytometry were used with monoclonal antibodies to bovine (Bo) CD molecules to identify lymphocyte subpopulations. Few ileal mucosal lymphocytes were present in calves at birth. However, by 1.5 weeks of age, the villi were populated with large numbers of lymphocytes, and by 3 weeks of age, the numbers had increased further. These included a prominent subpopulation of γδT cells. Several subsets of γδT cells populated ileal mucosa after birth. The predominant subset coexpressed BoCD2, and a smaller subset coexpressed BoCD8. $WC1^+$ γδT cells comprised the smallest subset. All γδT cell subsets coexpressed ACT2, a molecule expressed on activated $WC1^+$ and $WC1^-$ γδT cells from cattle.

Keywords: Mucosa; Ileum; $TcR1^+$ lymphocytes; Calves

1. Introduction

Bovine lymphocytes are composed of three major subpopulations. B lymphocytes express a surface form of IgM and several non-immunoglobulin molecules (Morrison et

* Corresponding author. Tel.: (509) 335-6046; fax: 509-335-8529.

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al., 1988; Griebel et al., 1992; Mukwedeya et al., 1993). T lymphocytes that express the $\alpha\beta$ T cell receptor (TcR2) coexpress either BoCD4 (T helper lymphocytes) (Baldwin et al., 1986), or BoCD8 (T cytotoxic/suppressor lymphocytes) (Ellis et al., 1986). $\gamma\delta$ TcR^+ (TcR1) lymphocytes comprise the third major subpopulation. $TcR1^+$ cells are a prominent subpopulation, especially in young animals. $TcR1^+$ cells in cattle 1 week to 2 years of age can comprise 40–80% of total lymphocytes in peripheral blood and 35% of lymphocytes in spleen (Hein and Mackay, 1991; Wyatt et al., 1994). The prominence of $TcR1^+$ cells in peripheral blood is attributable to a subset of $TcR1^+$ cells that expresses a high molecular weight molecule (workshop cluster 1, WC1; Wijngaard et al., 1992; Crocker et al., 1993; MacHugh et al., 1993b). The prominence of $TcR1^+$ cells in spleen is attributable to a $WC1^+$ subset that coexpresses BoCD2 and BoCD6 (Davis et al., 1990; Sopp et al., 1991). The $WC1^-$ subset represents a very small proportion of peripheral blood $TcR1^+$ cells, while the $WC1^+$ subset represents a very small proportion of splenic $TcR1^+$ cells. Neither subset is well represented in lymph nodes (McClure et al., 1989; Howard and Morrison, 1994; W.D. Davis, 1994, unpublished observations). Phenotypic heterogeneity of peripheral blood $TcR1^+$ cells derives, in part, from the expression of the WC1 molecule as either of two monoclonal antibody (mAb) defined isoforms (MacHugh et al., 1993a). In addition, the forms of TcR1 on $WC1^+$ lymphocytes are positive for mAb-defined determinants expressed on all TcR1 molecules or expressed on subsets of the TcR1 molecule (Morrison and Davis, 1991).

Phenotypic heterogeneity of splenic $TcR1^+$ lymphocytes derives from the coexpression of BoCD8 on a portion of the cells (Howard and Morrison, 1994). A small proportion of the $TcR1^+$ $BoCD8^+$ cells expresses a form of TcR1 positive for one of the mAb defined determinants; however, the majority of the $WC1^-$ $TcR1^+$ cells express TcR1 currently identifiable only by molecules expressed on all TcR1.

Information about the distribution of $TcR1^+$ lymphocytes in intestinal mucosa in neonatal animals is limited. The available information suggests that $TcR1^+$ lymphocytes are numerous in both lamina propria and epithelium. Ileal intraepithelial lymphocytes (IEL) from calves 4–8 weeks of age contain a prominent subpopulation of $TcR1^+$ lymphocytes (approximately 25% of recovered cells); however, very few express WC1 (Waters et al., 1995). The lamina propria and epithelium of 1-week-old calves also contain a prominent population of $TcR1^+$ lymphocytes (Mackay and Hein, 1989). $BoCD8^+$ T lymphocytes are found within mucosal epithelium (Parsons et al., 1989; Howard et al., 1992), but it is unclear whether they express TcR1 or TcR2.

This study was undertaken to more closely examine the lymphocyte subpopulations recovered from ileal mucosa in neonatal calves. The data suggest that, at birth, ileal mucosa contains relatively few lymphoid cells. Within 1.5 weeks after birth, a large increase in the number of T lymphocytes occurs within villous epithelium and underlying lamina propria. A substantial proportion of the T cells in these compartments are $TcR1^+$ lymphocytes. Within the $TcR1^+$ subpopulation, a majority coexpress BoCD2, and a large proportion express the BoCD8α chain, probably as a homodimer. No BoCD8$\alpha\beta$ heterodiner expressing $TcR1^+$ lymphocytes are observed. A very small proportion of $TcR1^+$ cells coexpresses WC1. Taken together, the data suggest that calf ileal mucosa is populated after birth by several subsets of $TcR1^+$ cells.

2. Materials and methods

2.1. Animals

Newborn bull calves, obtained from the Washington State University (WSU) dairy facility, were maintained for up to 22 days in a pathogen-free environment, as previously described (Bjorneby et al., 1990). Newborn calf cecal contents were submitted after necropsy to the Washington Animal Disease Diagnostic Laboratory (WADDL) to test for commonly encountered enteric pathogens. These included F41 pilus positive *Escherichia coli*, *Salmonella* and *Pseudomonas* sp., coronavirus and rotavirus. Multiple fecal samples from older calves were submitted to WADDL to confirm absence of enteric pathogens.

Calves were cared for in accordance with accepted procedures of the American Association for Accreditation of Laboratory Animal Care. All animals were used as sources of ileal intraepithelial and lamina propria lymphocytes. Calves were used at 0–1 day of age ($n = 4$), at 9–12 days of age ($n = 5$), or at 20–22 days of age ($n = 5$).

2.2. Mucosal lymphocyte isolations

Calves were killed by intravenous pentobarbital injection. Beginning 1 cm from the ileocecal junction, 40 cm of ileum were removed for processing. The ileocecal junction, attached 1 cm of ileum, and a portion of the cecum were saved in 10% buffered formaldehyde. They were submitted to the WSU College of Veterinary Medicine Histology laboratory, sectioned, and stained with hematoxylin and eosin to confirm normal appearance.

Procedures for the isolation of mucosal lymphocytes have been previously published (Wilson et al., 1986; Myers and Schat, 1990; L. Ward,1993, personal communication). These procedures were followed, with minor modifications, as outlined below. Briefly, the ileum was flushed with cold phosphate buffered saline (PBS), and several small samples of ileum were removed and snap-frozen in O.C.T. compound (Miles, Inc., Elkhart, IN) for histological evaluation. The ileum was weighed, cut into three to four pieces of approximately equal length, and each piece tied at one end. The ileum pieces were then everted to expose the luminal surface, filled with Hank's Balanced Salt Solution (HBSS) (GIBCO/BRL, Grand Island, NY), and tied at the other end. These everted pieces were incubated in capped tubes in HBSS containing 5 mM dithiothreitol (DTT) (Sigma Chemical Co., St. Louis, MO) at 37°C for 12 min. After the incubation period, the tubes were gently inverted several times. The supernatants containing dislodged epithelial cells and associated lymphocytes (IEL) were collected. The incubation and collection steps were repeated three additional times in the absence of DTT. The resulting cell suspensions were pooled.

Each ileum piece was placed into Dulbecco's Modified Eagle's Medium (DMEM) (GIBCO/BRL) containing 5% fetal bovine serum (FBS) and Collagenase Type II (Sigma) at 10 U g^{-1} tissue. The pieces were incubated at 37°C for 30 min to digest the lamina propria membrane and release underlying lamina propria lymphocytes (LPL) and

associated cells. After incubation, the supernatants containing the dislodged cells were collected and pooled.

Epithelial and lamina propria cell populations were centrifuged through discontinuous gradients of 43% and 67% Percoll (Sigma), at $1500 \times g$ for 30 min at ambient temperature (approximately 20°C). On visual and microscopic inspection, the majority of epithelial cells banded at the supernatant–43% Percoll interface, and the lymphoid cells containing variable numbers of residual epithelial cells banded at the 43–67% Percoll interface. There were no visible pellets. The lymphoid cell bands were collected, washed three times in HBSS containing 10 mM *N*-[2-hydroxyethyl]piperazine-*N*′[2-ethanesulfonic acid] (HEPES) (GIBCO/BRL), and counted. Viabilities, as determined by trypan blue dye exclusion, were 80% or greater, and those cells with epithelial cell morphology took up the dye.

Small pieces of ileum were snap-frozen in O.C.T. compound after each processing step. These were later sectioned at 10 μm and examined to evaluate the completeness of the collection procedures. Only IEL preparations in which lamina propria remained intact, and LPL preparations in which underlying continuous Peyer's patch nodules remained intact, were included in the results. Based on microscopic examination of the pieces obtained during processing, the procedures generally resulted in suspensions that contained an estimated 85–95% of available IEL and 50–70% of available LPL.

2.3. Monoclonal antibodies and flow cytometry

MAbs to bovine leukocyte differentiation molecules were used with single and dual fluorescence flow cytometry to evaluate the lymphocyte populations. MAbs were obtained from the WSU Monoclonal Antibody Center. They included mAb to bovine (Bo) T lymphocytes: anti-BoCD2 (BAQ95A, IgG1) (Davis and Splitter, 1991), anti-BoCD3 (MM1A, IgG1) (Davis et al., 1993), anti-BoCD4 (CACT138A, IgG1) (Davis et al., 1990), anti-BoCD5 (CACT105A, IgG1) (Davis et al., 1990), anti-BoCD8α chain (CACT130A IgG3, CACT80C IgG1) (MacHugh et al., 1993a), anti-BoCD8β chain (BAT82A IgG1) (MacHugh et al., 1993a), anti-TcR1 (CACT61A, IgM; GB21A, IgG2b) (Parsons et al., 1993; MacHugh et al., 1996), anti-WC1 (B7A1, IgM) (MacHugh et al., 1993b), and anti-ACT2, expressed on $TcR1^{+}$ and $BoCD8^{+}$ bovine lymphocytes with modulatory activity for antigen-driven BoCD4 T lymphocyte proliferation (CACT26A, IgG1; CACT77A, IgM) (Park et al., 1993; Park et al., 1994). MAbs to B lymphocytes included anti-surface IgM (sIgM) (BIg73A, IgG1 clusters with anti-sIgM mAb in cattle (W.C. Davis, 1990, unpublished observations), and anti-B-B2, a non-immunoglobulin B lymphocyte molecule (BAQ44A, IgM) (Griebel et al., 1992). Myelomonocytic cells were identified with anti-G + M (DH59B, IgG1) (Davis et al., 1990). Nonrelevant isotype control mAbs were included to account for nonspecific labeling.

Cells were labeled for flow cytometry as previously described (Davis et al., 1987; Wyatt et al., 1994).

Fluoresceinated goat anti-mouse immunoglobulin antibody (CalTag, South San Francisco, CA) was used to label cells in single fluorescence assays. Fluorescein or phycoerythrin conjugated goat anti-mouse immunoglobulin isotype specific antibodies (CalTag) were used for dual fluorescence evaluations. Flow cytometry was performed

using a FACScan™ flow cytometer and LYSYS II software (Becton Dickinson Cytometry Systems, San Jose, CA). A minimum of 3000 events was collected per sample. The proportion of lymphoid cells recovered was determined by forward scatter and side scatter gating which excluded dead cells, clumps, and any residual epithelial cells. Data were analyzed using LYSYS II™ analysis software (Becton Dickinson Cytometry Systems). Dual parameter analysis was performed using dot plots with available statistics. The percentage of cells expressing a given molecule was determined from the average of two to four replicates. Background levels (generally less than 1%) were subtracted, and the number of recovered lymphocytes in a given subpopulation was calculated. Means, standard deviations, and pooled *t*-tests were performed using InStat (GraphPad Software, San Diego, CA). All data are expressed as mean ± 1 standard deviation.

3. Results and discussion

Because subsequent interpretation of mucosal lymphocyte population data depended upon confirmation that IEL and LPL were not contaminated by substantial numbers of lymphocytes from underlying locations, the ileum pieces snap-frozen at each processing step were carefully examined by light microscopy. Fig. 1 contains photomicrographs of representative hematoxylin stained ileum pieces obtained from 1.5-week-old calf ileum, with Peyer's patch designated P: intact ileum (panel A), ileum after IEL removal (panel B), and ileum after LPL removal (panel C). Before processing, ileum contained readily recognizable intact villi (panel A arrow). Removal of IEL resulted in retention of an essentially intact lamina propria compartment (panel B arrow).

Continuous Peyer's patch nodules also remained intact. Because nearly all of the IEL and most of the epithelial cells were removed, the villi appeared collapsed. Digestion of the lamina propria membrane released numerous underlying lymphoid cells. However, the process was not complete, as many LPL remained attached (panel C arrow). Continuous Peyer's patch remained intact as well; thus, the resulting LPL did not contain Peyer's patch cells. These results were consistent with those reported by other investigators for these procedures (Wilson et al., 1986; Myers and Schat, 1990), and indicated that the cell suspensions recovered from epithelium and lamina propria contained only the cells normally found in those locations in the intact ileum.

IEL and LPL from calves from the three age groups were compared to determine the extent to which the ileal mucosa became populated with lymphocytes after birth. Fig. 2 contains a representative set of photomicrographs of sections of hematoxylin and eosin stained ileum from a newborn calf (panel A) and a 1.5-week-old animal (panel B). The newborn calf ileum contained relatively few lymphoid cells, when compared with ileum from the older animal, suggesting incomplete development of the lymphoid compartment of calf ileal mucosa at birth. The number of lymphoid cells recovered from ileal mucosa was consistent with a paucity of lymphocytes in newborn calf ileum (Fig. 3). Over 2×10^7 lymphoid cells were recovered from villous epithelium of newborn animals, compared with 7×10^7 cells for 1.5-week-old calves. This represented a significant increase ($P = 0.034$) in the number of IEL recovered from 1.5-week-old

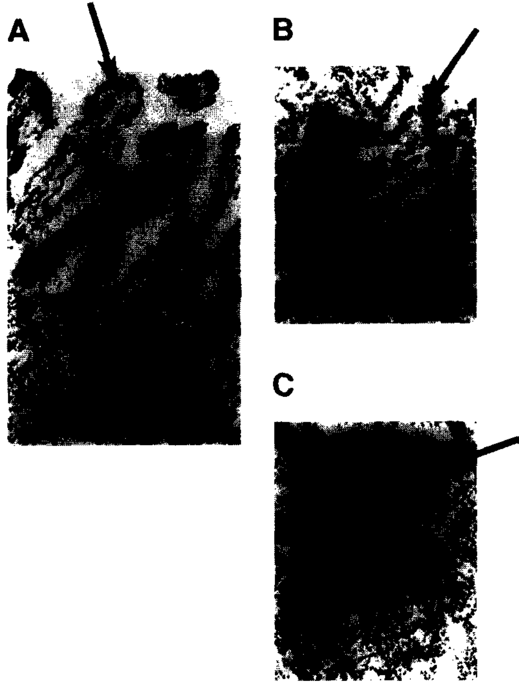

Fig. 1. Calf ileum sections obtained during processing. Ileum pieces were snap-frozen prior to processing (panel A), after collection of intraepithelial lymphocytes (panel B), and after enzymatic digestion (panel C). Sections 10 μm thick were examined for completeness of processing. Representative ileum samples are shown. Panel A arrow points to an intact villus. Panel B arrow points to an intact lamina propria area. Panel C arrow points to LPL remaining in tissue after digestion. Pieces from each ileum processed were examined. original magnification ×32.

calves compared with newborns. Approximately 1×10^8 lymphoid cells were recovered from villous epithelium of 3-week-old animals, a substantial, but not statistically significant, increase when compared with 1.5-week-old animals. The number of lamina

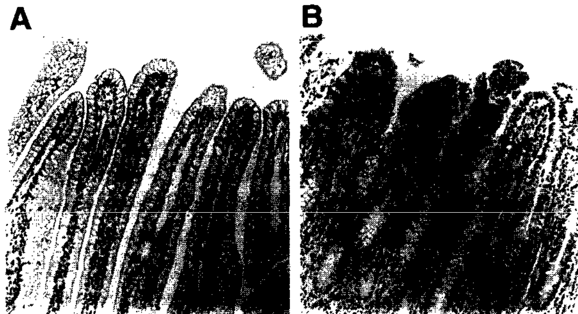

Fig. 2. Sections of ileum from calves of different ages. Representative formalin-fixed ileum from newborn calf (panel A) and 10-day-old calf (panel B) are shown. Sections were examined from each ileum obtained. Original magnification ×32.

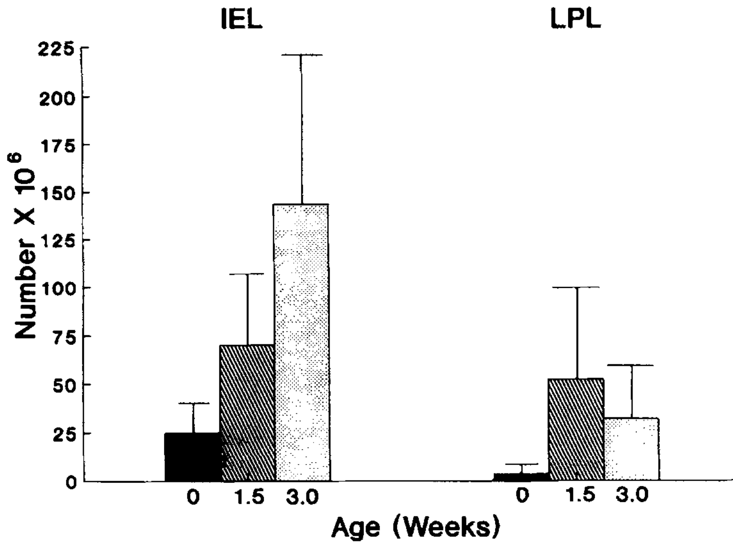

Fig. 3. Comparison of IEL and LPL recovered from calves of different ages. Means and standard deviations of total number of IEL and LPL recovered from 40 cm of ileum from newborn, 1.5, and 3-week-old calves are shown.

propria lymphoid cells was also consistent with the presence of more cells in older calves. However, fewer cells were recovered from 3-week-old calves than from younger animals. It is probable that the digestion times used to release LPL were insufficient to dislodge larger numbers of cells from the ilea of older calves. Approximately 5×10^7 cells were recovered from 1.5-week-old calves compared with 4×10^6 lymphoid cells from newborn calves, a significant increase ($P = 0.042$). In contrast, there was no significant increase observed in the total number of recovered LPL after 1.5 weeks. Thus, the epithelium and lamina propria became populated with lymphocytes by 1.5 weeks after birth.

Ileal IEL and LPL were phenotyped. Recovered T and B lymphocytes were compared to determine which lymphocyte subpopulations increased in number after birth (Table 1). Overall, there were no significant differences between the numbers of T and B lymphocytes recovered within a given age group. The differences that were found occurred between age groups. Among both IEL and LPL, T lymphocytes, as defined by BoCD3 expression, were substantially increased in number in older calves. There were significant differences between the number of $BoCD3^+$ lymphocytes recovered from newborn and 1.5-week-old calves, after which time no additional significant increases could be documented. Changes in the number of $BoCD5^+$ lymphocytes recovered from epithelium paralleled the changes seen with $BoCD3^+$ cells, with significant differences seen between newborn and older animals.

While the mean number of B lymphocytes within ileal mucosa appeared to be increased in older calves, there were no statistically significant differences. B-B2 was coexpressed on $sIgM^+$ lymphocytes, and the numbers of $B\text{-}B2^+$ cells paralleled $sIgM^+$

Table 1
Comparison of numbers of T and B lymphocytes recovered from calves of different ages

	Molecule expressed		
	BoCD3 [a]	BoCD5	SIgM
IEL			
Newborn ($n=4$)	9.0 ± 10.2 *	4.2 ± 4.7 **	11.8 ± 11.0
1.5 weeks ($n=5$)	32.0 ± 15.1	19.0 ± 6.2	20.6 ± 20.7
3 weeks ($n=5$)	50.4 ± 30.4	41.0 ± 21.6	46.1 ± 53.5
LPL			
Newborn ($n=4$)	1.6 ± 0.9 ***	1.4 ± 1.2	1.1 ± 1.1
1.5 weeks ($n=5$)	23.9 ± 22.3	15.5 ± 13.2	6.1 ± 4.0
3 weeks ($n=5$)	9.8 ± 4.1	6.1 ± 4.0	5.5 ± 8.1

[a] Expressed as number of cells $\times 10^6 \pm 1$ SD.
* Significant at $P = 0.036$ between newborn and 1.5 weeks, and between newborn and 3 weeks; ** significant at $P = 0.009$ between newborn and 1.5 weeks, and at $P = 0.016$ between newborn and 3 weeks; *** significant at $P = 0.022$ between newborn and 1.5 weeks.

cells when evaluated. However, cell numbers were occasionally limiting, especially for newborn calves; thus, anti-B-B2 was not always included in the mAb panels. The presence of B lymphocytes within ileal epithelium was an interesting observation, because of an absence of documentation of this subpopulation outside of traditional organized gut-associated lymphoid tissue. Immunohistochemistry performed on snap-frozen ileum sections using anti-B-B2 confirmed that, in underlying continuous Peyer's patch, B-B2$^+$ lymphocytes were in the expected locations within the nodules (Griebel et al., 1992) with especially large concentrations at nodule margins. B-B2$^+$ IEL were associated primarily with crypt epithelium, while B-B2$^+$ LPL were scattered in underlying lamina propria (C.R. Wyatt and E.J. Brackett, 1994, unpublished observations). These observations are consistent with the demonstration that the procedures used to isolate IEL and LPL did not result in significant contamination of B lymphocytes from other locations, and indicate that B lymphocytes are present within villous epithelium in neonatal calves.

Portions of the recovered cells did not express T or B cell molecules. Among intraepithelial cells, these accounted for $23 \pm 11\%$ of newborn cells, $25 \pm 10\%$ of 1.5-week-old calf cells, and $26 \pm 15\%$ of 3-week-old calf cells, and included a substantial proportion of myelomonocytic cells ($8 \pm 2\%$ in newborns, $13 \pm 7\%$ in 1.5-week-old calves, and $10 \pm 3\%$ in 3-week-old animals). Among lamina propria cells, they accounted for $26 \pm 8\%$ of newborn, $29 \pm 14\%$ of 1.5-week-old, and $28 \pm 13\%$ of 3-week-old animal cells, and included substantial proportions of myelomonocytic cells ($14 \pm 3\%$ in newborns, $5 \pm 4\%$ in 1.5-week-old calves, and $18 \pm 14\%$ in 3-week-old animals).

Among the T lymphocytes in intestinal mucosa, those that express TcR1 form a prominent subpopulation (Mackay and Hein, 1989; Taguchi et al., 1991). Because phenotypic heterogeneity is characteristic of this lymphocyte subpopulation, dual fluorescence flow cytometry was performed to identify subsets of TcR1$^+$ lymphocytes among IEL and LPL from the three groups of calves. Fig. 4 contains dot plot

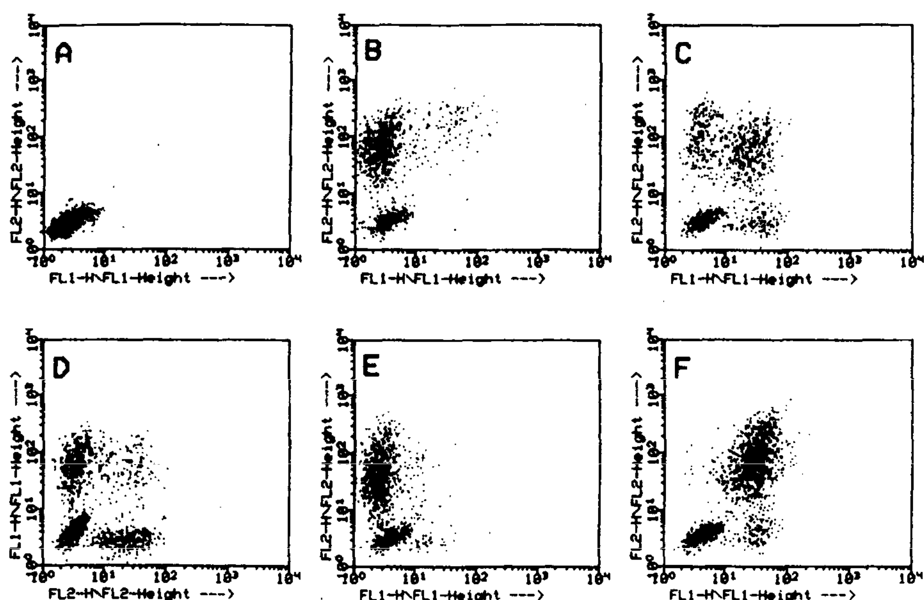

Fig. 4. Identification of ileal intraepithelial TcR1$^+$ lymphocyte subsets. Representative flow cytometric profiles from 10-day-old calf ileum. Label using anti-TcR1 mAb is depicted on *Y* axis; label using subset identifying mAb depicted on *X* axis. Panel A, isotype control mAb; panel B, WC1 mAb; panel C, BoCD2 mAb; panel D, BoCD8α mAb; panel E, BoCD8β mAb; panel F, ACT2 mAb.

representations showing the coexpression of molecules on TcR1$^+$ lymphocytes recovered from villous epithelium from a 1.5-week-old calf. Panel A depicts isotype control mAb. In the remaining panels, expression of TcR1 is shown on the *Y* axis, while expression of the other molecule being examined is depicted on the *X* axis. Panel B shows WC1, panel C shows BoCD2, panel D shows BoCD8α, panel E shows BoCD8β, and panel F shows ACT2. Of the TcR1$^+$ lymphocyte subsets seen, TcR1/BoCD2$^+$ cells comprised the largest subset, while TcR1/WC1$^+$ lymphocytes were the smallest subset. A small proportion of TcR1$^+$ cells coexpressed BoCD8α, but TcR1/BoCD8β$^+$ cells were rare among IEL, suggesting the TcR1/BoCD8$^+$ cells expressed the BoCD8α homodimer. No TcR1/BoCD4$^+$ lymphocytes were found (data not shown). ACT2 was expressed on all of the TcR1$^+$ lymphocytes recovered from villous epithelium. All of these observations are consistent with results of others that indicate TcR1$^+$ lymphocytes in calf ileum are phenotypically different from other lymphoid compartments (Waters et al., 1995).

The numbers of TcR1$^+$ lymphocytes recovered from epithelium and lamina propria were compared among the three age groups to determine whether differences could be identified (Table 2). The number of TcR1/BoCD2$^+$ cells was significantly increased in older calves compared with newborns. Among the TcR1$^+$ IEL, BoCD8α$^+$ and WC1$^+$ lymphocytes from older calves comprised significantly larger subsets compared with newborns. Overall, however, these cells represented relatively small subsets of the TcR1$^+$ subpopulation at birth, and remained relatively low in number compared with

Table 2
Comparison of $TcR1^+$ lymphocyte subsets recovered from calves of different ages

	Molecules coexpressed			
	TcR1/BoCD2 [a]	TcR1/BoCD8α	TcR1/WC1	TcR1/ACT2
IEL				
Newborn (n = 4)	3.0 ± 2.8 *	1.7 ± 1.7 +	0.7 ± 0.7 **	4.5 + 4.4 ***
1.5 weeks (n = 5)	13.2 ± 4.3	4.7 ± 4.1	3.2 ± 1.8	22.0 ± 12.9
3 weeks (n = 5)	22.2 ± 12.1	10.3 ± 4.7	6.6 ± 3.6	39.8 ± 23.1
LPL				
Newborn (n = 4)	0.6 ± 0.4 †	0.5 ± 0.4	0.7 ± 1.1	1.1 ± 0.7 ‡‡
1.5 weeks (n = 5)	10.6 ± 7.7	3.0 ± 2.9	3.0 ± 2.8	18.7 ± 17.2
3 weeks (n = 5)	4.0 ± 1.2	1.7 ± 1.1	1.1 ± 0.5	7.2 ± 3.4

[a] Expressed as number of cells $\times 10^6 \pm 1$ SD.
* Significant at $P = 0.012$ between newborn and 1.5 weeks, and at $P = 0.018$ between newborn and 3 weeks; ** significant at $P = 0.046$ between newborn and 1.5 weeks, and at $P = 0.016$ between newborn and 3 weeks; *** significant at $P = 0.037$ between newborn and 1.5 weeks, and at $P = 0.021$ between newborn and 3 weeks; + significant at $P = 0.011$ between newborn and 3 weeks; † significant at $P = 0.040$ between newborn and 1.5 weeks, and at $P = 0.005$ between newborn and 3 weeks; ‡‡ significant at $P = 0.026$ between newborn and 3 weeks.

the $BoCD2^+$ subset. Consistent with increased numbers of $TcR1^+$ cells in older calves, the number of $ACT2^+$ $TcR1^+$ cells was significantly increased in older animals compared with newborns. These data indicate that $TcR1^+$ lymphocytes that express BoCD2 represent the predominant subset in ileal mucosa. Only a small subset of $WC1^+$ $TcR1^+$ cells is present. Further, the majority of $TcR1^+$ cells populate the ileum after birth.

Our findings in calf ileum are similar to reports on $TcR1^+$ lymphocytes in mouse small intestine. $TcR1^+$ lymphocytes populate mouse small intestine after birth. A large increase in the number of $TcR1^+$ and $TcR2^+$ IEL occurs around weaning (about 3 weeks of age in mice) (Bandeira et al., 1991). Both of these observations are consistent with our results.

Mouse IEL are thought to be of extrathymic origin, since they have been reported in athymic mice (nude and thymectomized) (Lefrançois, 1991). While a similar situation appears to exist for most IEL subsets in calves, at least the $TcR1/WC1^+$ subset could be of thymic origin. WC1 has been demonstrated in fetal thymus in ruminants where it is expressed on $TcR1^+$ cells late in differentiation (Hein, 1994). Thus, the $WC1^+$ $TcR1^+$ cells found in calf ileum might have matured within the thymus and migrated to ileal mucosa.

Very little is known of the functions of $TcR1^+$ lymphocytes. However, they might serve as regulatory cells in intestinal immune responses. Suppression of immune responses in the intestine might be important in preventing adverse immune responses to the large number of potential antigens delivered to the intestine during food consumption. It has been suggested that ingested food proteins are presented to villous epithelial cells, rather than via M cells to organized lymphoid tissue (Kraehenbuhl and Neutra, 1992), and that this alternate antigen presentation pathway induces suppressive T

lymphocytes in intestinal mucosa (Bland and Warren, 1986). Suppressive $TcR1^+$ lymphocytes have been demonstrated in cattle, both in blood and in mammary gland secretions. They express ACT2, and can be either $WC1^+$ or $WC1^-$. These $TcR1/ACT2^+$ cells downregulate antigen-specific $CD4^+$ T lymphocyte responses (Chiodini and Davis, 1992; Park et al., 1994). In this context, it is of interest that ACT2 is expressed on all $TcR1^+$ IEL and LPL from birth. The presence of ACT2 on all of the $TcR1^+$ lymphocytes in calf ileal mucosa is consistent with an immunoregulatory function for this subpopulation.

This study indicates that T lymphocytes, including several subsets of $TcR1^+$ cells, populate calf ileal mucosa after birth. This information is important because it will help provide a basis for evaluating mucosal immune responses to enteric pathogens important in young animals. The findings also suggest cattle might prove useful as a model for comparative studies on the function of TcR1 cells in the mucosa.

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