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VIEWPOINT

Numerical Distribution of Lymphoid Nodules in the Human Sigmoid Colon, Rectosigmoidal Junction, Rectum, and Anal Canal

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There is little information on the numerical distribution of lymphoid nodules (LN) in distal segments of the human large bowel. A novel approach was therefore developed to assess the number of LN in the sigmoid colon, the rectosigmoid segment, the rectum, and the anal canal in humans. The distal large bowel from five cadavers was selected for quantitative study. The number of LN was scored macroscopically from the proximal sigmoid colon to the distal anal canal. A numerical distribution, previously unreported, consisting of two circular bands of LN was observed in each of the five cadavers. One band was located 3 cm proximal from the pectinate line and the other was located at the rectosigmoid segment. Significantly more LN occurred 3–5 cm proximal to the pectinate line compared to areas distal or proximal to this band of LN. This band of LN has not been reported previously in humans. Clin. Anat. 19:164–170, 2006. © 2006 Wiley-Liss, Inc.

Key words: lymphoid nodule; large bowel; chromoendoscopy; lymphoid follicles

INTRODUCTION

In the small intestine of humans, the lamina propria and the submucosa contain aggregates of lymphoid nodules (ALN) called Peyer's patches, which are located on the antimesenteric side of the small intestine. There are about 30 such patches in the human small intestine (Bloom and Fawcett, 1968). In contrast, lymphoid nodules (LN) in the large bowel of humans occur as isolated nodules, not as aggregates. The large bowel epithelium over the LN of young healthy human specimens consists of a dome of columnar epithelium. Surrounding this central dome of columnar epithelium are the mouths of intestinal glands (crypts) that extend into the mucosa. Adjacent to the crypts is a ring-shaped protrusion of mucosa.

The authors of two past studies reported counts of LN in the colon. Dukes and Bussey (1926) reported on the number of LN in the large intestine of 117 individuals who ranged in age from 7 months to 88 years at death. Dukes and Bussey's results on

the spatial distribution and counts of LN did not include the rectum or the anal canal. More recently Langman and Rowland (1986) gave evidence that Dukes and Bussey's numerical density counts may have been low. Langman and Rowland proposed that the low count of LN as reported by Dukes and Bussey was probably due to their removal of large portions of mucosa and scoring only the submucosal surface of specimens.

Langman and Rowland (1986) reported data on the numbers of LN in the colon and the rectum of five human accidental death victims who ranged in age from 19–58 years. They scored LN in random

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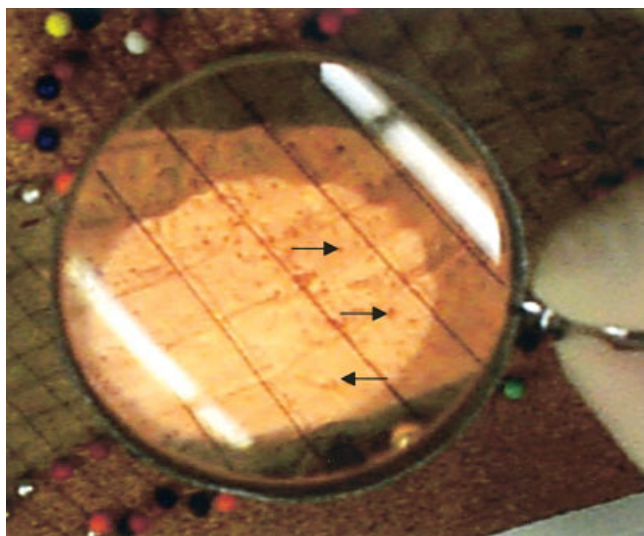


Fig. 1. Photograph of the large bowel mucosal surface pinned to cork board and photographed through a magnifying lens. The arrows point to LN in the large intestine. The dark lines are surgical suture strings spaced 1-cm apart to facilitated scoring of number of nodules per cm segment of bowel mucosa. These lines start from the pectinate line and end at the proximal sigmoid colon. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

samples along the length of the large intestine. Their method of estimating the LN numbers in the colon and in the rectum was to overlay opened areas of the intestinal surface with a grid of 1 cm squares drawn on a transparent piece of plastic. They did not score every LN along the length of the large bowel, which did not allow them to establish a precise spatial distribution of LN in the large bowel. Their counts of LN per cm² surface area in the colon were four to five times higher than those of Dukes and Bussey probably because Langman and Rowland did not separate the mucosa from the submucosa before scoring LN.

Langman and Rowland's data indicate that the number of LN per cm² remained constant along the length of the colon, but that the numerical density of LN was greatest in the rectum. Langman and Rowland did not distinguish the sigmoid colon as a distinct anatomical segment. Finally, neither Langman and Rowland nor Dukes and Bussey scored LN in the anal canal.

A novel approach was developed to assess the number and the spatial distribution of LN in the sigmoid colon, in the rectosigmoid segment, in the rectum, and in the anal canal. Unlike the prior reports of Dukes and Bussey and Langman and Rowland, this new approach allowed determination of specific distributional patterns of LN in these segments of the large bowel.

MATERIALS AND METHODS

Cadavers were obtained from the University of Texas Health Science Center at San Antonio "Willed Body" program. All individuals used in this experiment willed their bodies for medical research and educational purposes before their death. The lower end of the bowel from 12 cadavers was excised with minimum damage to the mucosa, and the feces were washed out of the specimens using a tap water rinse. A longitudinal incision was made from the proximal to the distal end of the specimen, and the bowel segment was opened serosa side down. The mucosal surface was then cleaned with Scope mouthwash (Proctor and Gamble, Cincinnati, OH) to remove surface mucus. Each specimen had to meet the following specific criteria for inclusion in the study: cause of death should not be associated with a disease of the large bowel, the specimen must be intact from the descending colon to the anus, and the specimen must be normal in gross appearance and in size (there should not be any locations in the

TABLE 1. Basic Data From Five Human Distal Large Bowel Specimens

Case No.	1	2	3	4	5	Average
Gender	M	M	M	M	M	—
Age	89	57	94	65	61	73.2
Length of sigmoid colon (cm)	13	15	16	14	9	13.4
Ave. width of sigmoid colon (cm)	7.7	5.4	6.6	5.9	6.8	6.5
Area of sigmoid colon (cm ²)	99.1	81	105.5	82.0	62	85.9
Length of rectosigmoid segment (cm)	5	5	4	6	5	5
Ave. width of rectosigmoid segment (cm)	6.9	7	7.13	5.9	7.8	6.9
Area of rectosigmoid segment (cm ²)	34.5	35	28.5	35.4	39	34.5
Length of rectum (cm)	11	8	9	8	10	9.2
Ave. width of rectum (cm)	9.95	9.31	7.77	8.06	8.05	8.63
Area of rectum (cm ²)	109	74.5	70	64.5	80.5	79.7
Length of anal canal above pectinate line (cm)	1	1	1	1	1	1
Ave. width of anal canal above the pectinate line (cm)	5.5	10	8	8.5	6	7.6
Area of anal canal above the pectinate line (cm ²)	5.5	10	8	8.5	6	7.6

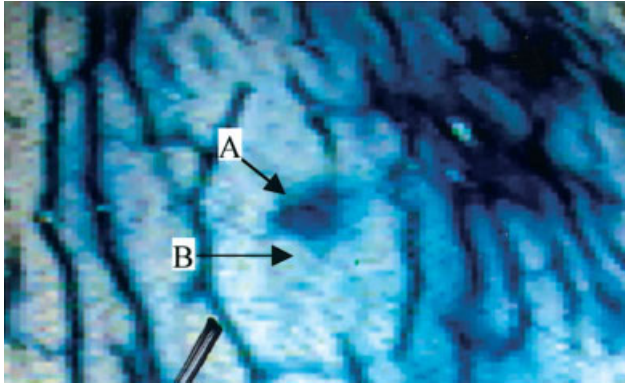


Fig. 2. A photomicrograph of a single LN taken through an endoscope. Arrow A indicates the location of the dome of columnar epithelium over a LN. Arrow B indicates the location of the mound that surrounds the location of a dome of columnar epithelium. This photograph field is about 3 mm². [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

large bowel that are collapsed, stretched, or that contain diverticula). Five bowel specimens met all criteria for inclusion in the study.

Once the specimens were selected, longitudinal, and vertical incisions were made through the tunica muscularis about 0.5 cm apart. This procedure was done to facilitate removal of the tunica muscularis from the mucosa. After carefully making incisions through the tunica muscularis, surgical scissors were used to remove the 0.5 × 0.5 cm squares of muscle

from the external surface of the submucosa. The remaining submucosa and mucosa were then pinned to a cork board with just enough tension to remove folds in the mucosal surface. This allowed better visualization and scoring of LN. Care was taken to avoid mucosal tearing while the mucosa was pinned to the cork board. Examples of LN, as seen by gross observation and with the aid of a handheld magnifying lens in such a mucosal preparation, are illustrated in Figure 1.

Surgical suture was used as gridline to mark transverse 1-cm segments along the entire length of the mucosal preparation, from pectinate line to the sigmoid colon as illustrated in Figure 1. The width of the specimen at each 1-cm transverse segment was measured to allow calculation of the numbers of LN per cm² and total number of LN in each 1-cm segment in: the anal canal above the pectinate line, in the rectosigmoid segment, in the rectum and in the sigmoid colon. In the five different specimens studied, these four anatomical segments varied slightly in length between individuals as reported in Table 1. The following is a brief descriptive summary of the four bowel segments that were scored. The anal canal continues above the pectinate line for 1 cm. The next segment above the anal canal is the rectum with its three sub-segments (distal rectum, ampulla, and proximal rectum). The rectum continues proximal for 8–11 cm. Above the proximal

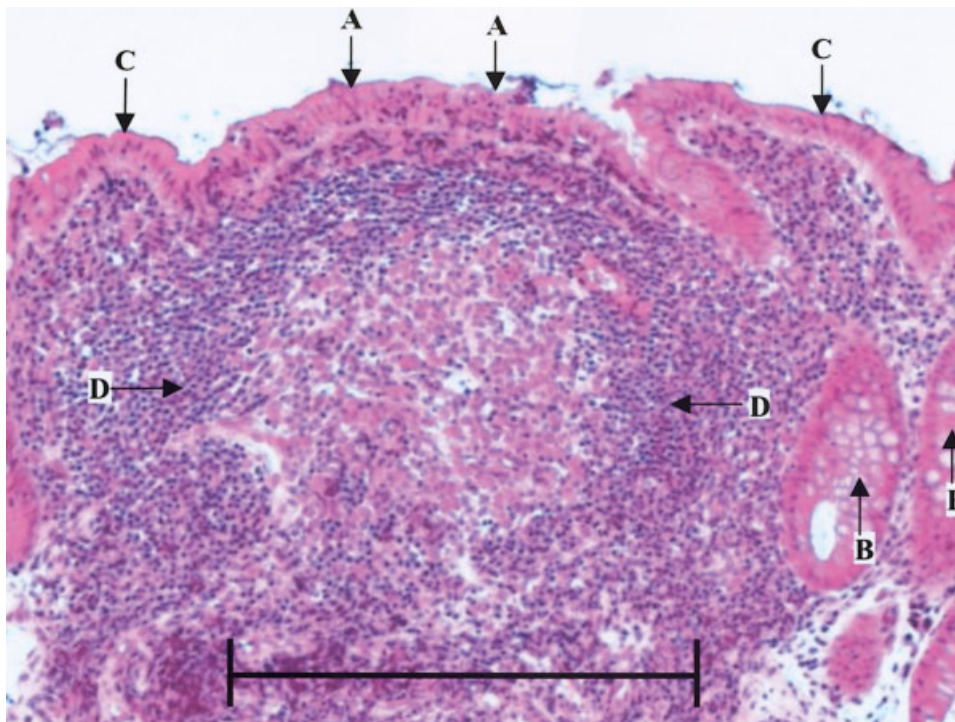


Fig. 3. Photomicrograph of an H&E stained histological cross-section of LN of a biopsy taken from the large bowel of a healthy adult. The arrows at A indicate a sheet of columnar epithelium in the form of a dome over the lymphatic tissue. The arrows at B indicate mucosal crypts extending into the mucosa, surrounding the dome of columnar epithelium. The arrows at C indicate the protrusion of mucosa, which looks like a circular mound when viewed from the luminal surface (Fig. 2). The arrows at D indicate the LN proper with the germinal center (scale bar = 1,842 μm in length). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

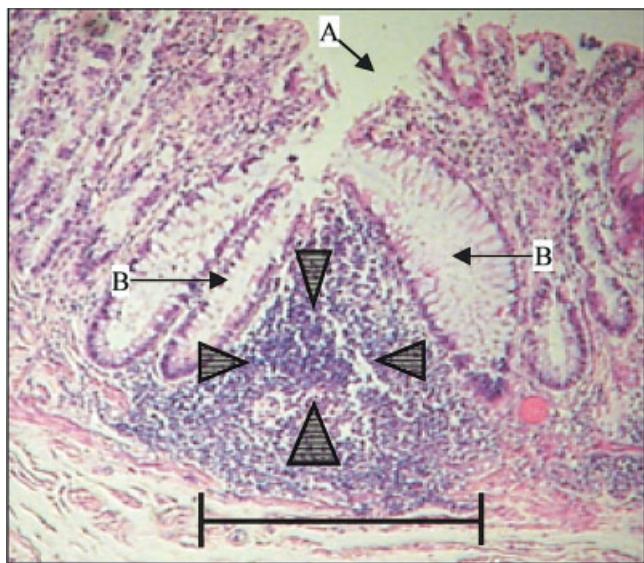


Fig. 4. A histological cross-section of a LN taken from a cadaver in this study. Arrow A indicates the depression in the mucosa. The dome of columnar epithelium commonly observed in biopsy specimens of healthy adults (Fig. 3) is not observed in the cadaver specimen illustrated here. The arrows at B indicate the mucosal crypts immediately adjacent to the LN. Arrowheads point to nodule of densely packed lymphoid cells (scale bar = 1070 μm in length; H&E stain). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

rectum, the bowel makes a turn to the left and continues for 5–7 cm. This region is defined as the rectosigmoid segment. From the rectosigmoid segment, the sigmoid colon segment proper continues for 9–16 cm to the descending colon. After scoring was complete, one to three grossly observed LN were dissected from each specimen and were prepared for histology. Hematoxylin and eosin (H&E)-stained histological cross sections of these LN from the five individuals used in the study were prepared to confirm the presence of a lymphoid nodule. Eleven LN were taken for histological examination.

The human large bowel biopsy specimens used for this endoscopic study were obtained under a protocol (LAB94-032) approved by the Institutional Review Board of The University of Texas MD Anderson Cancer Center at Houston. Informed consent was obtained from each volunteer. Subjects scheduled for routine endoscopy for detection and removal of colorectal polyps were screened for entry into the protocol. Suspected LN specimens from four subjects (mean age = 53 years) were used. No further demographic data on these patients was available. None of these subjects displayed evidence of large bowel disease. A Go-Lytely enema (Braintree Labs, Braintree, MA) was given before colonoscopy. Fine detail of the mucosal surface was visual-

ized using an Olympus ZX 200 magnifying video-colonoscopy. Imaging was enhanced by the application of a chromoendoscopic technique that included the endoscopic application of a fine spray of Indigo Carmine dye to the mucosal surface as a contrast agent (Fig. 2). Biopsy specimens from suspected LN were taken using 5-mm pinch forceps, and the specimens were fixed in formalin for routine histology.

An analysis of variance (ANOVA) statistical test, followed by a Student-Newman-Keuls (SNK) multiple range test, was done to determine significant differences in numerical density of LN.

RESULTS

Gross and Microscopic Appearances of LN in the Large Bowel of Humans

Figure 3 is a photomicrograph of a histological section of a LN of a biopsy specimen taken from the large bowel of a healthy adult. A dome of columnar epithelium on the luminal surface over the surface of the LN is visible. Surrounding the central dome of columnar epithelium are the mouths of crypts. A crypt is seen extending into the mucosa. Adjacent to the crypts is a peripheral protrusion of mucosa. This protrusion surrounds the dome of columnar epithelium and contains the mouths of crypts.

In contrast, the LN that were dissected from cadavers of older individuals (age range = 57–94) did not have a dome of columnar epithelium above the LN as observed in histological section (Fig. 4) and were visualized on the mucosal surface as a depression in the mucosa. The histological section of these depressions in the mucosa did, however, show a nodule of densely packed lymphoid cells. Histological analyses of LN from each of the five specimens showed that 10 of 11 suspected LN sampled contained a nodule of densely packed lymphocytes. These findings confirmed that the depressions scored in the mucosa of non-diseased gross specimens were LN.

Distribution of LN in the Distal Large Bowel

The LN counts were expressed in two ways: per cm^2 (Table 1, Fig. 5) and per 1-cm segment (Figs. 6,7). Macroscopically, the LN ranged in diameter from 0.5–2 mm in diameter. Any depression within this range was counted as a nodule. These data indicate that the highest numerical counts of LN occurred in the rectal segment, and the second highest numerical counts of LN occurred in the rectosigmoid segment in all five specimens (Figs. 5,6). LN at each of these two sites formed a circular band in

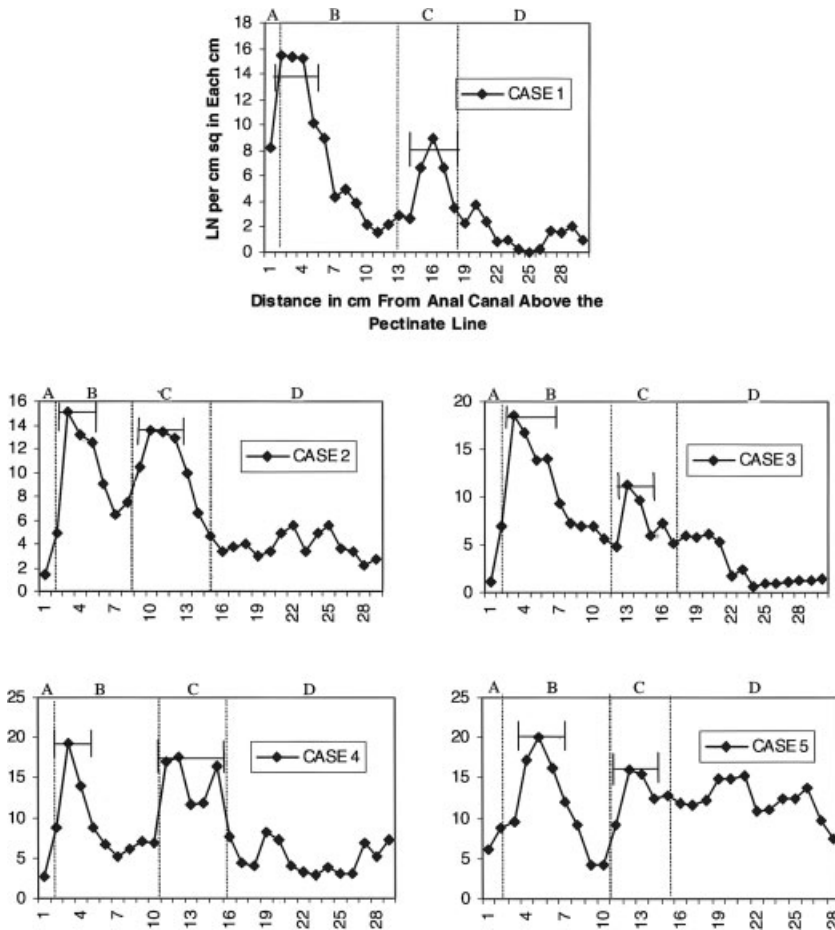


Fig. 5. Distribution of LN per cm² in each one segment of the five individual cadaver specimens. Note in all five specimens there are two peaks in the number of LN. The first peak is at the distal end of the rectum and the second is at the rectosigmoid junction represented in bars. The low count area between the two peak count areas was coincident with the rectal ampulla. The region labeled A is the anal canal above the pectinate line. The region labeled B is the rectum. The region labeled C is the rectosigmoid segment and the region labeled D is the sigmoid colon. The x-axis is distance in cm from the pectinate line and the y-axis the total number of lymphatic nodules per cm².

all five individual specimens studied. The numerical density of LN scored in the first band, located at the distal end of the rectum, was always highest. Above this distal region of the rectum, the numerical density of LN sharply decreased for a distance of about 5 cm, and then the numerical density of LN again increased creating a second band of LN at the rectosigmoid segment. No LN were observed below the pectinate line.

An analysis of variance statistical test followed by a Student-Newman-Keuls multiple comparison test was done to test for significant differences in the mean number of LN in each cm segment from the pectinate line to the proximal sigmoid colon using data from all five specimens (Fig. 7). The results of these analyses showed that there was a significantly higher number of LN in the 3–6 cm segments (highest peak in the first band) at the distal end of the rectum than in any of the other one cm segments located immediately proximal to or distal to this first band. A second peak of LN is observed between the 11- and the 14-cm segment above the pectinate line (Fig. 7). The results of the SNK multiple tests did not show this second peak to have a significant

higher number of LN than segments 7–10-cm. In all five cases the counts of LN in the 11–14-cm segments were observed to be higher than in the segments immediately distal or proximal to this second band of LN (Figs. 5,6).

DISCUSSION

Numerical Distribution of LN in the Large Bowel

Our novel approach for scoring LN allowed determination of the spatial distribution of LN in the sigmoid colon, the rectosigmoid segment, the rectum, and the anal canal. The results showed a distributional pattern of LN not previously reported. Specifically it was found that LN exist in higher numbers at two specific sites in each of the five specimens studied. These sites of higher numbers of LN occurred as circular bands, one at the distal end of the rectum and the other band at the rectosigmoid segment.

A band or ring of higher density of lymphoid tissue at the orifice of the distal gastrointestinal tract seems logical because the orifice at the proximal end of the digestive tract (the oral cavity) also has lym-

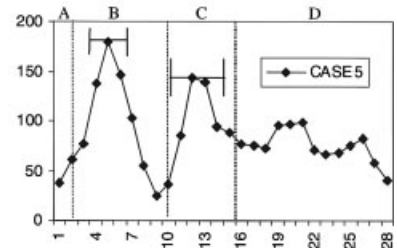
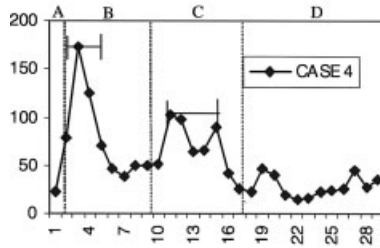
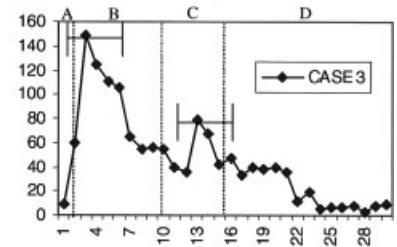
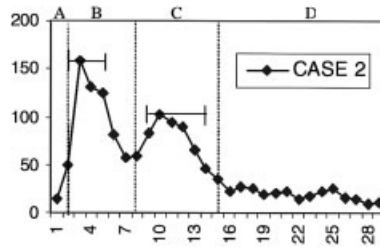
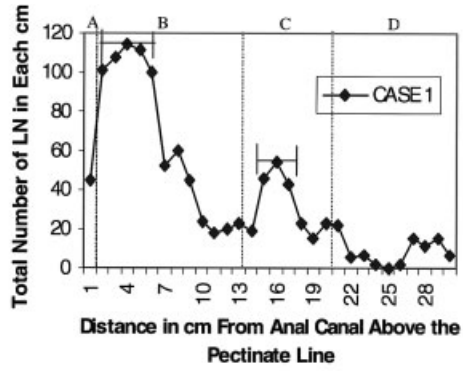


Fig. 6. Distribution of total number of LN in each one segment of the five individual cadaver specimens. Note in all five specimens there are two peaks in the number of LN. The first peak is at the distal end of the rectum and the second highest peak is at the rectosigmoid junction represented in bars. The low count area between the two peak count areas was coincident with the rectal ampulla. The region labeled A is the anal canal above the pectinate line. The region labeled B is the rectum. The region labeled C is the rectosigmoid segment and the region labeled D is the sigmoid colon. The x-axis is distance in cm from the pectinate line and the y-axis is the number of lymphatic nodules per cm.

phoid tissues arranged in a ring, referred to as Waldeyer's tonsillar ring consisting of pharyngeal, palatine, and lingual tonsils. The tonsillar ring of Waldeyer has been promoted as the first line of immunological defense against invasion of microorganisms and foreign substances that enter the body through

the oral cavity. It follows that the increased numbers of LN observed at the external orifice of the large bowel might also be strategically located to defend against invasion of microorganisms and foreign substances that may enter the body through the distal end of the gastrointestinal tract.

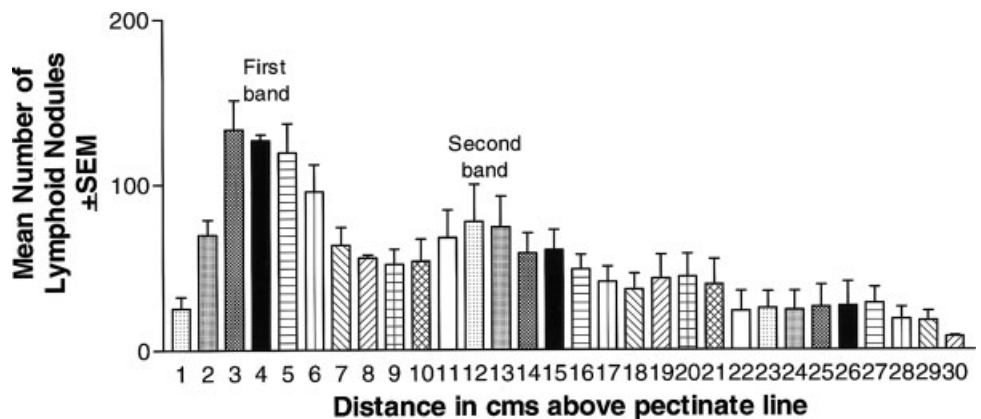


Fig. 7. A graph of the mean \pm SEM number of LN in 1-cm segments above the pectinate line of the five cases scored. The cm segments 3-5 above the pectinate line were significantly higher ($P < 0.05$) in number of LN than all other segments.

Numerical Differences Due to Methods of Detection of Lymphoid Nodules

Lymphoid nodules, also referred to as follicles, have been detected in the large bowel of humans using air-contrast-barium enema radiology (Bronen et al., 1984; Glick et al., 1988). Using this radiology method, Bronen et al. reported that it was unusual to demonstrate any lymphoid follicles in patients in the 59–75 age group. In a more extensive study to detect lymphoid follicles using the air-contrast-barium enema radiology methods, Glick et al. (1998) reported finding radiology evidence for lymphoid follicles in 184 of 3,399 patients older than 40 years of age. Based on comparisons between these radiographic findings on frequency of lymphoid follicles and on the findings of numerous lymphoid follicles observed in human specimens by direct morphological identification, the radiographic detection method clearly fails to identify most of the lymphoid nodules. It may be that the radiology method detects only the very large lymphoid nodules.

Link Between Adenocarcinoma (AC) Incidence and Spatial Numerical Density of LN in the Large Bowel

The second most common site for cancer in men and women in the U.S. is the colon and rectum (colorectal cancer). It is estimated that 106,370 new cases and 56,730 deaths will have occurred from this disease in 2004 (American Cancer Society, 2004). An individual's risk for developing colorectal cancer over a lifetime is 6%. Studies have shown that a higher percentage of AC occur in the rectum than anywhere else in the large bowel (American Cancer Society, 2004). What might account for this uneven distribution of large bowel cancer?

Cameron et al. (1996) have reported that a significant positive relationship exists between the numerical distribution of gut associated LN and the numerical distribution of AC along the length of the large bowel of the rat. These authors provided direct evidence that LN stimulated hyperplasia of adjacent crypts and that LN are promotional to development of de novo AC in carcinogen-treated rats (Hardman and Cameron, 1994).

In humans, Langman and Rowland (1986) reported that LN are in higher numerical density in the sigmoid colon and rectum than elsewhere in the large bowel. In addition, it is a fact that AC occur in higher frequency in the sigmoid colon and in the rectum than anywhere else in the large bowel (Massacesi et al., 2002). Thus, in humans, there is also concordance between the numerical density of LN and incidence of AC in the large bowel.

Currently, there are not enough published data on the precise location of large bowel AC to establish a precise relationship with the specific spatial distribution patterns of LN as observed in the lower large bowel of humans (this study).

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