Original article

Effect of appendicectomy on colonic inflammation and neoplasia in experimental ulcerative colitis

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Background: **Ulcerative colitis (UC) promotes cancer, and can be ameliorated by early appendicectomy for appendicitis. The aim of the study was to explore the effect of appendicectomy on colitis and colonic neoplasia in an animal model of colitis and a cohort of patients with UC.**

Methods: **Five-week old IL10/Nox1^{DKO} mice with nascent colitis and 8-week-old IL10/Nox1^{DKO} mice with established colitis underwent appendicectomy (for experimental appendicitis or no appendicitis) or sham laparotomy. The severity and extent of colitis was assessed by histopathological examination, and a clinical disease activity score was given. From a cohort of consecutive patients with UC who underwent colectomy, the prevalence of appendicectomy and pathological findings were collected from two institutional databases.**

Results: **Appendicectomy for appendicitis ameliorated experimental colitis in the mice; the effect was more pronounced in the 5-week-old animals. Appendicectomy in the no-appendicitis group was associated with an increased rate of colonic high-grade dysplasia (HGD) or cancer compared with rates in sham and appendicitis groups (13 of 20 *versus* 0 of 20 and 0 of 20 respectively; $P < 0{\cdot}001$). Fifteen of 232 patients who underwent colectomy for UC had previously had an appendicectomy, and nine of these had colonic cancer or HGD. Thirty (13·8 per cent) of 217 patients with the appendix *in situ* had colonic neoplastic lesions. Multivariable analysis showed that previous appendicectomy was associated with colorectal neoplasia (odds ratio 16·88, 95 per cent c.i. 3·32 to 112·69).**

Conclusion: **Appendicectomy for experimental appendicitis ameliorated colitis. The risk of colorectal neoplasia appeared to increase following appendicectomy without induced appendicitis in a mouse model of colitis, and in patients with UC who had undergone appendicectomy.**

Surgical relevance

Appendicectomy for appendicitis protects against UC. In this murine model of colitis, appendicectomy for experimental appendicitis protected against colitis, but appendicectomy without appendicitis promoted colorectal carcinogenesis. In patients with ulcerative colitis who underwent colectomy, absence of the appendix (proof of previous appendicectomy) in the resection specimen was independently associated with colorectal neoplasia.

Although patients with UC and a history of appendicectomy represent a small subset, they may need closer monitoring for colorectal neoplasia.

Presented to a meeting of Journées Francophones d'Hépato-gastroentérologie et d'Oncologie Digestive, Paris, France, October 2015, a meeting of the Association Française de Chirurgie, Paris, France, September 2015, and the Tenth Congress of the European Crohn's and Colitis Organisation, Barcelona, Spain, February 2015

Paper accepted 5 April 2016

Published online 8 August 2016 in Wiley Online Library (www.bjs.co.uk). **DOI:** 10.1002/bjs.10209

Introduction

Appendicectomy for appendicitis may have a protective effect on the occurrence of ulcerative colitis (UC) and has also been suggested as a possible therapy for ulcerative proctitis[1]. The appendix could be considered as a priming organ in the pathogenesis of UC[2]. Appendix-derived cytokines have been proposed to modulate immunological responses in the colon. The pathogenesis of UC, however, remains poorly understood, and animal studies[3–6] have provided conflicting results. Recently, a mouse model (interleukin10/NADPH oxidase 1 double knockout mice, IL10/Nox1^{DKO}) was established. Starting at the age of 6 weeks, these mice develop colitis that mimics the histological features, epithelial dysfunctions and natural course of human UC[7]. Interestingly, 40 per cent of these mice develop multifocal colonic high-grade dysplasia (HGD) or cancer by the age of 8 months[7]. It is already known that UC is associated with an increased risk of colorectal cancer[8]. A recent study reported that appendicectomy is associated with an increased risk of colorectal neoplasia in patients with UC[9], although this observation has not yet been replicated.

The aim of this study was to investigate the effects of appendicectomy in IL10/Nox1^{DKO} mice on the severity of colitis and the development of colonic neoplasia. In addition, a cohort of patients with UC was studied to assess the association between appendicectomy and colorectal neoplasia.

Methods

Animals

Male C57/BL6 IL10/Nox1^{DKO} mice at aged 5 and 8 weeks were obtained from hosted pathogen-free conditions (specific pathogen-free zones; Centre de Recherche sur l'Inflammation, Paris, France). The phenotype, features and development of colitis in IL10/Nox1^{DKO} mice have been described previously[7]. All experiments were approved by the local ethics committee and the protection of laboratory animals committee of the university (reference numbers 17/773-0132 and 17/773-0133). The experiments were conducted in accordance with European legislation (Directive 2010/63/EU).

Three groups of wild-type (WT) and IL10/Nox1^{DKO} mice were created: an appendicitis group that underwent appendicectomy; a no-appendicitis group that had appendicectomy; and a sham group that had laparotomy only (*Fig. 1a*). In each group, surgical procedures were performed at the ages of 5 weeks (10 young mice with dawning colitis) and 8 weeks (10 older mice with established colitis), with random allocation of the mice. The number of mice used for the study was defined according to the resource equation method[10].

Until the end of the experiment, mice were monitored daily for weight, stool consistency, presence of blood in the stool, and rectal prolapse. The mice were killed 35 days after surgery.

Surgical procedure

All mice had two surgical procedures, on day 0 and 7 (*Fig. 1a*). All procedures were performed by the same surgeon under sterile conditions. The animals were anaesthetized with intraperitoneal ketamine (100 mg/kg) and xylazine (5 mg/kg) reconstituted in sterile phosphate-buffered saline (PBS). The skin was prepped with an antiseptic solution (70 per cent ethanol). A 1-cm laparotomy incision was made in the left iliac fossa, and the caecum was identified, positioned outside the abdomen and kept moist via the periodic application of PBS until it was reintroduced into the peritoneal cavity. The caecal patch (a unique lymphoid structure in the murine colon that corresponds to the human appendix lymphoid cellular structure) was identified as a 2-mm white ovoid structure on the antimesenteric side of the caecum (*Fig. 1b*).

The induction of experimental appendicitis in the appendicitis group was performed by suctioning the caecal patch with a 1-ml plastic syringe and ligation at the base on day 0 (*Fig. 1b*). Appendicectomy in appendicitis and no-appendicitis groups was performed as follows: the caecal patch (inflamed in the appendicitis group and macroscopically normal in the no-appendicitis group) was ligated and resected on day 7.

Sham laparotomy in the no-appendicitis and sham groups included mobilization and positioning of the caecum outside the abdomen on day 0 and/or day 7. Another control group was constituted to study the role of appendicular inflammation: six IL10/Nox1^{DKO} mice underwent experimental caecal inflammation, by caecal ligation distant from the caecal patch, followed by resection.

At the end of each surgical procedure, the caecum was repositioned in the peritoneal cavity and the abdomen closed. Animals were given food starting 2 h after surgery. They were killed by cervical dislocation on day 35.

Disease activity index

The consistency of stools, presence of blood in the stool and occurrence of rectal prolapse were scored to obtain the disease activity index (DAI) (1 point for each condition), which ranged from 0 to 3 points.

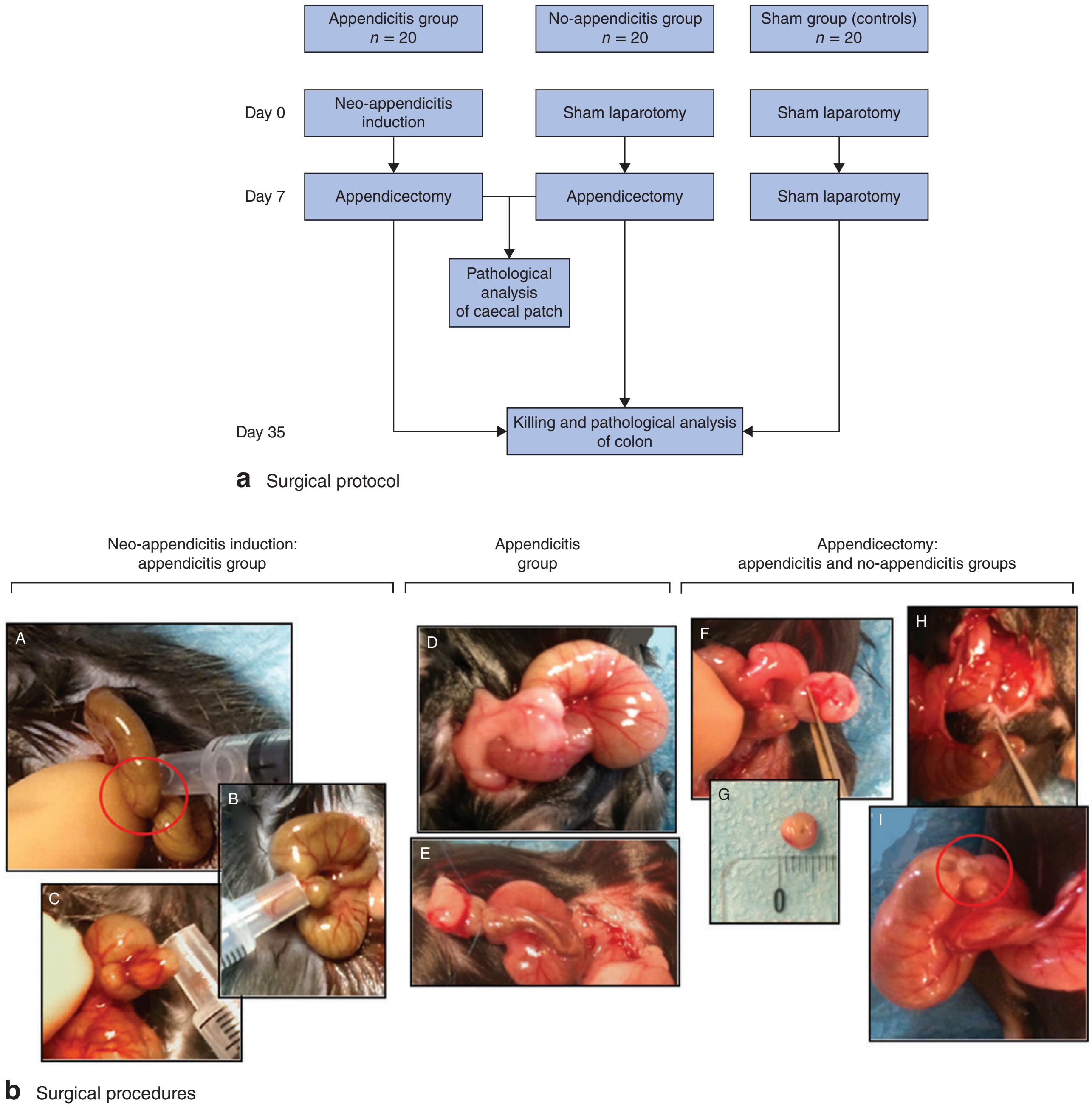

Fig. 1 Surgical protocol for the IL10/Nox1^{DKO} murine model of colitis. **a** Flow chart of the surgical protocol. **b** Surgical appendicitis/appendicectomy procedures in the IL10/Nox1^{DKO} mice. **A** Caecal patch identification (red circle); **B** suction of a caecal patch with 1-ml plastic syringe; **C** occlusive ligation of caecal patch at the base; **D** adhesion of omentum to the inflamed caecal patch (appendicitis); **E** ligation at the base of the 'appendicitis'; **F** resection of the 'appendicitis'; **G** macroscopic aspect of the 'appendicitis'; **H** caecal patch resection after ligation at the base in the no-appendicitis group; **I** macroscopic aspect of the caecum after caecal patch resection (red circle) in the no-appendicitis group

Pathological examination

The resected caecal patch was fixed in 10 per cent neutral formalin solution and then embedded in paraffin for histological analysis. After cutting paraffin blocks, the caecal patch was stained with haematoxylin and eosin, and Alcian Blue.

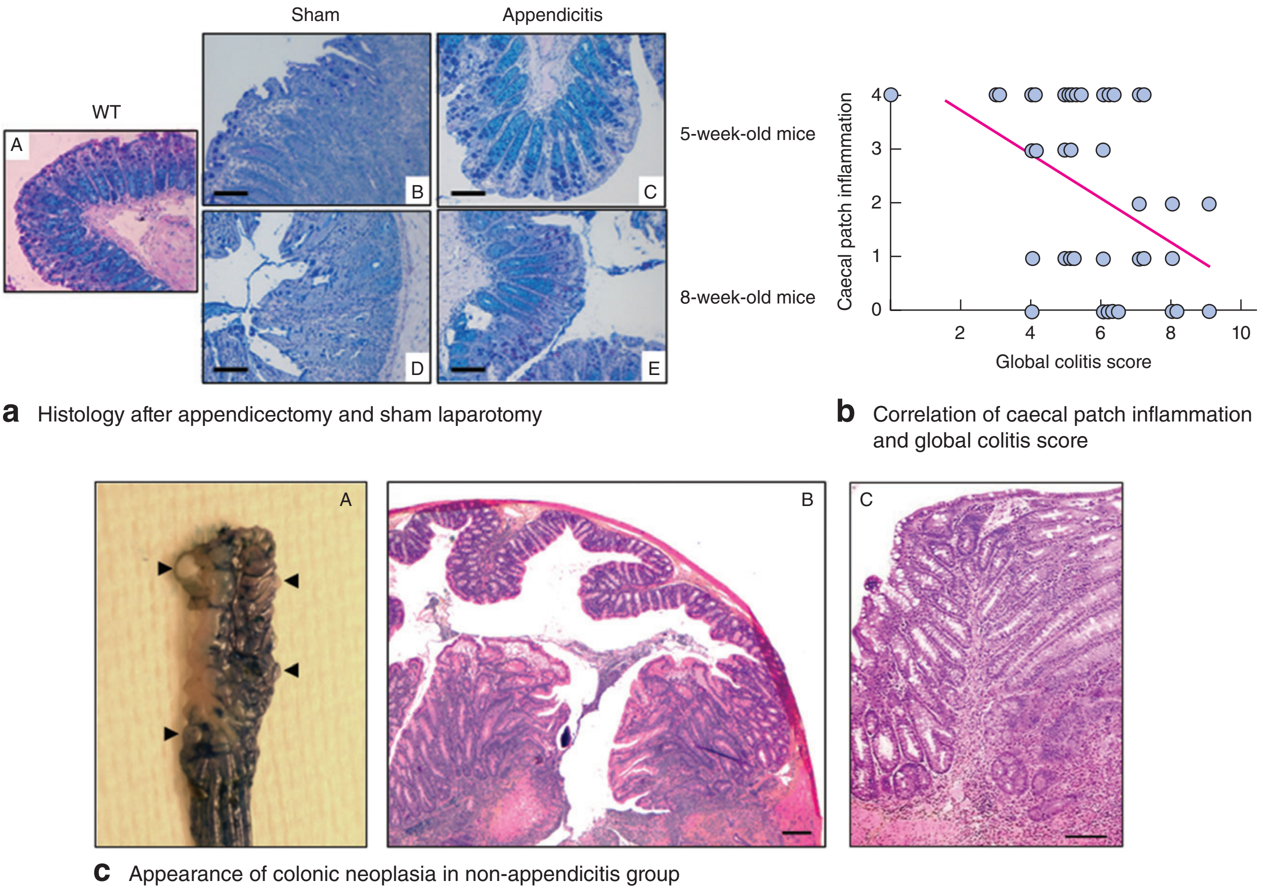

Fig. 2 Results after appendicectomy and sham laparotomy in the IL10/Nox1^{DKO} murine model of colitis. **a** Histological appearance of 5- and 8-week-old IL10/Nox1^{DKO} mouse colons following appendicectomy for 'appendicitis' or sham laparotomy compared with wild-type (WT) mice (stained in Alcian Blue): **A** WT mouse colon exhibiting normal epithelial architecture, no infiltration, and normal secretion of mucus; **B** 5-week-old sham mouse exhibiting colitis with epithelial infiltration of neutrophils, abnormal epithelial architecture with hypertrophied crypts, and loss of secretion of mucus; **C** 5-week-old mouse with 'appendicitis' exhibiting no infiltration, normal epithelial architecture, and secretion of mucus; **D** 8-week-old sham mouse exhibiting severe colitis with neutrophil infiltration and cryptitis, abnormal epithelial architecture, and loss of secretion of mucus; **E** 8-week-old mouse with 'appendicitis' exhibiting lack of infiltration and cryptitis, a quasi-normal epithelial architecture, and a quasi-normal secretion of mucus. **b** Correlation of caecal patch inflammation with global colitis scores in IL10/Nox1^{DKO} mice ($n = 40$) with and without appendicitis that underwent appendicectomy ($r_s = -0{\cdot}43$, 95 per cent c.i. $-0{\cdot}67$ to $-0{\cdot}12$; $P = 0{\cdot}007$). **c** Macroscopic and microscopic appearance of colonic neoplasia in IL10/Nox1^{DKO} mice in the no-appendicitis group: **A** macroscopic view of multiple tumours (arrows) in the proximal colon stained with indigo carmine dye; **B** pathological aspects of disorganized epithelial architecture with T1 adenocarcinoma; **C** pathological aspects of hypertrophied and disorganized epithelial architecture with multifocal high-grade dysplasia (scale bars 1 μm)

After killing the animals on day 35, the entire colon was removed, irrigated with PBS and divided into three segments (proximal, mid and distal). Each colonic segment was fixed in formalin and embedded in paraffin, and processed in the same manner as the caecal patch.

Histological assessment was performed by an expert pathologist, who was unaware of the phenotype and treatment of the mice. The severity of colonic mucosal inflammation and the presence of HGD or invasive neoplasia in the colon were analysed systematically. The caecal patch and colonic inflammation were graded as follows: 0, no acute inflammation with no neutrophils, absence of epithelial disruption; 1, presence of a few neutrophils in the lamina propria with no epithelial disruption; 2, cryptitis composed of neutrophils with no epithelial ulceration; 3, acute epithelial ulceration and/or abscesses and/or neutrophils into the submucosa; 4, purulent necrosis of the epithelium or transmural infiltration of neutrophils. Caecal patch inflammation was then scored from 0 to 4. A global colitis score was obtained by adding

Table 1 Disease activity index and global colitis score after appendicectomy or sham laparotomy in IL10/Nox1^{DKO} mice

	Control group (laparotomy only)	Appendicectomy in no-appendicitis group	*P versus* control†	Appendicectomy in appendicitis group	*P versus* control†
5-week-old mice	*n* = 10	*n* = 10		*n* = 10	
Weight (× 10^{-3} kg)*	22·6(0·5)	19·8(0·6)	0·004	21·2(1·1)	0·761
DAI*	1·5(0·8)	1·3(0·5)	0·373	0·25(0·2)	0·006
Global colitis score*	6·3(0·9)	7·1(1·1)	0·104	4·1(0·6)	0·004
HGD or cancer	0	6	0·015‡	0	–
HGD	0	6	0·015‡	0	–
Cancer	0	0	–	0	–
8-week-old mice	*n* = 10	*n* = 10		*n* = 10	
Weight (× 10^{-3} kg)*	25·1(0·4)	24·1(0·6)	0·424	24·3(0·3)	0·531
DAI*	2·1(0·9)	1·0(0·7)	0·021	0·5(0·3)	0·008
Global colitis score*	6·9(1·2)	5·9(1·7)	0·163	5·1(0·5)	0·032
HGD or cancer	0	7	0·005‡	0	–
HGD	0	3	0·210‡	0	–
Cancer	0	4	0·094‡	0	–
5 + 8-week-old mice	*n* = 20	*n* = 20		*n* = 20	
DAI*	1·8(0·9)	1·2(0·6)	0·019	0·4(0·2)	< 0·001
Global colitis score*	6·5(1·1)	6·5(1·5)	0·974	4·7(0·4)	< 0·001
HGD or cancer	0	13	< 0·001‡	0	–
HGD	0	9	0·003‡	0	–
Cancer	0	4	0·114‡	0	–
T1sm1	0	3	0·230‡	0	–
T1sm2	0	1	1·000‡	0	–

*Values are mean(s.d.). DAI, disease activity index; HGD, high-grade dysplasia. †Mann–Whitney *U* test, except ‡χ^2 test with Yates' correction.

the colitis scores for the proximal, mid and distal colon. For each segment, the most severe item was chosen (maximum of 4 points/segment, giving a maximum of 12 points for the entire colon) (*Fig. S1*, supporting information). Colonic neoplasia was classified according to the TNM classification of colonic cancer (Tis, T1sm1/2/3, T2, T3 and T4)[11].

Patients

Consecutive patients with UC who underwent colectomy at Beaujon Hospital and Centre Hospitalier Universitaire Hôpital Pontchaillou, Rennes, France, between January 2001 and December 2011 were included. Patients with a secondary diagnosis of Crohn's disease were excluded. Data on age, smoking, indication for surgery, UC characteristics (maximum extent of disease, treatments received, family history of colonic neoplasia and primary sclerosing cholangitis (PSC)) and history of appendicectomy were retrieved from the institutional database. All resection specimens were reviewed by a pathologist. Presence or absence of the appendix and presence of colorectal HGD/cancer in the specimens were recorded. Only patients with no appendix in the resection specimen were included in the appendicectomy group. Ethical approval for this part of the study was obtained from the Assistance Publique des Hôpitaux de Paris Institutional Review Board (number 12·739).

Statistical analysis

Animal model

Five- and 8-week-old appendicitis and no-appendicitis groups were compared with sham laparotomy groups (controls) to determine differences in clinical, morphological and pathological observations. Categorical data are reported as the number of cases and compared with the χ^2 test with Yates' correction. Quantitative variables are shown as mean(s.d.) values and compared using the Mann–Whitney *U* test. Correlations between DAI, caecal patch inflammation and global colitis scores were determined with the Spearman test.

Patients

Comparison of categorical variables between patient groups was performed using Fisher's exact test. Unadjusted odds ratios (ORs) and 95 per cent confidence intervals (c.i.) were calculated to estimate the association of various factors with HGD/cancer. A multiple logistic regression model was used to correct for the effect of potential confounders. Clinically relevant factors

Table 2 Characteristics of 232 patients with ulcerative colitis who underwent proctocolectomy

	Appendix *in situ* (*n* = 217)	Appendicectomy (*n* = 15)	*P*†
Age at colectomy (years)*	38·5 (27·2–49·8)	49·0 (35·2–63·7)	0·018‡
Sex ratio (M : F)	109 : 108	9 : 6	0·596
Duration of UC (months)*	41 (14–107)	151 (113–242)	< 0·001‡
Cumulative UC spread			0·727
E1/E2	28	2	
E3	189	13	
Indication for colectomy			
Acute severe colitis	83	1	0·029
Refractory UC	92	6	1·000
Colonic stricture	16	1	0·681
Dysplasia/cancer	26	7	0·002
Active smoker	66	2	0·266
Familial history of colonic cancer	10	0	0·847
Primary sclerosing cholangitis	47	1	0·291
Cumulative treatment exposure			
Steroids	204	11	0·016
Salicylates	165	15	0·067
Purine analogues	100	3	0·089
Ciclosporin	26	2	0·699
Infliximab	75	7	0·014
Inflammatory lesions			
None	9	3	0·038
Rectum	203	10	0·004
Left colon	202	10	0·005
Right colon	176	9	0·088
Colorectal neoplasia	30	9	< 0·001
Age at colectomy (years)*	41 (36·6–73·7)	44 (30·6–66·6)	0·204‡
Duration of UC (months)*	144 (42–210·5)	212 (113–252·5)	0·474‡
High-grade dysplasia	18	4	0·019
Cancer	12	5	< 0·001
Tis	5	1	0·851
T1	1	0	0·076
T2	3	2	0·031
T3	3	1	0·621
T4	0	1	0·076
N+	0	1	0·076
M+	0	0	–
Location			
Rectum	14	3	0·151
Left colon	7	3	0·015
Right colon	10	4	0·004
Multiple sites	7	1	0·980

*Values are median (i.q.r.). UC, ulcerative colitis. †Fisher's exact test or χ^2 test with Yates' correction when appropriate, except ‡Mann–Whitney *U* test.

(including maximum extent of colonic lesions and preoperative treatment exposure) and those with $P \leq 0{\cdot}100$ in univariable analysis were selected for the multivariable analysis. A backward stepwise procedure was used (MASS package) and all analyses were performed using R software version 2.12.2 (The R Project, University of Auckland, Auckland, New Zealand). $P < 0{\cdot}050$ was considered statistically significant.

Results

Effect of appendicectomy on colitis in IL10/Nox1^{DKO} mice

The surgical induction of experimental appendicitis was efficient and reproducible in 5- and 8-week-old mice (*Table S1*, supporting information). In all groups (appendicitis, no-appendicitis and controls) there was a statistically significant correlation between the DAI and global colitis score ($r_S = 0{\cdot}43$, 95 per cent c.i. 0·18 to 0·63; $P = 0{\cdot}001$) (*Fig. S2*, supporting information). All mice survived until they were killed at day 35.

The global colitis score was lower in the appendicitis group than in the control group, irrespective of the age of the mice (*Fig. 2a*). Induction of appendicitis followed by appendicectomy was associated with an improvement in colitis in the majority of mice (*Table 1*; *Fig. S3*, supporting information); this effect was more pronounced in 5-week-old mice. In 5-week old mice, appendicectomy in the no-appendicitis group was not statistically significantly associated with improvement of colitis. In 8-week old mice, DAI was significantly lower in the no-appendicitis group, but the global colitis score did not differ (*Table 1*).

There was an inverse correlation between the severity of caecal patch inflammation and the global colitis score ($r_S = -0{\cdot}43$, 95 per cent c.i. −0·67 to −0·12; $P = 0{\cdot}007$) in IL10/Nox1^{DKO} mice that underwent appendicectomy for appendicitis and no appendicitis (*n* = 40) (*Fig. 2b*).

Appendicectomy for appendicitis and no appendicitis performed in 5- and 8-week-old WT mice did not result in changes in the colon at either the macroscopic or microscopic level (*Table S2*, supporting information).

The induction of caecal inflammation outside the patch by ligating and removing a caecal non-immune area, following a similar protocol to that performed in the appendicitis group in IL10/Nox1^{DKO} mice, had no effect on colitis (*Table S3*, supporting information).

Effect of appendicectomy on colonic neoplasia in IL10/Nox1^{DKO} mice

Of 20 mice in the no-appendicitis group that underwent appendicectomy, 13 developed colonic lesions (*Fig. 2c*) at 35 days (10–13-week-old IL10/Nox1^{DKO} mice). Pathological examination revealed that nine mice had HGD and four had category T1 tumours (*Table 1*). No neoplastic lesions were observed in either the appendicitis or sham IL10/Nox1^{DKO} group, or in WT mice.

Table 3 Univariable and multivariable analysis of risk factors associated with high-grade dysplasia or cancer in patients with ulcerative colitis

	Univariable analysis		Multivariable analysis	
	Odds ratio	*P*	Odds ratio	*P*
Male sex	2·55 (1·23, 5·44)	0·011	2·84 (1·11, 7·99)	0·036
Ever smoked	0·47 (0·18, 1·08)	0·075	–	
Duration of UC before colectomy	1·01 (1·006, 1·014)	< 0·001	1·01 (1·00, 1·01)	< 0·001
Family history of CRC	2·40 (0·50, 9·22)	0·248	–	
UC extension (E3 *versus* E1/E2)	1·36 (0·49, 4·83)	0·575	–	
Inflammatory lesions				
Rectum	0·74 (0·25, 2·71)	0·616	–	
Left colon	0·52 (0·18, 1·69)	0·258	–	
Right colon	0·80 (0·36, 1·91)	0·593	–	
Primary sclerosing cholangitis	2·89 (1·34, 6·16)	0·007	2·80 (1·07, 7·27)	0·034
Indication for colectomy*	23·61 (10·43, 57·69)	< 0·001	–	
Appendicectomy before colectomy	9·35 (3·15, 29·72)	< 0·001	16·88 (3·32, 112·69)	0·001
Preoperative treatment				
Salicylates	4·87 (1·40, 30·82)	0·009	–	
Purine analogues	1·23 (0·60, 2·56)	0·570	–	
Infliximab	0·46 (0·19, 1·03)	0·061	0·33 (0·10, 0·91)	0·043
Ciclosporin	0·91 (0·25, 2·57)	0·866	–	

Values in parentheses are 95 per cent confidence intervals. Multivariable regression analysis was performed on 205 patients without missing data; 33 had high-grade dysplasia or cancer. *Univariable analysis of indications for colectomy: acute severe colitis + refractory ulcerative colitis (UC) *versus* stricture + dysplasia/cancer. CRC, colorectal cancer.

Association between appendicectomy and colorectal neoplasia in patients with ulcerative colitis

Of the 232 patients with UC included in the study who underwent proctocolectomy, 39 (33 with indication for dysplasia/cancer, 6 with other indication but found to have dysplasia/cancer on pathological examination) had either colorectal cancer (17 patients) or HGD (22) (*Table 2*). All neoplastic lesions were found in areas that showed signs of active inflammation or previous episodes of colitis.

Fifteen patients had no appendix in the resection specimen, indicating they had undergone an appendicectomy before colectomy. Nine of these patients had a colorectal neoplastic lesion, including five with cancer and four with HGD. Thirty (13·8 per cent) of the remaining 217 patients, with the appendix *in situ*, had colonic neoplastic lesions, including 12 with cancer and 18 with HGD. Among the 48 patients with PSC, one had previously undergone appendicectomy (2·1 per cent) and 15 had colorectal neoplasia.

Patients in the appendicectomy group were older and had a longer duration of UC (*Table 2*). For patients with colorectal neoplasia, there was no difference in age at colectomy or duration of UC between patients with the appendix *in situ* and those with appendicectomy.

Factors associated with HGD/cancer in the univariable analyses are shown in *Table 3*. After adjustment, appendicectomy was independently associated with colorectal neoplasia (OR 16·88, 95 per cent c.i. 3·32 to 112·69). Other independent risk factors for colonic neoplasia were male sex, PSC and disease duration.

Discussion

It is generally accepted that appendicectomy is protective for the development of UC[12]. However, the underlying mechanisms remain poorly understood. This study sought to characterize better the interplay between the appendix, colonic inflammation and colorectal neoplasia.

IL10/Nox1[DKO] mice spontaneously develop colitis with progressive colonic spread and increasing severity, which enables the performance of dynamic experiments at different stages of the disease[7]. The present study demonstrated that appendiceal inflammation is necessary to improve the colitis. Additionally, this effect of experimental appendicitis followed by appendicectomy was greater in 5-week-old mice with nascent colitis than in 8-week-old animals. These observations are consistent with several studies[2,13,14] in humans. Moreover, endoscopically observed inflammatory appendiceal orifice, or periappendiceal red patch, is commonly observed in patients with distal UC.

However, the mechanisms responsible for the effect of appendicitis on UC have yet to be determined. Mizoguchi and colleagues[4] reported that appendicectomy performed in young T-cell receptor α mutant mice suppresses gut inflammation development and is associated with a reduced number of mesenteric lymph nodes. Older

T-cell receptor α mutant mice are not protected from the development of colitis by appendicectomy, suggesting that the appendix is a site of priming for the immune cells that control colonic inflammation in the early stage of UC[4]. Watson and co-workers[5] studied immune cell population changes following the induction of appendicitis in WT mice. These authors observed that the appendiceal inflammatory process followed an induction sequence of polymorphonuclear cells and then CD4 and CD8 lymphocytes, with an increase of 66 per cent in the proportion of CD25 + FoxP3 + regulatory cells, mostly in mice less than 10 weeks old[5]. In this model of appendicitis followed by appendicectomy in WT mice (without colitis), the distal colonic mucosa exhibits T helper 17 cell (Th17) cytokine-related gene alterations[15] and a pronounced autophagy gene suppression[16]. The IL10/Nox1^{DKO} mouse model in the present study demonstrates that the degree of appendiceal inflammation is inversely correlated with the severity of colitis. In line with the human situation, this phenomenon was most pronounced in the young mice. Characterization of the immunological and cellular mechanisms associated with this colonic anti-inflammatory effect of appendicitis requires further investigation.

Almost 40 per cent of IL10/Nox1^{DKO} mice develop spontaneous colitis-related neoplasia at 8 months[7]. In the authors' animal husbandry conditions, no spontaneous HGD or cancer was observed before 28 and 30 weeks of age respectively. However, appendicectomy in the no-appendicitis group was associated with early onset of colonic HGD/cancer in 10–13-week-old IL10/Nox1^{DKO} mice. This acceleration of carcinogenesis was not associated with an increased severity of colitis: the colitis scores of mice in the no-appendicitis group were not significantly increased compared with controls. This observation suggests that the increased colonic carcinogenesis following appendicectomy is not linked only to inflammatory processes. Interestingly, the increased risk of colonic neoplasia following appendicectomy in UC has been suggested previously in an Australian study[9]. Unfortunately, appendicectomy was not included in the analysis of previous studies that evaluated the risk of colonic cancer in UC[8,17,18].

Limitations of the present study in patients who underwent colectomy for UC are its retrospective design and the fact that indications for the appendicectomy were not known. However, a prospective long-term study does not seem feasible. Restricting the study population to patients with UC who required surgical treatment also limited the external validity of the study. However, by including all consecutive patients who underwent colectomy for indications reflected in the guidelines, a large cohort of patients with UC who developed colonic neoplasia from two centres could be created. This allowed the authors to check for the presence or absence of the appendix in the surgical colectomy specimens and thus avoid bias in the collection of information regarding the history of appendicectomy. Thus, factors were identified that were previously associated with an increased risk of carcinogenesis in UC, including male sex, PSC and disease duration[18]. Moreover, the strength of the association between appendicectomy and colorectal neoplasia in UC is so high that, even in the presence of residual confounding factors that might have been ignored, one could not ignore the real association. At the mechanistic level, the present translational study highlights the protective role of the appendix in colonic carcinogenesis in a chronic inflammatory background. Appendicectomy is not associated with sporadic colonic cancer in humans without inflammatory bowel disease[19]. Similarly, histological modification of the colon of WT mice following appendicectomy in the presence or absence of appendicitis was not seen. These observations suggest that the appendix may be the priming site of the immune cells that are responsible for antitumour activity in the inflamed colonic mucosa. These cells remain to be identified.

Some authors[14] have proposed prophylactic appendicectomy (without appendicitis) for the treatment of UC. The largest prospective case series of patients with ulcerative proctitis who were treated with elective appendicectomy was reported in 2009 by Bolin *et al.*[1]. Appendicectomy (without appendicitis) is currently being evaluated in a European multicentre trial considering the treatment of distal UC after achieving remission with medical treatment.

Acknowledgements

E.O.-D. and X.T. contributed equally to this publication. This paper is written in memory of B. Larroque (Department of Epidemiology and Clinical Research, Pôle Santé Publique, Recherche Clinique et Information Médicale Unité de Recherche Clinique Paris Nord, Hôpital Bichat, Paris, France).

The authors thank the following: V. Desfourmeaux-Denis for analysing the results and editing the manuscript; A. Marah, Y. Ladeiro and F. Daniel for their contribution to mouse experiments and data analysis; L. Peyrin-Biroulet for manuscript editing and advice on study design; and B. Larroque for statistical analysis.

Grant funding for this study was received from the Association François Aupetit and the Société Française de Chirurgie Digestive.

Disclosure: The authors declare no conflict of interest.

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Supporting information

Additional supporting information may be found in the online version of this article:

Table S1 Comparison of caecal patch inflammation with and without induced appendicitis in IL10/Nox1^{DKO} mice (Word document)

Table S2 Results in wild-type mice following a similar study protocol to that used in IL10/Nox1^{DKO} mice (Word document)

Table S3 Induction of caecal inflammation outside the patch in IL10/Nox1^{DKO} mice (Word document)

Fig. S1 Histological grading of murine caecal patches and colonic inflammation (Word document)

Fig. S2 Distribution of disease activity indices according to global colitis scores in all IL10/Nox1^{DKO} groups (Word document)

Fig. S3 Effects of appendicectomy for appendicitis on disease activity and global colitis scores in IL10/Nox1^{DKO} mice (Word document)