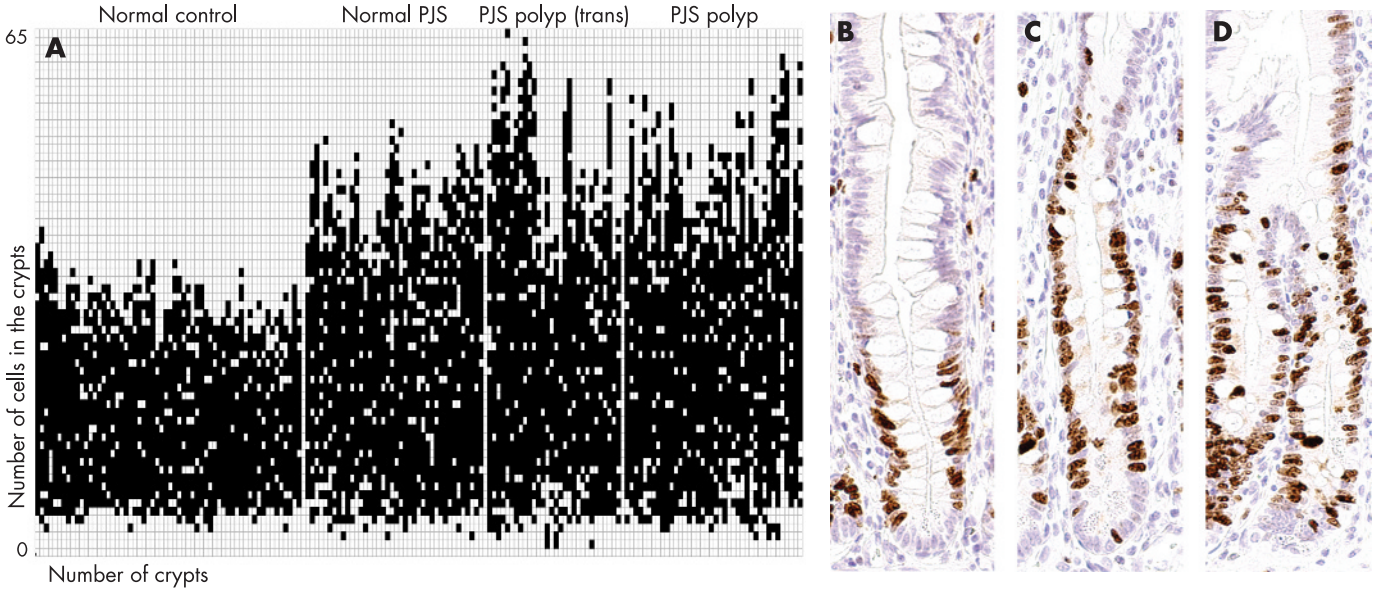

Figure 1 Ki67 staining pattern in intestinal crypts of normal and polyp tissue from patients with Peutz–Jeghers syndrome (PJS) and controls. In all samples the number and location of Ki67 positive cells was scored in 10 completely visible and longitudinally sectioned crypts. The proliferative compartment was defined as the cellular compartment extending between the cell positions of the lowermost and uppermost Ki67 positive labelled cell using the bottom of the crypt as the reference cell position 0.[8] (A) Schematic overview of Ki67 staining, black = positive Ki67 staining. (B) Ki67 staining on normal control intestine. (C) Ki67 staining on PJS normal intestine. (D) Ki67 staining on PJS polyp.

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Peutz–Jeghers syndrome polyps are polyclonal with expanded progenitor cell compartment

Peutz–Jeghers syndrome (PJS) is an autosomal dominant cancer susceptibility syndrome characterised by mucocutaneous melanin pigmentation, hamartomatous polyps, and an 18-fold increase in intestinal and extraintestinal cancer risk.[1] PJS is caused by a germline mutation in *LKB1*, a gene that plays a role in cellular polarity.[2] Proper cellular polarity is critical for accurate asymmetrical stem cell division.[3] The pathogenesis and neoplastic risk, if any, of hamartomatous Peutz–Jeghers polyps remain unclear.

Based on rare observations of neoplastic changes in PJS polyps and the finding of biallelic inactivation of the gene involved in PJS, the existence of a unique hamartoma–carcinoma sequence has been proposed in this disorder.[4 5] This concept suggests that PJS polyps are clonal premalignant lesions responsible, at least in part, for the high rates of gastrointestinal cancer in these patients. However, dysplastic changes have been found rarely. Clarification of the risk of neoplastic transformation in PJS polyps would assist in the design of polyp surveillance strategies for PJS patients.

We studied the clonality of laser microdissected epithelium from two fresh frozen PJS polyps obtained from each of two female PJS patients from different well characterised PJS families, employing X chromosome inactivation of the *HUMARA* gene.[6] Diagnosis of PJS in these two patients was confirmed by identifying the *LKB1* germline mutations—two new mutations resulting in premature truncations (c.829-830insGGGCG, p.Asp277Glyfs12 and c.718C→A, p.Ser240X). The clonality assay showed no significant change in the shift of ratios between the small and large *HUMARA* allele for all polyps after digestion of the unmethylated allele, indicative of a polyclonal nature of these polyps. Furthermore, a loss of heterozygosity (LOH) analysis of four markers surrounding *LKB1* showed no LOH in any of the polyps. As LOH would be indicative of monoclonal cell expansion, its absence is consistent with the polyclonal nature of the polyps as found in the clonality assay.

The polyclonal nature and lack of *LKB1* LOH is compatible with our previous hypothesis that elongation of the progenitor zone, caused by a germline defect in asymmetrical stem cell division and subsequent mucosal prolapse, may play a role in polyp formation.[3 7] To investigate whether the length of the progenitor zone in PJS patients is altered compared with the length of the progenitor zone in normal intestinal mucosal controls, immunohistochemical Ki67 analysis was undertaken to obtain proliferation indices. Six PJS polyps from the PJS patients studied in the clonality assay were divided into three areas: (1) normal flat intestinal mucosa adjacent to the stalk of the polyp; (2) the area that forms the transition between flat intestinal mucosa and hamartomatous mucosa; and (3) the head of the polyp consisting of hamartomatous mucosa. The length of the proliferative zone in PJS flat intestinal, transitional, and polyp head mucosa combined was elongated compared with the crypts in the normal intestinal mucosa of the controls ($p<0.001$) (table 1, fig 1). However, the labelling index—defined as the percentage of positive Ki67 cells within the proliferative compartment—was not statistically different, showing that the percentage of proliferating cells in the crypt remains unchanged, but that in PJS mucosa the length of the proliferative zone is expanded.

In conclusion, we have shown that PJS polyps are polyclonal expansions, arguing against the presence of a hamartoma–carcinoma sequence in PJS. Furthermore, the progenitor zone in intestinal crypts in PJS patients is expanded compared with normal individuals. We postulate that this is caused by disruption of the delicate balance between symmetrical and asymmetrical stem cell divisions, possibly leading to altered stem cell lineage turnover rates and protracted clonal

Table 1 The length of the progenitor zone in cell positions and the labelling index for normal intestine and Peutz–Jeghers syndrome (PJS) polyps and statistical analysis (*t* test)

	n	Mean length ofprogenitor zone	SD	Labelling index (,%)	p Values	
Normal	60	30	3	73.30		
PJS						
total	110	46	7	74.10	Normal vs PJS intestine:	<0.001
flat	30	43	5	73.30	PJS flat vs transition:	0.023
					PJS flat vs head:	0.012
transition	40	48	9	75.50	PJS transition vs head:	0.655
head	30	47	7	73.50		

Flat, normal flat intestinal mucosa adjacent to polyp stalk; head, head of polyp; transition, transition zone between flat mucosa and hamartomatous mucosa.

evolution, thereby accelerating progression to cancer.[9 10] These findings have practical consequences for surveillance strategies in PJS patients. Removal of the gastrointestinal hamartomatous polyps may not necessarily reduce cancer risk.

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Lymphogranuloma venereum proctocolitis: mucosal T cell immunity of the rectum associated with chlamydial clearance and clinical recovery

Lymphogranuloma venereum (LGV) is a sexually transmitted disease caused by *Chlamydia trachomatis* serovars L1 to L3. At present there is an epidemic of LGV proctitis in the Western world among men who have sex with men. HIV seropositivity and other sexual transmitted infections are the main risk factors.[1] Moreover, a concurrent HIV infection seems to be associated with a more severe course of LGV proctitis, indicating that LGV may behave as an opportunistic infection.[2 3] Animal studies have shown a predominant role of CD4 lymphocytes in clearing chlamydial infection, including LGV proctitis, but knowledge of the human rectal immune response in LGV is limited.[4–6] This is the first report of a patient with T cell immunodeficiency describing the rectal immunopathological response during prolonged LGV proctocolitis.

In April 2005, a 33 year old man was referred to our clinic because of resistant Crohn's disease despite treatment with sulfasalazine, corticosteroids, and azathioprine. The patient was known to have sex with men and he assured that HIV serology was recently performed and was negative. Endoscopy revealed deep aphthoid and linear discontinuous ulcers from rectum to sigmoid (fig 1). Histology showed indeterminate colitis. Other infections (*Treponema pallidum*, *Neisseria gonorrhoeae*, herpes simplex virus, cytomegalovirus, *Mycobacterium* sp, *Clostridium difficile*, *Entamoeba histolytica*, and common enteric bacterial pathogens) were excluded by culture or polymerase chain reaction (PCR). Crohn's disease was additionally treated with the anti-TNFα agent infliximab. Three months later the colitis deteriorated and the possibility of HIV infection was reconsidered. At this time, and retrospectively until at least 1 year before the onset of intestinal symptoms (July 2002), HIV serology was positive. The CD4 count was 69×10^6/l and the HIV-RNA load was 229 000 copies/ml plasma. All available rectal biopsy specimens were reviewed and PCR was carried out for *C trachomatis* detection and typing, as described previously.[7 8] L-type *C trachomatis* was present in all specimens, confirming the diagnosis of LGV proctocolitis (fig 2). The diagnosis of Crohn's disease was rejected, and mesalazine, azathioprine, and infliximab were stopped while prednisone was tapered off. The LGV was treated with a four week course of azithromycin (1 g loading dose followed by 250 mg daily) as tetracyclines were contraindicated. In September 2005 a subsequent rectal biopsy specimen remained positive for *C trachomatis* DNA (serovar L2 by RFLP genotyping). By two months, the clinical symptoms improved and highly active antiretroviral therapy (HAART: tenofovir, lamivudine, and nevirapine) was started. Endoscopy in January 2006 showed normal mucosa of the left colon except for some superficial ulcers and submucosal scarring in the rectosigmoid. *C trachomatis* PCR on rectal biopsy specimen was negative. HAART resulted in well suppressed HIV replication (HIV-RNA load, <50 copies/ml) and an increase in the CD4 count (406×10^6/l). Histological examination of all rectal biopsy specimens stained with immunohistochemical markers showed that CD4 lymphocytes (fig 2, A, B) and macrophages (fig 2, E, F) appeared during immune reconstitution and clinical recovery while CD8 lymphocytes disappeared (fig 2, C, D).

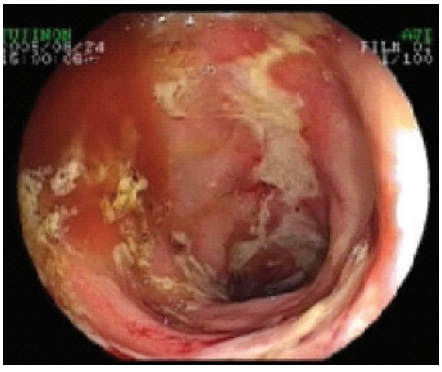

Figure 1 Endoscopy of the rectosigmoid showing multiple deep linear discontinuous ulcerations.

HIV causes a rapid depletion of most CD4 lymphocytes in gastrointestinal mucosal surfaces.[9] This might explain the particular susceptibility of HIV infected people to the development of LGV proctocolitis. As is illustrated by this case, the close association over time between the appearance of CD4 lymphocytes and macrophages and chlamydial clearance strongly suggests that the immunological recovery was the underlying key in controlling rectal chlamydial infection, as antibiotic treatment alone was not sufficient.

In conclusion, rectal CD4 lymphocytes and macrophages seem to play a significant role in mucosal host defence against LGV proctitis. It is possible therefore that the vulnerability of HIV infected people to developing LGV proctitis is not only explained by sexual behaviour but also by mucosal T cell immunodeficiency of the rectum. This may contribute to the development of chronic symptomatic LGV proctocolitis which can be indistinguishable from resistant Crohn's disease.

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