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The aetiology of colonic suture-line recurrence

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Summary

Cell viability studies have been performed on human desquamated colonic cancer cells obtained by both in-vivo and ex-vivo techniques, and on desquamated colonic cancer cells from an experimental animal model. There was no evidence of cell viability and I conclude that the hypothesis that suture-line recurrence occurs as a result of the implantation of desquamated tumour cells is of questionable validity.

Field change in the colonic mucosa has been studied by examining the reactions of the mucosubstances in the goblet cells. A potential correlation between transitional mucosa at the anastomosis and the development of suture-line recurrence was found and warrants further study.

The clinical problem has been investigated by the clinicopathological study of 16 patients developing suture-line recurrence in an attempt to discern the aetiology of each. In all but one the recurrence was due to incomplete excision of cancer or, in one instance, a second primary growth.

Introduction

John Hunter was a dedicated investigator of anatomical, physiological, and surgical problems. Although he recognised cancer of the large bowel, the problem of suture-line recurrence was unknown to him as surgery for such cancer was not performed in his day.

Suture-line recurrence occurs usually 1–2 years after a distal colonic resection which may have been thought curative[1-3]. Sometimes the cause of anastomotic recurrence is obvious (expected) in that there is a massive pelvic recurrence that has grown into the bowel lumen, but at other times it may be more difficult to explain (unexpected)[2]. In these patients it has been suggested that viable cancer cells are exfoliated from the surface of the tumour into the lumen of the bowel and that these desquamated cells are implanted into the wall of the colon by the needle of the suture used for the anastomosis, the cells growing and dividing to form the recurrence at the suture line[4,1,2]. Although there is circumstantial evidence favouring this hypothesis, there has been no experimental work to assess the viability of desquamated colonic cancer cells. Nevertheless, the hypothesis has become widely accepted.

Other suggested causes for unexpected suture-line recurrence include field change as applied to the colonic mucosa and retrograde lymphatic permeation of malignant cells.

In this paper I will describe experiments assessing the viability of the desquamated colonic cancer cell both in humans and in an animal model, an investigation into possible field change as a cause of suture-line recurrence, and the clinicopathological study of a number of patients with suture-line recurrence in an attempt to ascertain the cause of the recurrence.

Implantation

The aim of this study has been firstly to develop techniques for obtaining suspensions of cells desquamated from the intraluminal surface of colonic cancers and secondly to develop tests to be performed on these cell suspensions to show whether the cells are alive or dead.

PATIENTS WITH COLONIC CANCER

Three cell suspensions were obtained from patients with colonic cancer; two were desquamated cell suspensions and the other was a tumour homogenate cell suspension from the excised tumour[5].

Preparation of exfoliated colonic cancer cell suspension

(*a*) *Colonic exfoliative cytology* As previously described[6], a suspension of exfoliated colonic cells could be obtained from a patient with cancer of the colon by a technique of exfoliative cytology.

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(*b*) *Ex-vivo exfoliation* I used a lavage technique on the surgically excised specimens of colonic cancer to provide a suspension of cells desquamated from the intraluminal surface of the growth[5].

Tests of cell viability

The following tests of cell viability were used on the cell suspensions. The methods are fully described and discussed elsewhere[5].

(*a*) *Trypan blue exclusion* This is the standard test of viability and will indicate definite cell death if the cell is unable to exclude the supravital stain. Recent cell death may enable the cell to retain cell-membrane activity and exclude the dye. If no cells were seen to exclude the dye the whole smear was carefully examined before concluding total non-viability of that particular suspension.

(*b*) *Non-specific esterase activity*

(*c*) *Tritiated thymidine uptake* For each slide obtained 500 cells were counted and the results obtained were expressed as the percentage of cells demonstrating incorporation of the radioactive label. An autoradiograph was only considered negative after the whole smear had been examined and found to show no labelled cells.

(*d*) *Tissue culture*

Results

Patients and surgical specimens studied

Ten patients with cancer of the colon provided exfoliated colonic cancer cell suspensions. Twenty-seven resected specimens of carcinoma of the colon were used for ex-vivo exfoliation studies. Twenty-five of these 27 specimens were used to prepare tumour homogenate cell suspensions.

Results of viability tests

(*a*) *Tumour homogenate suspensions* Table I shows the results I obtained with tumour homogenate suspensions, which are known to contain viable cells. The results indicate that the ex-vivo exfoliation was performed on viable tumours and also that the viability tests themselves were workable.

TABLE I *Tumour homogenate results*

Total	25
Trypan blue exclusion	23/25
Esterase activity	6/10
Thymidine uptake	8/12
Tissue culture	7/16

TABLE II *Exfoliated cell viability results*

	Colonic exfoliative cytology	*Ex-vivo exfoliation*
Total	10	27
Trypan blue exclusion	0/10	0/27
Esterase activity	0/10	0/11
Thymidine uptake	0/10	0/27
Tissue culture	0/4	0/16

(*b*) *Exfoliated colonic cancer cell suspensions* All 37 cell suspensions (10 by in-vivo and 27 by ex-vivo techniques) were shown by microscopy to contain malignant colonic cancer cells. The results of the cell viability tests are shown in Table II. In no instance was there evidence of any cell being viable. In particular, none of the cell suspensions contained any cell which excluded trypan blue. This is a strong indication of cell death and is evidence against the hypothesis of implantation recurrence.

EXPERIMENTAL COLONIC TUMOUR IN RATS

Preparation of cell suspensions

Wistar rats from an inbred colony were used throughout. Colonic tumour induction was achieved by treating the animals with 1,2-dimethylhydrazine hydrochloride in a weekly dose of 20 mg/kg body weight plus 20 mg subcutaneously. Tumours are usually apparent 20 weeks after starting these injections[7,8].

Two tumour-bearing rats provided cell suspensions. After induction of general anaesthesia I performed midline laparotomy, divided the proximal colon, and cleansed the colon. The proximal end of the defunctioned colon and the anus were each intubated. Tissue culture medium was perfused through the proximal tube into the colon and left for 5 minutes, during which time the tumours were agitated. The fluid was removed via the anal tube into a container at 4°C. This gave a suspension of cells desquamated from the surface of the colonic tumours in the living vascularised state. By performing a cell count on this cell suspension and resuspending in the calculated volume of medium 5 samples of 10^6 cells in 0.5 ml were prepared for each exfoliated cell suspension.

On completion of the exfoliated cell collection I excised the growths from the bowel

and a tumour homogenate cell suspension was prepared[5]. Again 5 samples of 1 million cells in 0.5 ml were prepared from each rat.

The remainder of the cell suspensions were used for tests of cell viability. Two tests only were used—the trypan blue exclusion test and the tritiated thymidine uptake test—as there were insufficient cells for them all.

Attempted implantation of cell suspension

Twenty syngeneic rats were used, 5 for each of the 4 cell suspensions. On each, under general anaesthesia, I performed midline laparotomy. A 10–15-cm length of colon was isolated between a pair of non-crushing clamps. The aliquot of the cell suspension under test (10^6 cells in 0.5 ml) was injected into the isolated colon lumen. A suture line was fashioned after cutting across half the isolated colon circumference on its antimesenteric border and repairing the deficit with interrupted 5/0 silk sutures in a single inverting layer. In this way I was able to fashion a suture line in a loop of bowel in which tumour cells were known to be present. After wound closure the animal was allowed to recover from the anaesthetic.

An autopsy was performed on every animal that died. All surviving rats were killed 6–8 months after their operation.

Results

Table III shows the results of the experiments on the 4 cell suspensions from the 2 tumour-bearing rats (A and B). There is no evidence of viable cells in either of the exfoliated cell suspensions, but both tumour homogenates are shown to contain viable cells.

There were no successful implants in the 10 rats in which exfoliated cell suspension was used. Only 9 of the rats in which implants of tumour homogenate were attempted were assessed as 1 died under the anaesthetic. Seven showed no evidence of tumour implantation. The eighth rat died 5 months after the operation. Autopsy performed the following morning revealed an intraluminal colonic warty nodule 5 mm in diameter situated exactly on the suture line. Microscopic examination of this mucosal nodule was limited by the postmortem autolysis of cells which prevented the cell architecture being clearly seen. However, there was a definite warty nodule at the anastomosis and microscopy showed that there was hyperplasia of the mucosa (Fig. 1); this is good evidence of tumour. The other rat was killed 7 months after the attempted implantation. At autopsy a loop of ileum was stuck to the colonic suture line which on attempted separation left a defect in the colon. On microscopic examination of this loop of ileum the ileal glands looked normal, but there were colonic glands invading the ileum. The colonic glands were abnormal, with irregular, deeply staining, crowded cells (Fig. 2); this was well-differentiated adenocarcinoma and therefore implanted growth at the suture line.

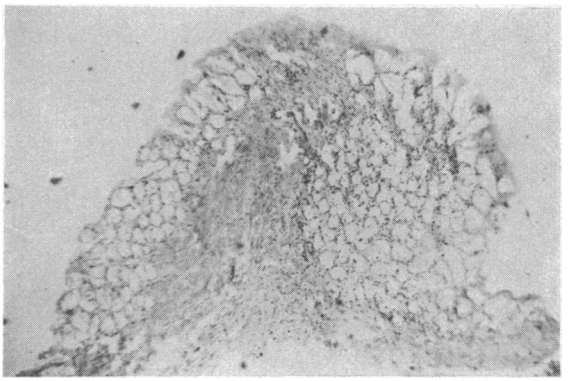

FIG. 1 *Low-power (×23) photomicrograph of anastomotic nodule in the rat which died in the night showing hyperplasia of the mucosa, but cell architecture is not visible owing to autolysis.*

Discussion

Cancer cell implantation into a colonic suture line has previously been demonstrated in several

TABLE III *Results of implantation experiments*

	Exfoliated cells		*Tumour homogenate*	
	Rat A	*Rat B*	*Rat A*	*Rat B*
Cell count (cells/ml)	13.5×10^6	10×10^6	30×10^6	25×10^6
Trypan blue exclusion	0	0	40%	30%
Uptake of tritiated thymidine	0	0	2%	1%
Suture-line implantation	0/5	0/5	2/5	0/4

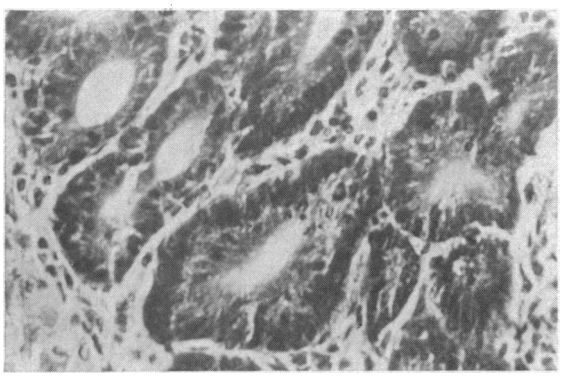

FIG. 2 *Photomicrograph (×174) of the colonic glands attached to the ileum in the rat. These are abnormal cells showing well-differentiated adenocarcinoma and therefore suture-line implantation.*

animal tumour models[9-12], my main criticism of them all being that the tumour was very different in type and behaviour from human colorectal adenocarcinoma. It can be argued that these more aggressive tumours may permit successful implantation, which the slower-growing human colonic cancer would not.

The dimethylhydrazine-induced colonic tumour in the rat is a more realistic model for suture-line implantation as the histological characteristics, the growth rates, and the natural history of these induced cancers are very similar to those of cancer of the colon in man. This is well illustrated by the behaviour of the tumour. After the development of the tumour the animal usually has diarrhoea and bleeding per rectum and eventually may develop large-bowel obstruction and metastases to lymph nodes, the liver, and intraperitoneally, leading to ascites.

It therefore appears that my finding that suture-line implantation can occur with known viable colonic cancer cells (tumour homogenate suspension) is of relevance as it may be deduced that in man suture-line recurrence can similarly occur by the implantation of viable cells. As with the human cell viability tests, I found that the rat exfoliated-cell suspensions contained no viable cells. These results cast further doubt on the hypothesis that suture-line recurrence is due to the implantation of intraluminal viable desquamated colonic cancer cells.

Field change

The hypothesis of field change as a cause of cancer may apply to the colonic epithelium. Some or all of the colonic mucosa may be initiated into a field of growth and some of these altered colonic cells may be promoted to undergo complete malignant change. If the area of cancer is resected but an area of field change is left, then the tendency to malignancy may not have been completely eradicated. This is another possible way that suture-line recurrence can occur. The anastomosed ends of bowel, while not actually cancerous, may contain cells that have been altered. The actual stimulus of the anastomosis with the subsequent sepsis and healing promotes the field change into overt cancer.

Filipe[13,14] has developed a technique of staining the mucosubstances in the goblet cells of the colonic mucosa and has been able to demonstrate areas of change, the so-called transitional epithelium in areas of seemingly normal bowel close to a tumour. Using her techniques, I have investigated 15 specimens of surgically excised colonic cancer.

If the resected specimen was large enough biopsy specimens of the mucosa from the following 8 areas were studied: (i) distal resection edge; (ii) 5 cm from distal edge of tumour; (iii) 3 cm from distal edge of tumour; (iv) 2 cm from distal edge of tumour; (v) 1 cm from distal edge of tumour; (vi) 5 cm proximal to proximal edge of tumour; (vii) 10 cm proximal to proximal edge of tumour; (viii) proximal resection edge. Obviously in smaller specimens all 8 specimens were not obtained.

The biopsy specimens were stained with haematoxylin and eosin, the standard histological stain, and then by each of the histochemical techniques described by Filipe for the demonstraton of the mucosubstances: periodic-acid–Schiff (PAS), alcian blue—periodic-acid–Schiff (AB-PAS), and high iron diamine—alcian blue (HID-AB).

I found that really only the HID-AB sections were useful in deciding whether the mucosa was of normal or transitional type. The difference is quite obvious. In normal mucosa the goblet cells in the lower two-thirds of the crypts contain a predominance of sulphomucins (staining brown), while in the upper third there is a mixture of sulphomucins and sialo-

mucins (staining blue). Transitional mucosa shows a decrease or even absence of sulphomucins and a marked increase in sialomucins, making the mucosa stain mostly blue over all the crypt.

Figure 3 shows a pictorial representation of the results on each of the 15 colonic cancer specimens studied. It is at once apparent that 2 cm from the growth would seem to be the critical distance, for up to and including this distance from the growth the majority of the mucosae were of the transitional type, whereas by 3 cm distal they were all normal.

Six patients (1, 5, 6, 11, 12, and 15 in Figure 3) had the suture lines fashioned with transitional mucosa and their subsequent progress is obviously of importance: Patient 1 developed suture-line recurrence 2 years later and subsequently had an abdominoperineal excision of the rectum. Patient 5 died 2 months after surgery. Patient 6 developed extensive pelvic recurrence, although none at the suture line. Patient 11 has recently returned $2\frac{1}{2}$ years later with suture-line recurrence. Patient 12 is well with no sign of recurrence 2 years later. Patient 15, whose original growth developed in an unstable mucosa in an area of villous papilloma and whose operation was a Hartmann's resection, has developed recurrent villous papilloma in the rectal stump.

Thus of the 6 patients whose suture line was fashioned with transitional mucosa, 5 are available for follow-up; only 1 remains tumour-free and 3 developed suture-line recurrence. Although I am unable to make a definite conclusion on these results, they certainly are suggestive of the influence of field change being of importance. As a prospective investigation a distal and proximal resected margin block is now obtained on all cases of colonic cancer; in any patient developing suture-line recurrence the type of mucosa with which the suture line was fashioned can be ascertained. I hope this long-term project may provide a clearer answer in the future.

Patients developing suture-line recurrence

I am much indebted to Professor J C Goligher, who has allowed me to study his large, well-documented series of distal colonic resections with anastomosis. From this series I have been able to analyse each case of suture-line recurrence and suggest a cause for the recurrence on the evidence of the clinicopathological features.

During the period 1955–73 there were 304 patients who underwent a left-sided colonic resection and anastomosis; 16 (5.3%) of the patients developed suture-line recurrence, 14 (8.5%) of 163 who underwent low anterior resection, 1 (0.9%) of 109 with high anterior resection, and 1 (3.1%) of 32 with abdomino-anal pull-through resection.

Of the 16 patients with suture-line recurrence, it is at once apparent that in 11 there was residual growth left attached to the pelvic side wall, and the recurrence in these patients was not unexpected and was due to extensive pelvic recurrence growing into the bowel at its weakest point, the suture line. Similarly, 3 of the 5 operations considered

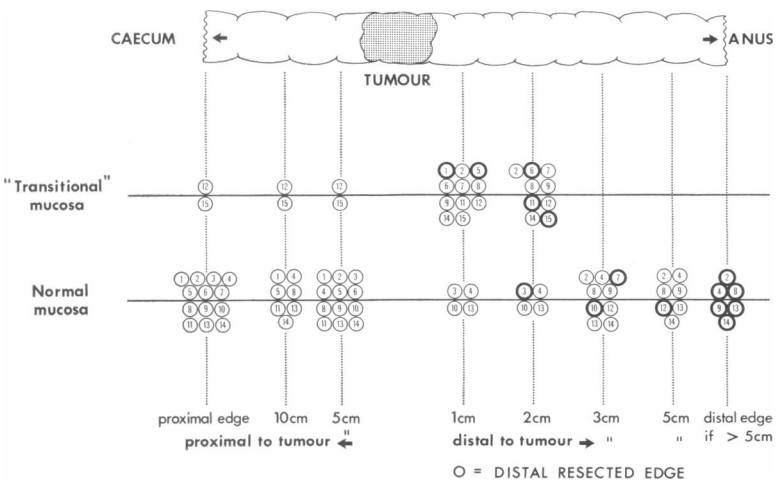

FIG. 3 *Diagrammatic representation of all the specimens whose histochemical reaction of the mucosubstances were studied. Each specimen has a number (1–15) and each distal resected edge is shown by the heavier ring around the number.*

curative can be shown on retrospective analysis to have been optimistically so labelled, as in 1 there was pelvic residual disease macroscopically and in the other 2 there was microscopic local extension.

It therefore seems that only 2 of the suture-line recurrences followed genuinely curative resections. One of these 'recurrences' was a second primary tumour. This leaves just the 1 patient whose suture-line recurrence is not so easily explained, and oddly enough this was the only suture-line recurrence following high anterior resection. There was seemingly an ample margin of resection distally (6 cm) and no local extension or vascular or lymphatic involvement. Microscopical examination of the growth showed it to be a well-differentiated adenocarcinoma. At reoperation for suture-line recurrence a radical low anterior resection could be performed, although in this second specimen lymph-node involvement was found. This is the sort of perplexing suture-line recurrence which the implantation hypothesis was designed to explain. However, my feeling is that in view of the second specimen having lymph-node metastases, maybe nodal metastases were missed by the pathologist in the first specimen.

Certainly this particular case puts the clinical problem in perspective. It is difficult to pinpoint the cause of the recurrence, but it is the only one of the 16 which cannot be readily explained.

Discussion and conclusions

Most patients developing a recurrence at the suture line do so because of an incomplete excision of their original tumour; they develop recurrence in the pelvic space and this grows into the bowel lumen at its weakest point, the suture line.

The hypothesis of implantation as the cause of suture-line recurrence seemed a convenient explanation for those patients who had had a seemingly complete cancer clearance. Despite the sound circumstantial evidence favouring it, the more direct evidence I have presented really excludes any possibility of desquamated cancer cells having any seeding potential. I have, however, been able to show in the experimental animal model that suture-line recurrence could occur if viable cells were in the lumen of the bowel. This could only really occur if the surgeon were to fracture or cut into the tumour during the mobilisation, and obviously he always endeavours not to do this.

The phenomenon of field change as applied to the colonic mucosa is interesting, and certainly the work I have presented shows the possibility of some correlation between the type of mucosa with which the suture line was fashioned and suture-line recurrence.

What then is the cause of the unexpected suture-line recurrence if it is not implantation? I am attracted on theoretical and intellectual grounds to the 'lymphatic way' hypothesis of Gricouroff[15,16]. He suggests that cancer cells can freely flow to and fro in the lymphatic vessels and that during resection some viable cancer cells may be trapped and remain in the lymphatic vessels of the colon or the rectum not being resected; when the anastomosis is completed these cells, on resuming their to-and-fro migration, may be caught up on the anastomosis and grow, causing the recurrent growth. Certainly the observed facts could be explained on this hypothesis and he has performed elegant histological studies showing this to-and-fro migration occurring. Moreover, it is apparent that the hypothesis would also explain why lavage with cytotoxic solution reduced the occurrence of recurrence at the suture line, the cytotoxic agent being able to kill cells in the normal lymphatic vessels.

While devoting this paper to John Hunter, I should like to close with a quotation from another great man in this nation's history. I believe this quotation to be most appropriate for suture-line recurrence:

> 'We have scotch'd the snake, not kill'd it;
> She'll close, and be herself, whilst our poor malice
> Remains in danger of her former tooth'
>
> *Macbeth,* act III, scene 2

I should like to express my gratitude to Professor Geoffrey Giles, in whose department this work was carried out, for his constant advice and encouragement, to Professor J C Goligher for allowing me to study his patients, to the surgeons of St James's Hospital, Leeds, for allowing me full access to their patients and the resected specimens, and to Dr M K Mason and Dr G Hardy for their pathological advice and assistance. The experimental work could not have been efficiently carried out without the expert technical assistance of Miss Carol Russell and the technicians in the Department of Surgery, St James's Hospital, Leeds.

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