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An analysis of the growth and differentiation of B cells isolated from follicles of the ileal Peyer's patch of sheep

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SUMMARY

We developed a method to isolate and culture cells from the lymphoid follicles of the ileal Peyer's (PP) patch of young sheep (6–12 weeks). These cells were 98% sIgM$^+$ B cells and 1% T cells. Cultured follicular cells were used to investigate B-cell proliferation and differentiation. Less than 50% of B cells were viable after 24 hr of culture and this decrease in B-cell viability also occurred following co-stimulation with pokeweed mitogen (PWM) and recombinant bovine interleukin-1 (rBoIL-1) or rBoIL-2. In contrast, co-stimulation with PWM and either rBoIL-1 or rBoIL-2 induced a marked proliferative response that was maximal on Day 4 of culture. Cytokine-induced proliferation of the B cells required PWM co-stimulation and proliferation induced by rBoIL-1 and rBoIL-2 was neither additive or synergistic. This suggests that PWM bound a molecule or molecules that signalled responsiveness to both rBoIL-1 and rBoIL-2. Culture of follicular cells with PWM and both rBoIL-1 and rBoIL-2 also resulted in B-cell differentiation. This differentiation was associated with decreased proliferation, an increased number of viable B cells, and increased expression of both surface IgM and non-Ig membrane molecules. Thus, co-stimulation of ileal PP follicular cells with PWM and rBoIL-1 and rBoIL-2 resulted in both B-cell proliferation and differentiation.

INTRODUCTION

Lymphoid follicles are distributed throughout the small and large intestines of many mammalian species including man, rodents, and many domestic mammals.[1-3] The lymphoid aggregates in different regions of the gut differ markedly in their morphology, life history and lymphocyte composition and these differences suggest that the gut-associated lymphoid tissue performs a variety of functions. The ileal and jejunal Peyer's patches (PP) of sheep have been well characterized as distinct lymphoid tissues that differ in their morphology and life history[1,2] as well as their lymphocyte composition.[4]

The ileal PP may function as a primary lymphoid organ[5] with a central role in the development of the B-cell pool and repertoire in sheep.[6] This conclusion is supported by the unique structure and life history of this lymphoid tissue,[2,7] a prolonged B lymphopaenia following foetal or neonatal ileectomy,[8] and a high rate of somatic mutation in the V genes of the Ig λ L chain.[6] Furthermore, production of B cells within the ileal PP follicles is characterized by rapid proliferation and extensive cell death[9] by apoptosis[10] and less than 5% of B cells emigrate from the follicles. A process of 'selection' may determine the survival of this small percentage of emigrant B cells.[9]

Little is known about the cellular interactions, cytokines, or other factors that may influence the proliferation, differentiation, and selection of B cells in the ileal PP follicles of young sheep. We have initiated an investigation of these aspects by developing a cell isolation method that yielded a population of cells representative of the follicular microenvironment. A culture system was developed for follicular cells that induced a period of rapid B-cell proliferation balanced by cell death. The phenotype of cells proliferating and surviving in these cultures was then characterized to determine if this was an appropriate model to study ileal PP follicular B-cell growth and differentiation.

Abbreviations: BrdU, bromodeoxyuridine; FALS, forward angle light scatter; FCM, flow cytometry; mAb, monoclonal antibody; MLN, mesenteric lymph node; PP, Peyer's patch; PWM, pokeweed mitogen; rBoIL, recombinant bovine interleukin; [^{3}H]dThd: titrated thymidine.

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MATERIALS AND METHODS

Animals and cell isolation from lymphoid tissues

Tissues were collected from outbred sheep of either sex and between the ages of 6 and 12 weeks. All tissues were collected in ice-cold Dulbecco's modified Eagle's medium (DMEM) supplemented with 25 mM HEPES and 100 U penicillin G/ml, 100 μg streptomycin/ml, and 250 ng amphotericin (DMEM/s). Medium and supplements were from Sigma (St Louis, MO).

Table 1. Monoclonal antibodies and their specificity

mAb designation	Ig isotype	Specificity	Reference
SBU-T4	IgG1	CD4	14
ST-1	IgG2a	CD5	15
CACT80C	IgG1	CD8	16
PIg45A	IgG2b	IgM	16
BAQ44A	IgM	B: 60*	17
BAS9A	IgM	B: 85	17
BAQ155A	IgG1	B: 25/85	17
TH14B	IgG2a	MHC class†	16

* B, B-cell lineage-specific antigen and M_r.
† MHC, major histocompatibility complex.

Ileal PP tissue was collected proximal to the ileocaecal ligament, cut into 2–4 cm lengths, and cleaned in ice-cold DMEM/s by gently scraping the mucosal and serosal surfaces. Lymphoid follicles were released by separating the mucosa and muscularis with a scalpel blade passed along the muscularis. Intact follicles were isolated by gravity sedimentation through a column of sterile, isotonic 25% Percoll (Pharmacia, Dorval, Canada) and follicles were then disrupted by repeated pipetting and passing the cells through a sterile 44 μm nylon mesh (Small Parts Inc., Miami, FL). Cell suspensions of ileal mesenteric lymph node (MLN), spleen, and jejunal PP were prepared by mincing or scraping tissues with a scalpel blade and passing the cell suspensions through a 44 μm nylon mesh. Cells were counted with a Coulter Counter (Coulter Electronics Ltd, Hialeah, FL) fitted with a Channelizer 256.

Cell culture, [^{3}H]thymidine incorporation, bromodeoxyuridine incorporation

The medium for all cultures was DMEM/s further supplemented with 2×10^{-5} M 2-mercaptoethanol (2-ME), 2 mM glutamine, 50 ng/ml gentocin, 250 μg/ml insulin, 250 μg/ml transferrin, 0·25 μg/ml sodium selenite, and 5% foetal bovine serum (FBS; Gibco Canada Ltd, Burlington, Canada). All culture supplements were from Sigma. Cells were cultured either in 6-well plates with 2×10^7 viable cells/well in 4 ml medium or in U-bottom, 96-well plates (Nunc, Gibco Canada Ltd) with 4×10^5 viable cells/well in 200 μl medium. Cultures were incubated at 39° in a humidified atmosphere with 5% CO_2. Cell proliferation in 96-well plates was quantitated by incorporation of [^{3}H]thymidine ([^{3}H]dThd; specific activity = 925 GBq/mmol; Amersham, Oakville, Canada) with 1 μCi/well added during the last 16 hr of culture. Results were expressed as c.p.m. and presented as the mean ± SD of quintuplicate cultures. Cells proliferating in 6-well plates were labelled with 5-bromo-2′-deoxyuridine (BrdU; Boehringer-Mannheim Canada Ltd, Laval, Canada) by incubating cells with 10 μM BrdU.

Cytokines and mitogens

The recombinant bovine interleukin-1 (rBoIL-1)[11] and rBoIL-2[12] were generous gifts of American Cyanamid (Princeton, NJ) and were provided as stock solutions of 10^6 U/ml. Pokeweed mitogen (PWM) (Gibco Canada Ltd) was reconstituted in 5 ml sterile distilled H_2O, aliquoted, and stored at −20°. Similar mitogenic activity was observed when comparing different lots of PWM.

Table 2. Lymphocyte composition of cell suspensions prepared from ileal PP follicles, jejunal PP, ileal MLN and spleen

Lymphocyte population	Per cent positive lymphocytes*			
	Ileal PP	Jejunal PP	Ileal MLN	Spleen
CD5	0·9 ± 0·25	30·2 ± 4·5	51·9 ± 5·9	45·5 ± 6·4
CD4	0·6 ± 0·17	22·4 ± 6·1	33·3 ± 3·9	13·4 ± 5·2
CD8	0·2 ± 0·06	6·34 ± 2·5	17·5 ± 2·9	12·5 ± 4·4
sIg$^+$	98·2 ± 2·3	63·5 ± 8·4	45·6 ± 6·6	50·9 ± 5·3
sIg$^+$ sIgM$^+$†	98·9 ± 1·1	78·2 ± 9·1	93·4 ± 6·3	93·3 ± 5·4
sIg$^+$ BAQ44A$^+$	28·1 ± 7·3†	86·4 ± 8·6	80·4 ± 5·6	88·4 ± 8·3
sIg$^+$ BAS9A$^+$	99·1 ± 0·8	92·8 ± 9·4	90·2 ± 7·2	95·6 ± 4·9
sIg$^+$ BAQ155A$^+$	95·2 ± 2·9†	63·3 ± 9·3	74·3 ± 8·3	62·7 ± 5·2
sIg$^+$ TH14B$^+$	98·7 ± 1·2	98·8 ± 2·1	98·6 ± 1·1	99·0 ± 0·9

* $n = 5$, 6–12-week-old lambs. Data presented as mean ± SD.
† Dual-labelling data presented as the per cent of sIg$^+$ cells co-expressing the second molecule. When compared with other lymphoid tissues there was a significant difference in the number of ileal PP follicular B cells co-expressing the BAQ44A molecule ($P < 0·01$) and the BAQ155A molecule ($P < 0·01$).

Flow cytometry, antibodies, cell size analysis and statistical analysis

Cells were labelled for use in flow cytometry (FCM) as described previously[13] with the following modifications. All monoclonal antibody (mAb; Table 1) labelling was developed using either fluorescein isothiocyanate (FITC)-conjugated or biotinylated isotype-specific goat anti-mouse-Ig (Southern Biotechnology, Birmingham, AL) and biotinylated antibodies were developed with strepavidin-phycoerythrin (Becton Dickinson, Mountain View, CA). Analyses of cultured cells were restricted to viable cells by excluding cells that stained with propidium iodide (Calbiochem-Behring, La Jolla, CA) at a concentration of 2·5 μg/ml. The protocol of Houck and Loken[18] was used for BrdU labelling and the FITC-anti-BrdU mAb was from Becton Dickinson. All FCM analyses were performed with a FACScan analyser (Becton Dickinson). Cell size analysis was completed using calibrated microspheres (Coulter Electronics, Hialeh, FL) to establish a standard curve for cell size versus forward angle light scatter (FALS). Differences in viable cell number, proliferative responses, mean cell size and mean fluorescence intensity were compared using a two-way, one-tailed Student's *t*-test.

RESULTS

Phenotype of ileal PP follicular cells

Ileal PP follicles are a unique microenvironment characterized by a predominance of B cells (98%) and a paucity of T cells (Table 2). Other lymphoid tissues, such as jejunal PP, ileal mesenteric lymph nodes (MLN) and spleen, contained a much greater number of T cells relative to B cells (Table 2). Furthermore, follicular B cells of the ileal PP were phenotypically distinct from B cells of the other lymphoid tissues examined. The follicular B cells were 99% sIgM$^+$ whereas the sIg isotype was more heterogeneous in other lymphoid tissues, particularly the jejunal PP (Table 2). Ileal PP follicular B cells also differed in the expression of non-Ig surface molecules

Table 3. Comparison of the level of antigen expression on lymphocytes isolated from ileal PP follicles, jejunal PP, ileal MLN and spleen

Surface molecule	Mean fluorescence intensity			
	Ileal PP	Jejunal PP	Ileal MLN	Spleen
sIgM	484±16·7*†	527±17·4	560±20·8	594±23·8
BAQ44A	491±24·2	713±35·7	729±21·9	712±28·5
BAS9A	579±27·2	601±20·5	604±24·2	612±29·0
BAQ155A	474±26·0†	609±30·5	665±39·9	645±29·0
TH14B	570±24·0	589±28·2	572±22·2	591±27·3

* Data presented as mean±SD. $n=3$, 6–12-week-old lambs. Log scale immunofluorescence.

† Mean fluorescence intensity of ileal PP follicular B-cell labelling was significantly lower for sIgM ($P<0·05$), BAQ44A ($P<0·01$), and BAQ155A ($P<0·01$) when compared with B cells from other lymphoid tissues. There was no significant difference in fluorescence intensity when comparing labelling of the five surface molecules among jejunal PP, ileal MLN, or spleen.

labelled by the BAQ44A and BAQ155A mAb. Approximately 30% of follicular B cells labelled with the BAQ44A mAb (Table 2). In contrast, over 80% of B cells in the lymphoid tissues examined were labelled by the BAQ44A mAb (Table 2). Furthermore, mean fluorescence intensity indicated that the level of BAQ44A labelling was markedly lower on follicular B cells than B cells of other lymphoid tissues (Table 3). The percentage of B cells labelling with the BAQ155A mAb was greater for the ileal PP than the other lymphoid tissues. However, mean fluorescence intensity indicated that the expression of this molecule was lowest on ileal PP follicular B cells (Table 3). The mean fluorescence intensity and percentage of B cells labelling with the BAS9A and TH14B mAb was similar for all lymphoid tissues examined. These two mAb served as controls when FCM analyses were compared among different cell populations. Macrophages and dendritic stromal cells were present in cell suspensions prepared from ileal PP follicles and were observed as plastic-adherent cells in cultures.

Ileal PP follicular cells were greater than 97% viable when isolated. However, in culture the number of viable cells decreased markedly during the first 24 hr and continued to decline during the 12-day culture period (Fig. 1). Addition of the polyclonal B-cell mitogen, PWM, alone or with either recombinant bovine interleukin-1 (rBoIL-1) or rBoIL-2 did not change this decline in viable B-cell number (Fig. 1). Only when the follicular cells were co-stimulated with PWM and both rBoIL-1 and rBoIL-2 was there a marked increase in viable cell numbers on Days 8 and 12 (Fig. 1). Several potential polyclonal B-cell mitogens, including anti-Ig, LPS and PMA, were screened for co-stimulatory activity with rBoIL-1 and rBoIL-2 but none of these increased the number of viable B cells recovered from cultures (data not shown).

Optimization of culture conditions for follicular B-cell proliferation

B-cell growth in ileal PP follicles was characterized by a high mitotic index balanced by extensive cell death.[9] Thus, follicular B-cell proliferation may not result in a net increase in cell

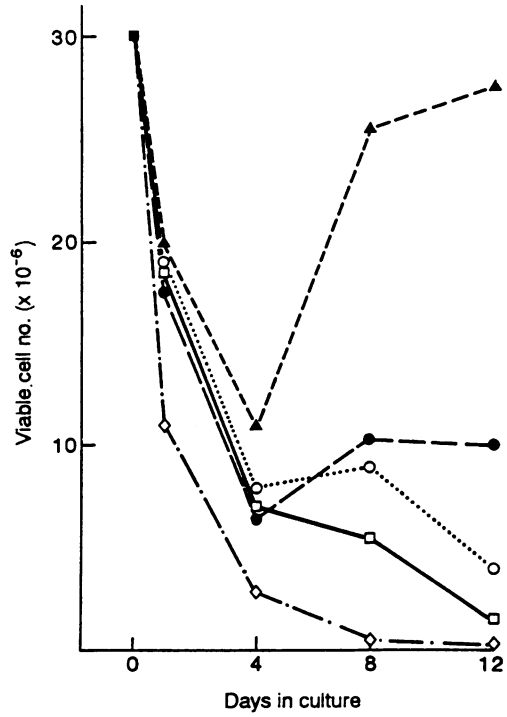

Figure 1. The effect of PWM and rBoIL-1 and/or rBoIL-2 co-stimulation on the number of viable cells recovered from cultures of ileal PP follicular cells. Cells were cultured in 6-well plates with 2×10^7 cells/well in 4 ml medium. Viable cell numbers were compared during culture in medium alone, with PWM, and following co-stimulation with PWM and cytokines. Viable cell number increased significantly when follicular cells were co-stimulated with PWM and both rBoIL-1 and rBoIL-2 (PWM/rBoIL-1/rBoIL-2) on Days 4 ($P<0·05$), 8 ($P<0·01$) and 12 ($P<0·01$). A similar comparison for PWM with rBoIL-1 and PWM with rBoIL-2 indicated a significant increase in viable cell number for both cultures on Day 8 ($P<0·05$) and for the PWM/rBoIL-2 culture on Day 12 ($P<0·05$). Data presented are the mean of three replicate experiments and the SD was less than 18% for each time point. Medium (◇); PWM (□); PWM/rBoIL-1 (○); PWM/rBoIL-2 (●); PWM/rBoIL-1/rBoIL-2 (▲).

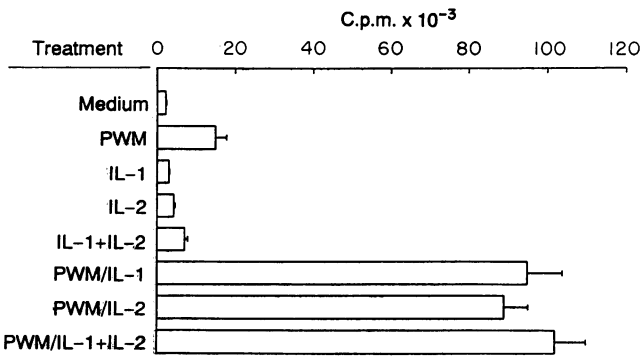

Figure 2. Co-stimulation with PWM and rBoIL-1 or rBoIL-2 is required for ileal PP follicular cell proliferation. Culture of follicular cells for 96 hr with mitogen or cytokine induced a small but significant ($P<0·05$) increase in proliferation compared with background (medium). In contrast, there was a markedly increased proliferative response following co-stimulation with PWM and rBoIL-1 and/or rBoIL-2 and there was no difference in the proliferative response induced by these cytokines alone or in combination. Data presented are the mean + SD of quintuplicate cultures and are representative of one of three replicate experiments.

Table 4. Phenotype of viable cells present in cultures of ileal PP follicular cells co-stimulated with PWM, rBoIL-1 and rBoIL-2

Culture time (days)	Cell viability		Phenotype of viable cell population						
			CD5	sIgM		BAQ44A		BAQ155A	
	% input	No. ($\times 10^{-6}$)	%+	%+	MFI*	%+	MFI	%+	MFI
0	97·8	58·7	0·85	98·9	475	28·2	500	98·2	497
1	48·6	28·8	0·60	98·7	458	36·5	484	88·6	476
4	37·7	22·6	0·57	98·7	577	44·0	467	94·1	450
8	84·7	50·8	0·74	97·6	584	68·4	442	96·2	529
12	89·0	53·4	0·59	96·6	593	82·9	422	94·9	570

* MFI, mean fluorescence intensity. Data presented as the mean of three experiments. The SD was less than 12% for all values.

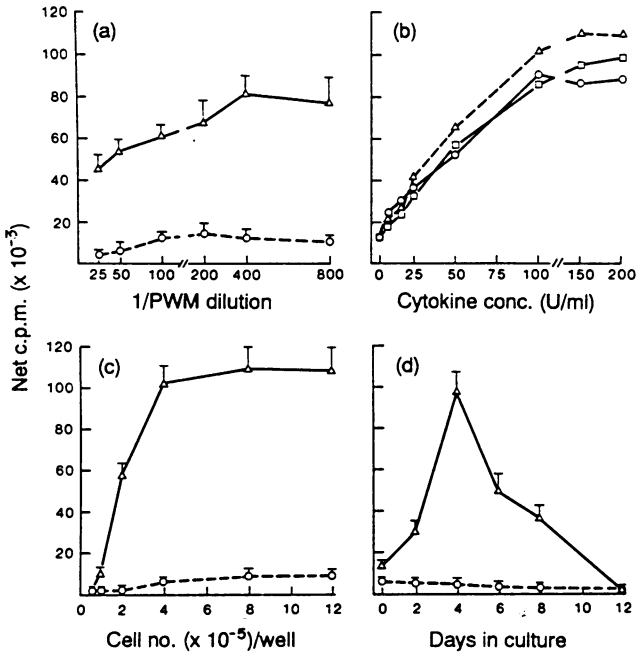

Figure 3. Optimal culture conditions for ileal PP follicular cell proliferation. The optimal PWM concentration [PWM (○); PWM/rBoIL-1/rBoIL-2 (△)] (a), recombinant cytokine concentration [PWM/rBoIL-1 (□); PWM/rBoIL-2 (○); PWM/rBoIL-1/rBoIL-2 (△)] (b), cell number/microtitre well [medium (○); PWM/rBoIL-1/rBoIL-2 (△)] (c), and incubation time [medium (○); PWM/rBoIL-1/rBoIL-2 (△)] (d) were defined for ileal PP follicular cells isolated from 6–12-week-old lambs. Net c.p.m. was calculated by subtracting background responses with either medium or PWM. Unless indicated otherwise cultures contained 4×10^5 cells/well, cells were co-stimulated with PWM (1:400 dilution) and rBoIL-1 or rBoIL-2 (100 U/ml), and cultures were incubated for 96 hr. Data presented are the mean ± SD of quintuplicate cultures and where not shown the SD was less than 10%. Data are representative of one of three replicate experiments.

number. To compliment the data on viable cell numbers in follicular B-cell cultures the proliferative response of these cells was determined. Co-stimulation of follicular B cells with PWM and either rBoIL-1 or rBoIL-2 induced a marked proliferative response (Fig. 2). This proliferative response was not increased by the addition of both rBoIL-1 and rBoIL-2 to PWM-stimulated follicular cells. Maximal ileal PP follicular cell proliferation was induced by co-stimulation with a 1:400 dilution of PWM (Fig. 3a) and 100 U/ml of both rBoIL-1 and rBoIL-2 (Fig. 3b). The optimal cell number per microtitre well was 4×10^5 cells with a marked decline in proliferation below this number (Fig. 3c). Proliferative responses were maximal on Day 4 of culture and thereafter declined rapidly (Fig. 3d). Optimal culture conditions for ileal PP follicular cells in 6-well plates were 2×10^7 cells/well in 4 ml of medium with PWM (1:400 dilution) and a combination of 100 U/ml rBoIL-1 and rBoIL-2 (data not shown). Follicular cells cultured in 6-well plates were used for FCM analyses of phenotype and these cells were transferred to new medium on Days 4 and 8 when cultured for 12 days.

Follicular B-cell phenotypic changes during culture

Co-stimulation of follicular cells with PWM, rBoIL-1, and rBoIL-2 induced a transient proliferative response that was maximal on Day 4 and declined sharply by Day 8 (Fig. 3). The proliferation on Day 4 was associated with a constant viable cell number relative to Day 1 of culture but the number of viable cells increased as the proliferative responses declined on Days 8 and 12 (Table 4). A FCM analysis of follicular cells was performed at intervals throughout the culture period to determine if B-cell phenotype correlated with proliferation. The number of T cells did not increase during the culture period (Table 4) and $sIgM^+$ B cells remained the predominant population (Table 4, Fig. 4). However, the phenotype of viable B cells changed during the culture period. On Day 4 an increased percentage of B cells labelled with the BAQ44A mAb and also expressed a higher level of sIgM (Fig. 4). Cell size analysis of Day 4 $sIgM^{Hi}$ and $sIgM^{Lo}$ B cells determined that the mean cell size of $sIgM^{Lo}$ cells was significantly greater ($P < 0{\cdot}01$) than $sIgM^{Hi}$ cells (Fig. 5). A decrease in cell size was also associated with both the $BAQ155A^{Hi}$ population and the $BAQ44A^+$ population (Day 4, 10·1 μm; Day 8, 9·80 μm) while the mean cell size of the $BAQ155A^{Lo}$ and $BAQ44A^-$ B cells remained relatively constant (Day 4, 12·1 μm; Day 8, 11·8 μm; Day 12, 10·8 μm). The predominant B-cell phenotype continued to change during the culture period. By Day 12 of culture most

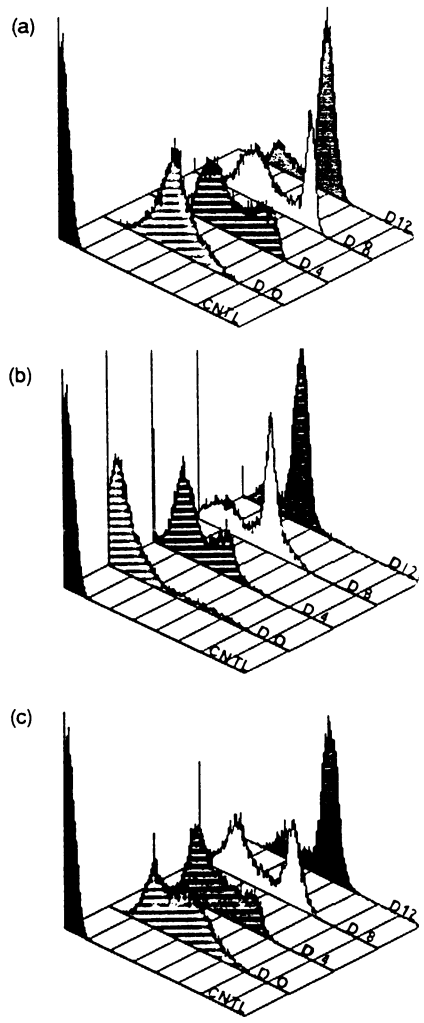

Figure 4. Analysis of follicular B-cell phenotype during a 12-day culture period. Labelling for sIgM (a), BAQ44A (b) and BAQ155A (c) was analysed every 4 days during culture and analysis was restricted to viable cells (propridium iodide negative cells). The phenotype of follicular B cells, prior to culture, is presented as Day 0 (D.0). On Day 4 (D.4) of culture the labelling for sIgM divided B cells into $sIgM^{Hi}$ and $sIgM^{Lo}$ subpopulations and a greater percentage of cells entered the $sIgM^{Hi}$ subpopulation on Day 8 (D.8) and Day 12 (D.12). Similar changes occurred for BAQ155A labelling during culture with B cells becoming predominantly $BAQ155A^{Hi}$ on Day 12. Few follicular B cells labelled with a detectable level of BAQ44A (D.0) but during culture there was a progressive increase in the $BAQ44A^+$ population.

B cells were sIg^{Hi}, $BAQ155A^{Hi}$ and $BAQ44A^+$ (Fig. 4) and again this phenotype was associated with decreased mean cell size (9·90 μm).

The association between decreased cell size and an increased level of expression for sIgM, BAQ44A and BAQ155A suggested that this cell phenotype was associated with a population of viable but resting B cells. A direct determination of the phenotype of proliferating B cells was not possible as both the BAQ44A and BAQ155A molecules were lost during the dual-labelling procedure for these molecules and BrdU (results not shown). However, with FALS it was possible to identify the S-phase B cells that had incorporated BrdU. Follicular cells were cultured in 6-well plates with PWM (1:400 dilution) and 100 U/ml of both rBoIL-1 and rBoIL-2. On Day 4 of culture the cells were transferred to fresh medium and incubated with 10 μM BrdU for various time intervals from 2 to 12 hr prior to terminating the cultures and analysing B cells in S-phase ($BrdU^+$) with FCM. These analyses determined that 23·3% of follicular cells had entered S-phase following a 12-hr incubation with BrdU and 97% of $BrdU^+$ cells had a FALS greater than the total cell population. Furthermore, 98% of follicular cells included by the upper FALS gate entered S-phase during a 12-hr period. Thus, an increased FALS was associated with 95% of the S-phase B cells. This confirmed that the phenotype of large B cells (sIg^{Lo}; $BAQ155A^{Lo}$; $BAQ44A^-$) also applied to the subpopulation of proliferating B cells. This B-cell phenotype was similar to that of the predominant B-cell population present in the lymphoid follicles of the ileal PP (Table 4, Day 0; Fig. 4, Day 0).

DISCUSSION

Lymphoid follicles isolated from the ileal PP of young lambs were used to prepare a population of cells representative of this unique microenvironment. Follicular cells were predominantly sIgM B cells that were phenotypically distinct from B cells in other lymphoid tissues. A low level of sIgM expression on ileal PP B cells has been reported by Miyasaka *et al.*[19] and Hein *et al.*[4] Also, immunohistochemistry has shown that $BAQ44A^+$ B cells and T cells were restricted to the dome region of the ileal PP[4] and that $BAQ155A^{Hi}$ B cells were in the inner follicle, while the $BAQ155A^{Lo}$ B cells were in the outer follicle (data not shown). Thus, the present FCM data are consistent with the isolation of cells from intact ileal PP follicles with associated dome regions. Approximately 50% of ileal PP follicular cells died during the first 24 hr in culture and this cell death occurred despite co-stimulation with PWM and rBoIL-1 and rBoIL-2. Motyka *et al.*[10] determined that the mechanism of ileal PP B-cell death was apoptosis and that 50–60% of cells died within 6 hr of culture. The constant number of viable B cells on Day 4 of culture, when the proliferative response was greatest, suggests that extensive cell death was also associated with PWM and cytokine-induced follicular B-cell proliferation. This *in vitro* association between proliferation and cell death is similar to that reported for the follicular microenvironment.[9] Thus, the isolated follicular B cells appeared to be both phenotypically and functionally similar to cells present in the follicles of the ileal PP.

The sharp decline in proliferative responses when fewer than 4×10^5 cells were cultured and as the culture period was extended suggests that a subpopulation of follicular B cells proliferated in response to PWM and rBoIL-1 and rBoIL-2 or either cell-to-cell contact or an accessory cell population limited proliferation. The BrdU pulse experiment determined that not all viable B cells were proliferating in response to co-stimulation with PWM and cytokines. The phenotype of S-phase B cells, or large B cells, was distinct from that of the small, resting B cells. The large B cells labelled less intensely with the PIg45A (sIgM) and BAQ155A mAb and did not label with the BAQ44A mAb. Thus, B cells proliferating *in vitro* were phenotypically similar to B cells isolated from the follicles of the ileal PP. In contrast, the increased level of sIgM and BAQ155A labelling and labelling by the BAQ44A mAb of small, resting B cells identified this population as phenotypically similar to that of B cells of spleen,

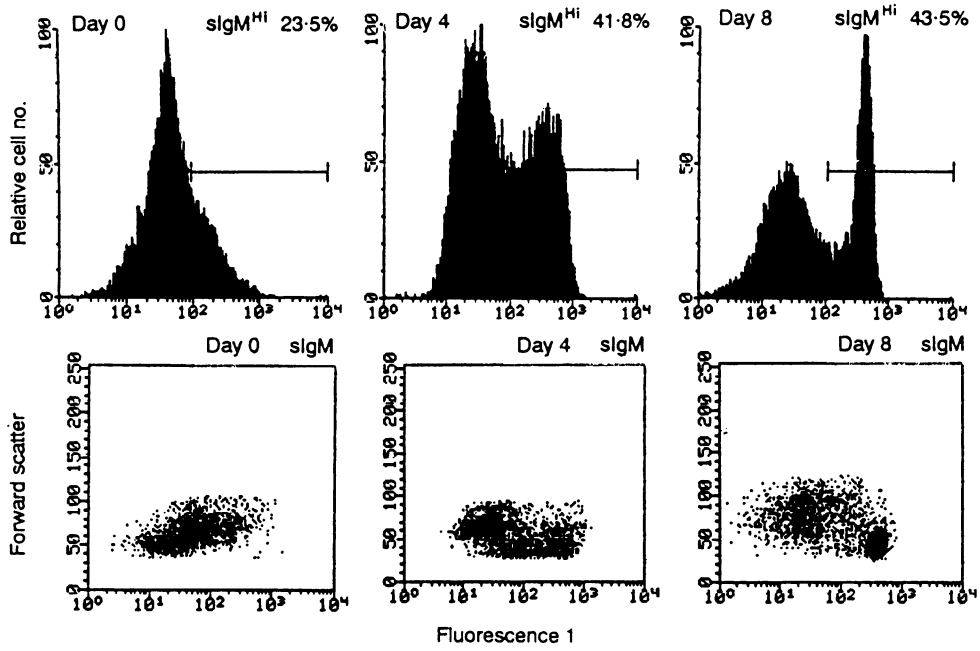

Figure 5. Comparison of the level of sIgM labelling and cell size for viable follicular B cells. An arbitrary demarcation has been used to define the $sIgM^{Hi}$ subpopulation of B cells. Follicular B cells (Day 0) contain 23·5% $sIgM^{Hi}$ B cells and mean cell size of $sIgM^{Hi}$ (12·05 μm) and $sIgM^{Lo}$ (11·55 μm) B cells was similar. On Days 4 and 8 of culture the $sIgM^{Hi}$ population increased to 41·8% and 43·5%, respectively. Furthermore, light scatter indicated that $sIgM^{Hi}$ B cells were significantly ($P < 0{\cdot}01$) smaller (Day 4, 9·74 μm; Day 8, 9·60 μm) than $sIgM^{Lo}$ B cells (Day 4, 12·8 μm; Day 8, 12·7 μm). Thus, there was an increased density of sIgM expression on the small B cells.

ileal MLN and jejunal PP. Thus, co-stimulation with PWM and rBoIL-1 and rBoIL-2 induced proliferation of immature ($sIgM^{Lo}$; $BAQ44A^-$) B cells and the production of mature ($sIgM^{Hi}$; $BAQ44A^+$) B cells.

The proliferation and differentiation of ileal PP follicular B cells in response to rBoIL-1 and rBoIL-2 is consistent with previous observations that purified or recombinant IL-1[20 23] and IL-2[24-26] can directly induce proliferation and differentiation of either mitogen or antigen-activated human and mouse B cells. A direct interaction was not demonstrated for rBoIL-1 and rBoIL-2 with ileal PP follicular cells. These cytokines may have acted indirectly through the induction of cytokine secretion by the small number of T cells, macrophages or stromal cells. However, a direct interaction of both rBoIL-1 and rBoIL-2 with follicular B cells provides the simplest explanation for the equivalent proliferative response to both cytokines and the absence of an additive or synergistic effect when both cytokines were added to cultures. Furthermore, T cells are unlikely to be a significant source of cytokines in these cultures in view of the small proliferative response induced by PWM, a T-dependent B-cell mitogen.[27]

Proliferation induced by rBoIL-2 may not be relevant to B-cell growth in ileal PP follicles. Immunohistochemical (Hein *et al.*, 1989) and FCM analysis (Table II) demonstrated a paucity of T cells in the ileal PP follicles. The T cells were restricted to the dome region[4] where few B cells proliferate.[28] This suggests that T-cell cytokines, such as IL-2, do not contribute to the growth of B cells in ileal PP follicles. However, the presence of both T cells and $BAQ44A^+$ B cells in the dome region of ileal PP follicles may be consistent with T-cell cytokines, such as IL-2 influencing follicular B-cell maturation. The development of $BAQ44A^+$ B cells in cultures co-stimulated with PWM and both rBoIL-1 and rBoIL-2 is consistent with IL-2 playing a role in follicular B-cell differentiation. In contrast, the rBoIL-1-induced proliferation of PWM-activated follicular B cells may be relevent to B-cell production in ileal PP follicles. Both stromal cells and macrophages may be a source of IL-1[29] and these cells are located throughout the follicle. However, the development of mature B cells during the culture period indicates that either rBoIL-1 also promoted B-cell differentiation or rBoIL-1 was not sufficient to maintain proliferation of the PWM-activated B cells. Other cytokines produced by macrophages or stromal cells may be necessary to sustain B-cell production in the ileal PP follicular microenvironment.

The present investigations demonstrated that PWM co-stimulation resulted in follicular B-cell proliferation and differentiation in response to more than one cytokine. These B-cell responses indicate that PWM interacts with a surface molecule or molecules that transduce a signal or signals that influence B-cell development. In conclusion, this culture system may be useful in the investigation of ileal PP follicular B-cell growth and differentiation and a characterization of B-cell surface molecules and the cellular interactions involved in these processes.

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