B cell attracting chemokine 1 (CXCL13) and its receptor CXCR5 are expressed in normal and aberrant gut associated lymphoid tissue

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Background and aims: In mice, the B lymphocyte chemoattractant (BLC) CXC chemokine ligand 13 (CXCL13) is sufficient to induce a series of events leading to the formation of organised lymphoid tissue. Its receptor, CXCR5, is required for normal development of secondary lymphoid tissue. However, the human counterpart, B cell attracting chemokine 1 (BCLA-1) has only been detected in the stomach and appendix and not in other parts of normal or diseased gut. Hence to elucidate the potential role of this chemokine and its receptor in human gut associated lymphoid tissue (GALT), we analysed their expression in normal intestine and ulcerative colitis (UC).

Methods: Frozen sections of surgical specimens were studied by multicolour immunofluorescence staining, in situ mRNA hybridisation, and reverse transcription-polymerase chain reaction.

Results: BCLA-1 mRNA was detected in all normal colonic and UC specimens. BCLA-1 was produced and accumulated in relation to peripheral dendritic elements of lymphoid follicles in Peyer’s patches and normal colon, as well as in irregular lymphoid aggregates in UC lesions. BCLA-1 was partially associated with the traditional follicular dendritic cell phenotype but also with extracellular fibrils in GALT structures. CXCR5 protein was expressed by mantle zone B cells and appeared at a high level on scattered germinal centre T cells.

Conclusions: BCLA-1 and CXCR5 are expressed in normal GALT structures as well as in irregular lymphoid aggregates in UC. This strongly suggests that BCLA-1 plays an important role not only in the formation of normal GALT but also in the generation of aberrant lymphoid tissue in inflammatory bowel disease.
development of murine GALT, might be restricted to certain parts of the human GALT, perhaps having a different and less important role in humans than in mice. To address this possibility, we examined normal human GALT specimens for BCA-1 and CXCR5 expression. Although BCA-1 was primarily described as a constitutively expressed chemokine, recent data have suggested that it might also contribute to the formation of malignant and inflammatory lymphoid aggregates. Hence we further examined the occurrence of BCA-1 and its receptor CXCR5 in colonic lesions of ulcerative colitis (UC) to reveal possible differences in the expression patterns between normal and aberrant lymphoid tissue in the human gut.

**MATERIAL AND METHODS**

**Patient characteristics**

The characteristics of the subjects are listed in table 1. Surgical specimens from 15 UC patients were screened for the presence of lymphoid aggregates by haematoxylin and eosin (H&E) staining of one section from each tissue block, and five specimens were selected for this study by their large number of lymphoid aggregates. These selected UC specimens were subjected to RNA extraction followed by new sections stained with H&E for evaluation of histological grade of inflammation, number of follicles, and to ensure an acceptable morphology of the sections before further serial cutting for immunohistochemistry. These sections were air dried overnight, fixed in acetone (10 minutes, 22°C), and stored at −20°C until use.

In addition, five 150 µm thick sections from each tissue block were cut from the normal colon and UC specimens and subjected to RNA extraction followed by new sections stained with H&E. Thereafter, 8 µm thick sections were cut and fixed in 4% paraformaldehyde/diethyl pyrocarbonate treated phosphate buffered saline (15 minutes) for in situ hybridisation. The Crohn's disease specimens were only prepared for immunohistochemistry.

Histological grading of inflammation in the UC specimens was performed according to the following 0–4 scale: 0, normal mucosa; 1, no active inflammation in the lamina propria; 2, mild active inflammation; 3, moderate active inflammation; and 4, severe active inflammation. All UC specimens showed histologically active inflammation (table 1).

**Reverse transcription-polymerase chain reaction (RT-PCR)**

RNA was isolated from 5×150 µm cryosections by means of QiAshredder columns and the RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. RNA primed with oligo (dT) and reverse transcribed with Superscript II (Gibco BRL, Life Technologies Inc., Rockville, Maryland, USA) was used for 20 µl reactions. Specific mRNA was amplified by real time KF-PCR with the Light Cycler (Roche Diagnostics, Indianapolis, Indiana, USA) and FastStart DNA master SYBR Green reagents, as described previously. Primers used were (5′ to 3′) BCA-1: TGCTAATGAGGAGCGAGAGAGGAAATG; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH): AAATCCCATACACCACTTCC, CATGATGCCTTCACGGATACC. The annealing temperature for both primer pairs was 60°C, and 35 cycles of PCR amplification were performed. The PCR products were separated by electrophoresis in 1.6% agarose gel and stained with ethidium bromide.

**In situ hybridisation**

A 436 bp digoxigenin (DIG) labelled riboprobe was generated from the coding region of human BCA with the DIG RNA labelling kit according to the manufacturer’s instructions.

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**Table 1**

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<th>Age (y)/sex</th>
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<th>Disease duration (y)</th>
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<th>No of BCA-1 CD21+ aggregates§</th>
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*UC, ulcerative colitis; NC, normal colon.

†Number of lymphoid aggregates per section judged by haematoxylin and eosin staining. Parallel sections from the same tissue block were evaluated (cut a distance of approximately 750 µm apart) and the numbers from the two sections were presented as a range.

‡Parallel sections 8 µm apart were examined for BCA-1+ follicles: one section was stained with polyclonal anti-BCA-1 in combination with anti-CD21; the other section was stained with monoclonal anti-BCA-1 in combination with the DRC antibody. Follicles were described as BCA-1+ when a reticular staining meshwork was seen; CD21+ or DRC+ aggregates were always surrounded by a BCA-1+ reticular meshwork.

§Extensive cell infiltration was seen but separate lymphoid aggregates were difficult to identify.

BCA-1, B cell attracting chemokine 1.
**Table 2** Characteristics of the immunoreagents used in the study

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BCA-1, B cell attracting chemokine 1; CXC5, CXC chemokine receptor 5; DRC, dendritic reticulum cell; vWF, Von Willebrandt factor; MAdCAM-1, mucosal addressin cell adhesion molecule 1; SMA, smooth muscle actin.

(Boehringer Mannheim, Mannheim, Germany). All incubations took place at room temperature unless otherwise stated. Briefly, after fixation, sections were washed twice (15 minutes each) in phosphate buffered saline containing 0.1% active diethyl pyrocarbonate (Sigma-Aldrich, St Louis, Missouri, USA). After 15 minutes of equilibration in 5% SSC, sections were hybridised overnight at 59°C with 250 ng/ml of riboprobe in hybridisation solution (50% formamide, 5× SSC, 50 µg/ml yeast tRNA, 100 µg/ml heparin, 1× Denhardt solution, 0.1% Tween 20, 0.1% CHAPS, and 5 mM EDTA) followed by high stringency wash. Detection of the hybridised probe was performed according to a protocol kindly provided by Bradley St Croix (Johns Hopkins Oncology Center, Baltimore, Maryland, USA).13 The sections were incubated (45 minutes) with horseradish peroxidase conjugated rabbit anti-DIG (1/50; Dako, Glostrup, Denmark) in blocking buffer (0.1% Boc-Bringer blocking agent dissolved in 100 mM Tris HCl (pH 7.5), 150 mM NaCl) followed by signal amplification with biotin-tetramide deposition (GenPoint kit; Dako). Subsequently, sections were incubated (20 minutes) with horseradish peroxidase conjugated rabbit anti-DIG (1/50 in blocking buffer; Dako), followed by an additional cycle of biotintetramide deposition. Signal was detected by incubation (20 minutes) with alkaline phosphatase conjugated rabbit antibiotin (Dako), followed by the alkaline phosphatase substrate Fast Red according to the manufacturer’s instructions (Ventana Medical Systems, Tucson, Arizona, USA). Finally, the sections were counterstained with haematoxylin.

**Two and three colour immunofluorescence staining**

Immunostaining on cryosections was performed as described previously,11 and primary as well as secondary antibody reagents are listed in table 2. Briefly, acetone fixed sections were first incubated with a mixture of primary reagents for one hour at room temperature. Either the monoclonal or polyclonal anti-BCA-1 was used at a concentration of 10 µg/ml in combination with different primary reagents detecting either follicular dendritic cells (FDCs), B cells, T cells, adhesion molecules, vessels, or extracellular matrix proteins. This combination was followed by incubation with the appropriate mixture of secondary antibody reagents and, when a biotinylated antibody was used, a final application with labelled streptavidin. The monoclonal anti-BCA-1 reagent was always visualised with a subclass specific secondary antibody reagent (goat antimouse IgG1; 1/2000). The monoclonal antibody to CXC5 was applied at 5 µg/ml and mixed with anti-CD3 followed by horse IgG antimouse IgG (1/200) and, subsequently, Alexa 488 conjugated goat IgG antirabbit IgG together with Cy3 labelled streptavidin.

As negative controls, we used tissue sections incubated in the first step with irrelevant isotype and concentration matched monoclonal antibodies, and concentration matched normal goat IgG (Sigma-Aldrich) and rabbit IgG (authors’ laboratory) purified from serum of non-immunised animals. As an additional control, recombinant BCA-1 (R&D Systems, Oxon, UK) was incubated at different concentrations together with antibody reagents against BCA-1 before the mixtures were applied to tissue sections in an attempt to block the staining reactions.

**RESULTS**

**Distribution of BCA-1 expressing cells in Peyer’s patches and normal colon**

BCA-1 was detected in the mantle zone of all lymphoid follicles in the gut (fig 1). The staining patterns obtained with the polyclonal and monoclonal antibody reagents were similar, often resembling a meshwork associated with cells that extended dendrites. Although this expression pattern was generally seen throughout the mantle zone, it was often most prominent peripherally, with positive dendrites outlining the
B cell zone (fig 1A). Conversely, in the germinal centres, a disparate staining pattern was obtained with the polyclonal versus the monoclonal antibody reagent. The latter often stained scattered cells in the germinal centres which appeared most prominently in PPs (fig 1B). This feature seemed to be related to the frequent large size of the germinal centres in these GALT structures. The polyclonal reagent did not decorate these scattered germinal centre cells but produced a relatively weak staining corresponding to the FDC network in some parts of the germinal centres, possibly representing the light zones (fig 1C, 1D).

Given the secretory nature of the chemokine BCA-1 and the disparate germinal centre staining pattern obtained with the two antibody reagents, we compared the distribution of BCA-1 protein to that of BCA-1 encoding mRNA transcripts. By means of in situ hybridisation, we detected BCA-1 transcripts in the mantle zone of all lymphoid follicles in PPs and normal colon (fig 1E, 1F). Thus the cellular nature of at least some of the reticular pattern observed with immunostaining was also supported by observing mRNA in a similar distribution (fig 1F, inset). Some scattered weak signals were furthermore observed in large germinal centres (fig 1E, inset: enlarged details) with predominately dendritic distribution of message in the mantle zone. (G, H) Two colour staining of BCA-1 in solitary follicle of normal colon detected by polyclonal (G) or monoclonal (H) antibody reagent combined with anti-CD21 (green) or anti-DRC (green) to reveal follicular dendritic cells shows some co-staining (yellow) of dendrites in the inner part of the mantle zone (arrows). The two sections were cut in parallel at a distance of 8 µm; some dendrites positive for BCA-1 but negative for DRC appear centrally as the follicle is cut closer to the mantle zone and therefore appears smaller (weak unspecific green staining of surrounding epithelial goblet cells is seen in (H)). (I) Two colour staining of BCA-1 and fibronectin (green) in PP shows extensive dendritic colocalisation (yellow) in the mantle zone (C=epithelial crypt). Original magnifications: ×200 (A–D, G, H); ×100 (E, F, and inset F); and ×400 (inset C, H, and I).

**Figure 1** Expression of B cell attracting chemokine 1 (BCA-1) in normal gut specimens. (A–D, G–I) Immunofluorescence and (E, F) in situ hybridisation analysis of frozen tissue sections with BCA-1 visualised by red signals in all panels. (A) Three colour staining of Peyer’s patch (PP) showing BCA-1 expressing elements between CD3\(^+\) T cells (green) and CD20\(^+\) B cells (blue). (B–D) Two colour staining of PP showing BCA-1 expressing cells detected by monoclonal (B) or polyclonal (C, D) antibody reagent combined with either anti-dendritic reticulum cell (DRC) (green) (B), anti-IgD (green) (C), or anti-CD23 (green) (D). BCA-1 protein is predominantly associated with a meshwork in the mantle zone while disparate staining patterns are produced in the germinal centre by monoclonal and polyclonal anti-BCA-1 (scattered central cells positive with the former, and weak positivity corresponding to follicular dendritic cells with the latter reagent). (E, F) Expression of BCA-1 mRNA in PP (E, inset: sense probe control) and solitary colonic follicle (F, inset: enlarged details) with predominantly dendritic distribution of message in the mantle zone. (G, H) Two colour staining of BCA-1 in solitary follicle of normal colon detected by polyclonal (G) or monoclonal (H) antibody reagent combined with anti-CD21 (green) (G) or anti-DRC (green) (H) to reveal follicular dendritic cells shows some co-staining (yellow) of dendrites in the inner part of the mantle zone (arrows). The two sections were cut in parallel at a distance of 8 µm; some dendrites positive for BCA-1 but negative for DRC appear centrally as the follicle is cut closer to the mantle zone and therefore appears smaller (weak unspecific green staining of surrounding epithelial goblet cells is seen in (H)). (I) Two colour staining of BCA-1 and fibronectin (green) in PP shows extensive dendritic colocalisation (yellow) in the mantle zone (C=epithelial crypt). Original magnifications: ×200 (A–D, G, H); ×100 (E, F, and inset F); and ×400 (inset C, H, and I).

**BCA-1 is associated with, but not restricted to, the traditional FDC phenotype in GALT**

In an effort to phenotype the BCA-1 expressing cells, we performed paired staining with two colour immunofluorescence for several FDC markers. In the inner part of the mantle zone, some colocalisation of BCA-1 with these markers was seen in both PPs and normal colonic follicles: dendritic reticulum cell (DRC) (fig 1B), CD23 (fig 1D), CD21 (fig 1G), CD35 (data not shown, but see fig 4D), and mucosal addressin cell adhesion molecule 1 (MAdCAM-1) (not shown). Thus the polyclonal reagent to BCA-1 produced co-staining with the different FDC
markers centrally in the germinal centres but generally not more peripherally (fig 1D). Even cells with the strongest expression of the two most restricted FDC markers, CD23 (fig 1D) and MadCAM-1 (not shown), displayed only partially overlapping staining for BCA-1.

FDCs were detected in the centre of most but not all follicles decorated for BCA-1 (table 1). Parallel sections were investigated for some specimens, and these revealed that the FDCs disappeared while prominent staining for BCA-1 remained in follicles when cut closer to the mantle zone (fig 1G, 1H). This finding showed that the appearance of BCA-1 in follicles without detectable FDCs could, at least partially, be explained by the differential localisation of BCA-1 and FDC markers within the follicle.

In contrast with the limited colocalisation of BCA-1 and FDC markers seen in the inner B cell zone, extensive overlapping fluorescence for BCA-1 and fibronectin occurred throughout the mantle zone meshwork (fig 1H). Conversely, immunostaining with antimuscle actin or antismooth muscle actin did not show any overlap with the BCA-1 positive meshwork but only occasional peripheral intermingling of separately decorated elements (data not shown). Based on the description of BCA-1 expressing vessels in human tonsils, we also co-stained for BCA-1 and von Willebrandt factor (vWF) and observed a small proportion of BCA-1 positive vessels both in the germinal centres and mantle zones of all specimens. In the mantle zones, the BCA-1 positive vessels were often bordered by strongly BCA-1 positive dendrites (see fig 5A, C).

The dendritic mantle zone pattern seen with both BCA-1 antibody reagents, and also the disparate staining features described for the germinal centres, were blocked by incubation with recombinant BCA-1. The monoclonal antibody was neutralised at an antibody: chemokine molar ratio of 1:2.5 while the polyclonal reagent was neutralised at a molar ratio of 1:0.25.

**Distribution of CXCR5 expressing B and T cells in GALT**

Expression of CXCR5 was seen mainly in lymphoid aggregates, both in the normal and diseased gut, while only scattered CXCR5+ cells appeared in the T cell zones of PPs as well as outside of the lymphoid aggregates in UC lesions. In an attempt to identify these positive elements as B cells, we used an IgG2B specific secondary antibody reagent to detect CXCR5 concurrently with staining for CD20. However, with this relatively insensitive costing protocol, CXCR5 expression was restricted to lymphoid follicles and aggregates (data not shown).

CXCR5 was detected mainly on mantle zone cells in PPs and normal colonic and UC follicles; the overall staining pattern clearly represented a general surface expression on B cells in this lymphoid compartment (fig 3A, 3B). When serial tissue sections were examined, CXCR5 expression was found in all follicles investigated. Such expression was also often seen in the centre of lymphoid follicles with no apparent germinal centre reaction; some of this staining reflected the fact that lymphoid follicles were cut close to the mantle zone. However, CXCR5 expression could also be found in follicles where no germinal centres appeared on serial sectioning, thus reflecting a general surface expression on B cells in primary follicles.

High expression of CXCR5 on T cells was restricted to lymphoid follicles. When CXCR5 staining occurred in overt germinal centres, it was not of even intensity but appeared particularly strong on T cells scattered throughout this compartment (fig 3C) and occasionally in the inner part of the mantle zone (fig 3A). This was the case in both the normal and diseased gut but most prominent in PPs, apparently being mainly associated with large germinal centres.

**Expression of BCA-1 in UC lesions**

By means of serial section analysis, we detected BCA-1 protein and transcripts in all aberrant lymphoid aggregates in UC (fig 4). Additional colonic and small bowel sections from a patient with Crohn’s disease were also investigated for BCA-1 protein, and a similar follicular meshwork positive for BCA-1 was

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**Figure 2** Reverse transcription-polymerase chain reaction (RT-PCR) analysis of B cell attracting chemokine 1 (BCA-1) expression in normal and inflamed colonic mucosa. BCA-1 mRNA is present in all samples of ulcerative colitis (UC) and normal colon (NC). Total RNA was isolated from frozen tissue samples of colonic mucosa, reversed transcribed, and subjected to RT-PCR with specific primers and a standardised numbers of cycles. PCR products were separated by electrophoresis in agarose gel stained with ethidium bromide. Comparable PCR with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers is shown as a control for the cDNA content of the individual samples.

**Figure 3** Localisation of CXC chemokine receptor 5 (CXCR5) protein in normal and inflamed gut. Two colour immunofluorescence staining of cryosections for CXCR5 (red) and CD3 (green). (A) Prominent CXCR5 expression is seen on B cells in the mantle zone of the Peyer’s patch and (B) solitary colonic follicle in normal mucosa. Strongly CXCR5+ T cells are seen scattered in the germinal centre (see inset in [A]) and the inner part of the mantle zone shown in (B). (C) CXCR5 is also abundantly expressed on B cells in irregular lymphoid aggregate in ulcerative colitis. Original magnifications: ×100 (A); ×200 (inset A and C); and ×200 (B).
tionally described as “follicular B helper T cells” (TFH cells) positive T cells that localise to B cell follicles have been functionally implicated to be FDCs, but this was not explicitly shown. The CXCR5 expression was probably a result of local receptor upregulation, which could explain why GC-Th cells reportedly show a stronger chemotactic response to BCA-1 than other T cells because they show all the characteristics required for efficient B cell help within the lymphoid follicles. A subpopulation of T<sub>Tfh</sub> cells has been identified as CD57<sup>+</sup> CXCR5<sup>+</sup> T cells and termed germinal centre T helper (GC-Th) cells because they appear to be essential for B cell differentiation and antibody production in lymphoid tissue<sup>17–19</sup>; these cells are localised only within the germinal centres. We observed quite prominent expression of CXCR5 on scattered T cells restricted to germinal centres and the inner part of the mantle zone in GALT, and such cells were also detected in tonsillar follicles (our unpublished observations). FACS analysis of isolated cells from murine PP and human tonsils<sup>20,21</sup> has revealed a much higher proportion of CXCR5<sup>+</sup> T cells, implicating the presence of a substantial fraction of such cells in the T cell zone of these tissues. Our observation of CXCR5<sup>+</sup> T cells being restricted to germinal centres in both PPs and tonsils most probably reflected the lower sensitivity of the applied in situ immunostaining method. In this location, the observed scattered T cells should belong to the GC-Th subset. Their prominent CXCR5 expression was probably a result of local receptor upregulation, which could explain why GC-Th cells reportedly show a stronger chemotactic response to BCA-1 than other CXCR5<sup>+</sup> T cells isolated from tonsils.<sup>22</sup> The fibrillar meshwork positive for BCA-1 protein detected in GALT mantle zones was a striking observation. Its colocalisation with the extracellular matrix protein fibronectin was extensive, suggesting that BCA-1 is deposited on reticular fibres in follicles where the fibrillar network is looser than in other compartments of lymphoid tissue. Also, BCA-1 mRNA was expressed in a pattern that strongly suggested a dendritic morphology of the cells producing BCA-1 in this compartment. BLC has been reported to be produced by non-lymphoid cells present in murine lymphoid follicles;<sup>23</sup> its origin was implicated to be FDCs, but this was not explicitly shown. The observed partial overlapping of our immunostaining for the chemokine with several traditional FDC markers might result from BCA-1 secreted from another cell type and deposited on peripherally located FDCs. However, electron microscopy has...
suggested considerable heterogeneity of FDCs in tonsillar germinal centres; possibly FDC related, peripherally located so-called germinal centre bordering cells with long extensions have been described in rat lymph nodes. Therefore, an FDC related subset of dendritic cells remains a potential but yet not proved source of BCA-1 in lymphoid tissue. This accords with in vitro stimulation of isolated human FDC-like cells in which no BCA-1 protein could be detected.

BCA-1 protein expression observed on scattered cells in GALT germinal centres was detected only by the monoclonal antibody but was supported by mRNA signals in the same compartment. In contrast, a more extensive germinal centre bordering cells with long extensions were scattered within the FDC meshwork. In the inner mantle zone of UC follicles however, some colocalisation of BCA-1 protein and the FDC markers CD21, CD23, CD35, DRC, and MAdCAM-1 was found while BCA-1 expressing dendritic elements mainly overlapped with extracellular fibrils. Hence the distribution of BCA-1 in UC follicles corresponded to our findings in normal GALT, thus suggesting a similar role of this chemokine in aberrant and normal GALT.

The so-called BLAs are a histopathologically defined feature indicative of IBD, although their relevance in the pathogenesis remains unknown. Experimental inflammation in mice can induce ectopic expression of BLC (and another lymphoid chemokine, namely SLC, also called 6Ckine, exodus-2, or CCL21). The first report on BCA-1 expression in human disease was from Helicobacter pylori induced gastric mucosa associated lymphoid tissue (MALT) in chronic gastritis and gastric B cell (MALT) lymphoma. The distribution of BCA-1 producing cells in such aberrant MALT was found to resemble that observed in human tonsils. Also, the reticular appearance of the immunostaining for BCA-1 was seen even in the absence of detectable FDCs. Rare expression of BCA-1 was also noted in the normal stomach in keeping with the original work of Legler and colleagues in which BCA-1 transcripts were found in the human stomach and appendix.

There are data on BCA-1 expression in other human diseases such as rheumatoid arthritis and Sjögren’s syndrome. Contrary to our findings in normal GALT and UC lesions, immunostaining for BCA-1 was reported to be associated only with FDCs in germinal centres in rheumatoid arthritis while the mantle zones were negative. In situ transcripts for BCA-1 and the cytokine lymphotoxin β suggested that BCA-1 and lymphotoxin β were necessary, but not sufficient, for the occurrence of FDCs in rheumatoid arthritis. This would be in keeping with our results in normal GALT and UC lesions where FDCs without exception were surrounded by a BCA-1 positive reticular meshwork.

The findings in experimental models and human diseases suggest that BLC/BCA-1 also has a function in aberrant lymphoid tissue. Our detection of both BCA-1 and CXCR5 in lymphoid aggregates in IBD lesions strengthens this notion. It has been proposed that induction of BCA-1 and other lymphoid tissue inducing chemokines at sites of inflammation could convert the lesion from an acute to a chronic state, and that blocking of chemokine activity therefore might be of therapeutic value. If this is true, our results point to IBD as a potential candidate for such chemokine blockade in the future.

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Figure 5 Vessel wall localisation of B cell attracting chemokine 1 (BCA-1) protein in normal gut associated lymphoid tissue and ulcerative colitis lesions. Three colour immunofluorescence staining of BCA-1 (red), von Willebrand factor (vWF) (green), and CD20 (blue) on frozen tissue sections from [A, C, and E] Peyer’s patch and [B, D, and F] ulcerative colitis lesion with single exposures identifying BCA-1 [B, D] and vWF [C, E]. Scattered vWF+ vessels in the mantle zones (identified by densely packed CD20+ B cells) are weakly positive for BCA-1 and sometimes bordered by strongly BCA-1+ vessels. In ulcerative colitis, larger BCA-1+ vessels are occasionally seen outside of follicles. Original magnifications: ×600 (A, C, and E); and ×400 (B, D, and F).
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