Lymphocytic infiltration and survival in rectal cancer

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SUMMARY Lymphocytic infiltration was assessed semiquantitatively in 447 specimens of rectal cancer. Corrected five year survivals for pronounced, moderate, and little lymphocytic infiltration were 92%, 65% and 36%, respectively. Grading was shown to be reproducible in an intraobserver study (observed agreement 81%, expected agreement 34%, kappa coefficient 0·72). In the multivariate survival analysis of grade and stage related variables lymphocytic infiltration was the only grade related variable to be accepted within the prognostic model. This model also included the number of lymph node metastases and the extent of tumour spread.

It is suggested that the conservation of the normal interactive traffic between epithelium and mucosa associated lymphoid tissue (MALT) signals a low grade growth. This is supported by the association between lymphocytic infiltration and the expression of secretory component by malignant epithelium.

The survival advantage of a pronounced lymphocytic infiltration in specimens of large bowel cancer has been known for many years. MacCarty[1] commented on the benefit of lymphocytic infiltration in 1931, and his observations have been endorsed and amplified in subsequent communications.[2-9] None of these studies has allowed the grade of lymphocytic infiltration to compete with more traditional variables by the method of multivariate survival analysis, using the proportional hazards regression model.[10 11] Such a statistical manoeuvre would allow the independent effect of lymphocytic infiltration on survival to be separated from other prognostic variables.

Material and methods

Sections from 447 unselected specimens of rectal cancer, which had been removed by radical surgery between 1960 and 1965, were examined. Cancers complicating chronic inflammatory bowel disease and familial polyposis coli were excluded, as were specimens removed from patients who had died within 28 days of surgery. Patients were followed up for at least 15 years, or until death. Details of follow up procedures and documentation have been published previously.[12]

Lymphocytic infiltration was graded as being pronounced, moderate, and little or none, and this assessment was made at the advancing front of the tumour. The lymphocytic infiltrate was usually distributed within a delicate connective tissue lamina at the growing tumour margin (Fig. 1). Other inflammatory cells were also represented. The lamina closely resembled normal lamina propria and followed the advancing edge of the tumour in a sleeve like manner. When the tumour invaded in a more nodular fashion the lymphocytic infiltrate was arranged in a cap. Little or no lymphocytic infiltration is self explanatory, and moderate infiltration implied a broken or imperfectly formed lamina with relatively few lymphocytes. Assessment was based on the worst area. Details of other discrete grade related variables (Table 1) have been published separately.[13] Three months after the completion of the study 50 consecutive cases were regraded and intraobserver agreement was measured by the kappa coefficient.[14]

Stage related data were obtained from the records of St Mark's Hospital (Table 1).

Variables were analysed by standard survival methods and by multivariate analysis using the proportional hazards regression model.[10 11] Deaths from causes other than rectal cancer were treated as censored observations at the time of death. In this way lymphocytic infiltration was directly linked with tumour behaviour. Variables were chosen by the forward stepwise regression model using the BMDP2L program. Using this method, the independent effect of lymphocytic infiltration on survival was separated from the other prognostic variables.

Twenty two tumours with pronounced lymphocytic infiltration and 23 with little or no lymphocytic infiltration were stained by secretory component (Da-
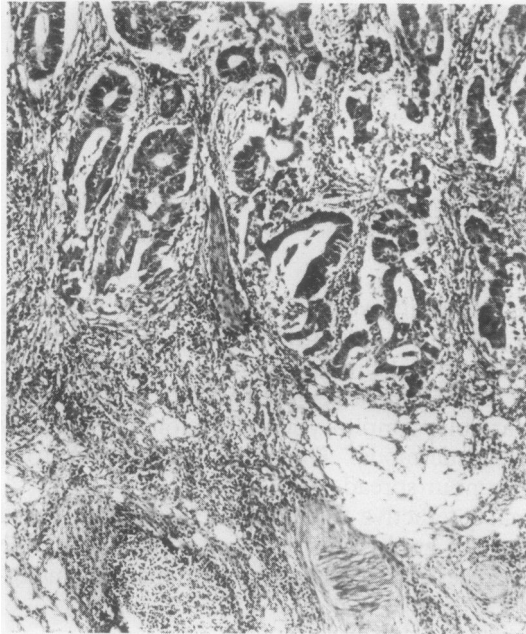
Fig. 1 *This section shows pronounced lymphocytic infiltrate that is not limited to advancing edge of tumour, but extends into stroma surrounding malignant epithelium (Haematoxylin and eosin) ×30.*

Table 1 *Pathological variables to compete with lymphocytic infiltration in proportional hazards regression model*

Grade related
Type: papillary, tubular, mucinous, signet ring
Tubule configuration: regular, irregular, none
Nuclear polarity: easily discerned, just discerned, lost
Pattern of growth: expanding, infiltrating
Fibrosis: little, average, pronounced

Stage related
No of affected nodes: 0, 1–4, ⩾5
Spread beyond wall: none, slight, extensive
Extramural venous invasion: no, yes

kopatts), using an indirect immunoperoxidase technique. Details of methodology and controls have been published previously.[15] Staining was graded positive or negative.

Results

The influence of lymphocytic infiltration on survival is shown by Kaplan-Meier survival curves (Fig. 2), and Table 2 shows the corresponding data. Lymphocytic infiltration was influenced by Dukes' stage, with 53%, 28%, and 13% of Dukes' A, B, and C cases (respectively), showing pronounced lymphocytic infiltration. An important independent effect of lym-
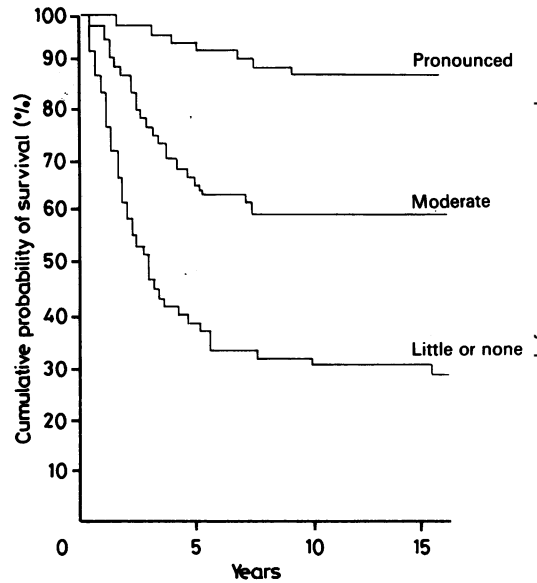

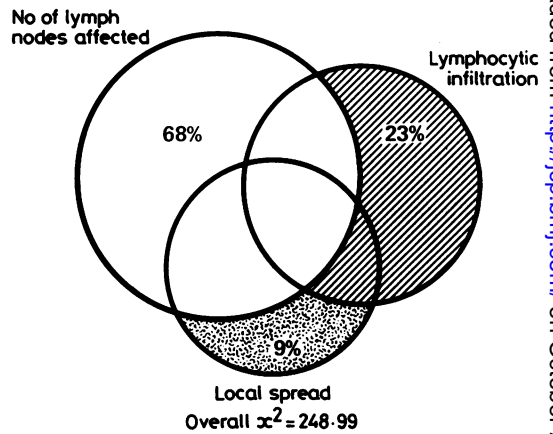Fig. 2 *Kaplan-Meier survival curves for lymphocytic infiltration.*

Overall $x^2 = 248.99$

Fig. 3 *Venn diagram depicting stepwise addition of variables and their independent contribution to overall likelihood ratio χ^2. Best fitting model was constructed by selecting variable with highest likelihood ratio χ^2 at step 0 (Table 3). On entering node status, χ^2 is recomputed to show only independent contribution of remaining variables. Highest χ^2 is again entered and process continues until no significant improvement in χ^2 occurs. Size of circles is proportional to individual χ^2 at step 0 of Table 3 (univariate analysis). Percentages refer to size of independent contribution within overall χ^2, as determined by multivariate analysis.*

Table 2 *Lymphocytic infiltration and survival rates in rectal cancer (figures in parentheses are numbers %)*

Lymphocytic infiltration	No of patients	Observed No of deaths	Deaths (O/E)	Corrected five year survival (%)	Corrected 10 year survival (%)
Pronounced	110 (25)	13	0·21	92	87
Moderate	152 (34)	55	0·80	65	61
Little or none	185 (41)	123	2·00	36	30

χ^2 trend = 99·6; p < 0·0001 (based on logrank analysis of entire survival curve); O = observed; E = expected.

Table 3 *Selection of regression model for grade and stage related variables*

Variables	Likelihood of ratio χ^2 to enter into model			
	Step 0	Step 1	Step 2	Step 3
Adenocarcinoma type	17·73	6·54	2·60	1·84
Tubule configuration	80·15	30·05	6·26	3·83
Pattern of growth	89·54	44·23	9·91	5·40
Nuclear polarity	38·38	15·38	2·13	1·13
Fibrosis	19·20	3·61	0·71	0·89
Lymphocytic infiltration	106·84	57·74	57·74*	32·70*
No of affected nodes	168·43	168·43*	119·33*	77·78*
Spread through bowel wall	112·14	47·87	22·82	22·82*
Vein invasion	29·93	10·84	2·27	0·76
		p < 0·0001	p < 0·0001	p < 0·0001
Overall likelihood ratio χ^2		168·43 (1df)	226·17 (2df)	248·99 (3df)

RMDP2L program calculates likelihood ratio χ^2 from survival analysis. Step 0 shows univariate analysis and forward stepwise entry follows in steps 1, 2, and 3 to achieve multivariate analysis.
*denotes that variable has been entered into model.

phocytic infiltration on survival was shown, however, by multivariate analysis (Table 3). The prognostic model is most easily appreciated by a Venn diagram (Fig. 3). This shows the magnitude of the contribution made by variables as they were chosen in steps, until no further clinical benefit was derived. Grading of lymphocytic infiltration was reproducible with an observed agreement of 81%, expected agreement of 34%, and kappa coefficient of 0·72. No pronounced case was regraded little or none (or vice versa).

Nineteen of the 22 cases with severe lymphocytic infiltration showed positive secretory component staining by malignant epithelium, whereas only six of the 23 cases with few or no lymphocytes gave a positive result ($\chi^2 = 16·55$; p < 0·01).

Discussion

This study confirms the survival advantage of a pronounced lymphocytic infiltrate in specimens of rectal adenocarcinoma.[1-9] It is the first study, however, to show that the effect is partly independent from other pathological variables. Practical applications of this observation have been presented in detail elsewhere.[13]

The role of immune mechanisms in the pathogenesis and spread of cancer has been debated for many years. There are two explanations for the results of this study that are not necessarily mutually exclusive. Firstly, the lymphocytic infiltrate could represent a specific response by the host against the tu-

mour. Secondly, the inflammatory lamina at the advancing front of the tumour may be closely related to the normal lamina propria in terms of its structure and function. The persistence of the normal channels of communication between epithelium and mucosa associated lymphoid tissue (MALT) might signal a high level of functional differentiation.

The proportions of lymphocyte subsets are changed in the malignant stroma of colorectal cancers compared with those of normal lamina propria, with an increased cytotoxic-suppressor:helper T cell ratio.[16 17] Reduced numbers of IgA secreting plasma cells have been observed, whereas IgG secreting plasma cells may be more numerous.[18] Macrophages have been detected in greatly increased numbers.[18] These modulations indicate that immune mechanisms were operating within the stroma surrounding neoplastic epithelium. No correlations, however, between lymphocyte subset numbers and stage of disease have been described.[18 19] Increased numbers of macrophages[18] and extensive fibrosis[13] have been found in advanced cases of rectal cancer. It is important to appreciate that most of these studies have relied on small samples of frozen tissue, which may not have been sufficiently representative.

Some in vitro studies support the existence of specific antitumour immunity. A leucocyte inhibition assay was positive in 41% of patients with colorectal cancer but only 6% of treated patients with no clinical evidence of residual disease. The incidence of positive results was highest in early cancers and lowest when

tumours were widely disseminated.[20] Specific cellular immunity has also been shown by a cell migration inhibition test. This was positive in 89% of patients with colorectal cancer, 63% of patients with adenomas, but only 10% of healthy control subjects.[21] A cytotoxic assay using tumour infiltrating lymphocytes showed that increased cytotoxicity was associated with longer survival.[22] Cytotoxic-suppressor T cells might destroy tumour cells that simultaneously express major histocompatibility (HLA) class I antigens and tumour associated neoantigens. The neoexpression of class II determinants (HLA-DR) might further increase or restrict the T lymphocyte response.[16 17] It has been found, however, that class I positive tumours are not associated with increased lymphocytic infiltration,[16] nor is T cell infiltration accompanied by overt evidence of cytodestruction.[16] Natural killer cells are conspicuous by their absence from colorectal cancer infiltrates.[16 19] Other mechanisms could be relevant including the production of lymphokines. γ Interferon slows tumour growth in vitro[23] and α and β interferon are known to influence the differentiation of cultured colorectal cancer cells.[24] This in turn could modulate tumour behaviour. On the other hand, α interferon stimulates tumour growth in human colorectal cancer transplanted into nude mice.[25]

Several studies have shown a correlation between reactive lymph node changes and survival in large bowel cancer. Hyperplasia of the paracortical zone,[26–28] hyperplasia of germinal centres,[27–30] and sinus histiocytosis[26] confer a survival advantage. Reactive hyperplasia in lymph nodes containing metastases identifies Dukes' C cases with a good prognosis.[30]

In this study the peritumoral lymphocytic infiltrate was often arranged within a delicate stromal lamina that followed the contour of the advancing front of the tumour. There was a distinct resemblance to the normal lamina propria. It is possible, therefore, that the observation of a pronounced lymphocytic infiltrate simply reflects the persistence of normal epithelial-stromal interaction. The ratios of lymphocyte subsets may be changed, and lymphocyte numbers may possibly be increased through the neoexpression of major histocompatibility complex class II antigens[31] and tumour associated antigens. Such modulations could, however, be epiphenomena with the survival advantage being due to the underlying preservation of epithelial-stromal communications. This would in turn signal a high level of functional differentiation and hence a low grade growth. One effector arm of the normal epithelial-stromal interactive unit is the cellular translocation of IgA (linked to secretory component). The loss of epithelial staining for secretory component accompanies loss of differentiation.[32 33] This observation provides indirect support for the second hypothesis. The association between lymphocytic infiltration and expression of secretory component by malignant epithelium, as shown by this study, adds further indirect support to the hypothesis. It is possible that the in vitro studies described above identified an epiphenomenon that could not arise without the persistence of epithelial-stromal interactions at the tumour interface. The same argument might apply to the lymph node changes.

The conclusion of this paper is that the presence of a peritumoral lymphocytic infiltrate is an important independent prognostic marker, second only to the number of lymph node metastases. This finding, however, is unlikely to indicate the successful limitation of growth by a specific immune response and probably represents the persistence of epithelial-stromal interactions, or both, that become secondarily modified and increased through the anomalous presentation of tumour associated and major histocompatibility complex class II antigens.

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