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Bovine lactoferrin and Crohn's Disease: A case study

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Abstract

A 22 year old male suffering from abdominal pain, repeated diarrhea, and weight loss visited the Digestive Disease Department of Nagoya City University Hospital on Dec. 19, 2011. He was hospitalized and diagnosed with Crohn's colitis. His Crohn's Disease Activity Index (CDAI) was 415. Treatment by granulocyte apheresis, mesalazine, and adalimumab was started. His CDAI was 314 on Dec 30 and 215 on Jan. 5. A colonoscopic examination on Jan. 19 showed almost complete remission in the transverse colon and marked remission in the rectum. Mesalazine therapy stopped on Feb 28, and the patient was instructed to self-inject 40 mg adalimumab every other week. His CDAI was 50 on April 10 indicating clinical remission. His last self-injection of adalimumab was April 24, 2012, and he started taking 1 g bovine lactoferrin (bLF) daily. His CDAI was 35 on Jan 08, 2013. He continued taking 1 g bLF daily without any other treatment for Crohn's disease. Laboratory blood tests on Sept 07, 2015, showed no sign of disease recurrence, and a colonoscopic examination on Oct 23, 2015, showed almost complete mucosal healing. This case indicates that ingestion of bLF to maintain Crohn's disease in a remissive state should be further explored.

Crohn's disease, Crohn's colitis, bovine lactoferrin

Introduction

Lactoferrin (LF) is abundantly expressed in tissues and fluids where the body interacts with external microbes: Neutrophils exocytose LF containing granules at sites of infection; LF in seminal fluid helps to protect sperm from the microflora of the vagina; LF present in the mucosa of the upper respiratory tract and tear film helps to protect these surfaces from invading pathogens; and LF in milk helps to protect the developing gastrointestinal tract of the suckling infant (Alexander et al. 2012). An essential feature of LF associated with its protective function against microbes is that LF inhibits attachment and invasion of target cells by microbes without killing them and generating microbial antigens, allowing microbial removal without causing inflammation and consequent tissue damage (Alexander et al. 2012). Thus, in the tissues and fluids where it is found, LF is a crucial component of the barrier that all multicellular organisms must maintain against microbes.

Crohn's Disease is an impaired barrier disease of the intestine (Antoni et al. 2014; Wehkamp et al. 2008). A striking characteristic of Crohn's disease is decreased expression of defensins: There is a strong association between defective α -defensin production by Paneth cells and ileal Crohn's Disease (Jager et al. 2013; Wehkamp et al. 2005; Wehkamp and Stange 2006), and colonic Crohn's Disease is associated with defective β -defensin production (Nuding et al. 2007; Wehkamp et al. 2005; Wehkamp and Stange 2006). A defective intestinal barrier allows adherence and invasion of the intestinal epithelium by luminal bacteria and consequent triggering of the host's immune response. In the absence of mucosal healing and restoration of the intestinal barrier, this will result in the chronic inflammation that is a hallmark of inflammatory bowel diseases.

While endogenous human LF is not part of the intestinal barrier (Alexander et

al. 2012), ingested LF does appear to have activity in the colon. For example, in a placebo controlled clinical trial, participants with colorectal polyps ingesting 3 g bovine LF (bLF) daily for 1 year had enhanced expression of type 1 interferons in the colon, an altered population of immune cells in their colorectal polyps, and decreased colorectal polyp growth (Alexander et al. 2014; Iigo et al. 2014; Kozu et al. 2009). Since ingested bLF is digested in the stomach and intestine of the adult and neither the bLF protein nor its digested fragments reach the colon in significant amounts or bind to receptors that would affect type 1 interferon expression or immune cell function ((Alexander et al. 2014): Supplementary Text S2), the effects of ingested bLF in the adult colon are likely to be indirect.

Materials and Methods

The patient took 3 bovine lactoferrin containing tablets orally every morning with a glass of water. The tablets were ingested at least 30 min before the patient consumed anything else. The bovine lactoferrin containing tablets were purchased from Nutricology (Item # 52080) (Alameda, CA. USA). Each tablet contained 350 mg bovine lactoferrin. Other ingredients included hydroxypropyl methylcellulose, microcrystalline cellulose, l-leucine. Nutricology did not respond to requests for additional information on the contents of the tablets.

Results

While residing in the USA, the patient (Patient A), a 22 year old male, had been suffering from abdominal pain, repeated diarrhea, and weight loss. In mid-December, he returned to Japan and on Dec. 19, 2011, he visited the Digestive Disease Department of the Nagoya City University Hospital. Physical examination showed

edema, anemia, and ascites, and computed tomography revealed extensive thickening of the wall of the sigmoid colon and rectum suggestive of inflammatory bowel disease. He was hospitalized as an emergency patient due to his symptoms and malnutrition. On Dec. 20, a colonoscopy from the rectum through the transverse colon showed extensive erosive and ulcerative lesions and erythematous (color) and edematous (swelling) changes in the mucosa from the sigmoid colon through the transverse colon (Fig. 1). Biopsy revealed the presence of a noncaseating epithelioid granuloma (see Turner et al. 2014). Tests for infectious diseases that could mimic Crohn's disease symptoms, including *Clostridium difficile*, were negative, and he was diagnosed with Crohn's disease of the colon (Crohn's colitis). His Crohn's Disease Activity Index (CDAI) was 415.

After confirmation that Patient A was free from infections such as Hepatitis B virus, Hepatitis C virus, HIV, HTLV, and tuberculosis that could be exacerbated by immunosuppressive therapy, treatment by granulocyte apheresis (granulocyte removal therapy: see Yamamoto et al. 2006), the anti-inflammatory agent Pentasa (mesalazine, 3000 mg/day: see Karagozian and Burakoff 2007), and Humira (the TNF-alpha antibody adalimumab: see Colombel et al. 2007) was started. The initial injection of Humira was 160 mg followed two weeks later by an injection of 80 mg. His CDAI was 314 on Dec 30 and 215 on Jan 5, 2012, when Patient A was discharged from the hospital. Pentasa therapy and maintenance doses of 40 mg Humira were administered every other week at the out patient clinic. A second total colonoscopic examination performed on Jan. 19 showed mucosal healing from the transverse colon to the cecum and marked improvement of ulcerative lesions from the sigmoid colon to the transverse colon (Fig. 2).

Mesalazine therapy was stopped on Feb 28. Patient A was instructed to self-

inject 40 mg Humira every other week. His CDAI was 50 on April 10 indicating clinical remission. Thus, after a course of granulocyte apheresis, approximately 2 months of mesalazine administration (3000 mg/daily from 22 Dec 2011 to 28 Feb 2012), and 9 injections of Humira (160 mg on 22 Dec 2011, 80 mg on 5 Jan 2012, 40 mg every other week thereafter) the disease was in remission.

Patient A's last self-injection of Humira was April 24, 2012 (a total of 10 injections). He left Japan and returned to the USA at the beginning of May. In the USA, due to the difficulty of obtaining medical insurance with a pre-existing condition and the high cost of medical anti-TNF-alpha antibodies such as adalimumab and infliximab, the patient was compelled to try to find an alternative. He started taking 1 g bovine lactoferrin (bLF) with a glass of water every morning at least 30 min before consuming anything else. He returned to Japan in 2013, and his CDAI was 35 on Jan 08, 2013. In Japan, the patient continued taking 1 g bLF daily, but did not restart medical anti-TNF-alpha antibody injections or any other Crohn's disease medication.

Laboratory blood tests on Sept 07, 2015, showed no sign of disease recurrence, and a colonoscopic examination on Oct 23, 2015, showed almost complete mucosal healing with only a few residual inflammatory polyps (Fig. 3). A fecal exam did not detect pathogenic bacteria, including *Clostridium difficile* (see Ananthakrishnan et al. 2010). Thus, Patient A while ingesting bLF has remained in clinical remission for more than 3¹/₂ years, and at the time of this writing Patient A has been symptom free for 4¹/₂ years.

Discussion

It is important to recognize that Patient A began taking bLF tablets after his disease was diagnosed as being in remission following treatment with granulocyte apheresis, Pentasa, and Humira, as described above. Therefore, the case study described in this report does not indicate that lactoferrin can be used to treat active Crohn's disease. However, this case study does suggest that ingestion of lactoferrin may be able to promote a healthy intestinal mucosa and maintain Crohn's disease in a remissive state.

How significant is the 3¹/₂ to 4¹/₂ year remission status of Patient A? Crohn's disease is a chronic, recurrent, inflammatory disease of the gastrointestinal tract. Two literature surveys found that only approximately 10% of patients being treated for Crohn's disease experience prolonged clinical remission (prolonged was not defined in these reports) (Loftus et al. 2002; Peyrin-Biroulet et al. 2010). A long-term follow-up of 185 Crohn's disease patients reported that after 5 and 10 years 93% and 99% of the patients had experienced at least one relapse (Binder et al. 1985). A more recent 10-year follow-up study of a cohort of 237 Crohn's disease patients reported cumulative relapse rates of 53%, 85%, and 90% at 1, 5, and 10 years (Solberg et al. 2007). A 35-year follow-up of 507 patients treated for colorectal Crohn's disease reported that of 407 patients who attained clinical remission without surgery and in whom further clinical course could be determined, only 29 had a non-relapsing course and all 12 of the non-treated patients who attained remission subsequently had a relapse; median follow-up time was 13.4 years, 0.5 - 319 months (Lapidus et al. 1998). The cumulative rates of relapse in these patients at 1, 2, 5, and 10 years after diagnosis were 20%, 40%, 67%, and 76%, respectively. While Patient A can not be directly compared with the patients followed in the studies noted here, since they were under clinical care, Patient A does compare favorably with these patients.

Table 1 lists several trials in which patients with colitis (Crohn's disease of the colon) and/or ileocolitis (Crohn's disease of the ileum and the colon) attained remission and were subsequently followed for maintenance of remission. The data shown is from patients who stopped taking medications after their disease was in remission and were followed-up (studies 19, 25-28) or entered the no treatment arm (studies 7, 8, 11, 13) or placebo arm (studies 1-6, 9, 10, 12, 14-18, 20-24) of clinical trials. The placebo arm of these studies did not receive concomitant medication. Studies that contained (1) only patients with Crohn's disease limited to the ileal or ileo-caecal region and/or ascending colon, (2) studies that contained only patients with surgically induced remission, and (3) studies in which medication was withdrawn before the patient had established remission are not listed in Table 1: The first two types of studies are not directly relevant to Patient A and the last type of study has high relapse rates, 70% or more within the first year (Colombel et al. 2007; Hanauer et al. 2002; Rutgeerts et al. 2004; Rutgeerts et al. 2012; Schreiber et al. 2007). Relapse rates vary markedly in the studies listed in Table 1, with most of these studies reporting relapse rates between 30% and 70% during the first year after medication withdrawal. The reason for this wide range of relapse rates between studies is unknown. There does not appear to be a relationship between disease location and short-term relapse rate; only study 23 reports a different relapse rate between patients with Crohn's colitis, ileocolitis, and ileitis. Study 28 found that younger age at diagnosis and male gender were associated with a higher risk of relapse, but study 12 did not find an association of relapse rate with any of the following parameters: age, gender, disease duration, previous surgery, previous steroid therapy, entrance CDAI, disease localization, extraintestinal manifestations, perianal lesions during acute disease, response to acute phase treatment, blood

sedimentation rate, or C reactive protein at time of randomization. Studies 19 and 26 also report that there were no predictive factors for relapse/remission after medication withdrawal. One factor that does appear to be associated with relapse is the time in remission before withdrawal of medication: see study 21. Studies 14 and 28 also found a decrease in the risk of relapse in patients with prolonged prestudy remission, and Holtmann, et al. (2006) report that after 3-4 years in complete remission while on azathioprine therapy, withdrawal of medication did not result in clinical deterioration for the remainder of the study. Finally, studies 20 and 27 report low relapse rates in the first 1 to 1.5 years, and both these studies followed patients with prolonged prestudy remission times. Time in remission, in turn, may be related to mucosal healing. However, while some studies have found a relationship between mucosal healing and relapse rates (Baert et al. 2010; Froslic et al. 2007; Papamichael et al. 2015; Schnitzler et al. 2009b), other studies haven't (Farkas et al. 2014; Landi et al. 1992; Lemann et al. 2005; Rutgeerts et al. 2006). Another possibility is that time in remission reflects disease severity, i.e., patients with extended prestudy remission times have lower disease severity, and hence, are less likely to relapse after medication withdrawal. This suggests that one reason for the prolonged remission experienced by Patient A may be low disease activity. However, at the time of diagnosis of Crohn's colitis in 2011, pathological examination revealed the presence of a noncaseating epithelioid granuloma, and this lesion is associated with increased clinical severity of Crohn's disease (Turner et al. 2014), indicating that the patient's prolonged remission is not due to initial low disease activity.

Studies 4, 21, 22, and 26-28 in Table 1 illustrate the well-known increase of cumulative relapse rates over time in Crohn's disease patients. Crohn's disease has both environmental and genetic components (Ostaff et al. 2013); consequently, it can

be controlled but not cured, which accounts for the steady increase in cumulative relapses over time. At 3¹/₂ to 4¹/₂ years in remission, Patient A's disease is well controlled.

Another point about the studies listed in Table 1 is that none of them induced remission using medical anti-TNF-alpha antibodies. This is because the placebo arm of trials testing anti-TNF-alpha therapy either started before the patients were confirmed to be in remission (Colombel et al. 2007; Hanauer et al. 2002; Rutgeerts et al. 2004; Rutgeerts et al. 2012; Schreiber et al. 2007) or they allowed use of concomitant medication. As noted above, the placebo arm of the first type of trial had high relapse rates, 70% or more within the first years. Table 2 lists several of the second type of study: Patients were treated with anti-TNF-alpha antibodies (infliximab or adalimumab: generally referred to as biological therapy in these studies), and concomitant with biological therapy steroid and/or immunosuppressive therapy was generally employed during initial treatment and was often continued after withdrawal of biological therapy. Two studies stand out in Table 2: Study 1, with a very high rate of relapse, and study 9, with a very low rate of relapse. There are no obvious differences between the 22 patients in remission in study 1 and the patients in remission in studies 2-8, suggesting that the high number of relapses within 1 year in this study was due to chance. On the other hand, the extraordinary low rate of relapse in study 9 is possibly due to patient selection. While all of the patients in Table 2 responded to biological therapy and were in clinical remission or clinical remission + mucosal remission at the start of the follow-up period, only patients with a follow-up period of 2 years or more were included in study 9.

Because the outcome of patients who did not receive concomitant therapy after biological therapy withdrawal was not specifically recorded in the studies listed in

Table 2, Patient A can not be directly compared with these patients; however, his time in remission does not contradict the possibility that ingestion of 1 g bLF daily is effective in maintaining remission after biological therapy withdrawal. Importantly, study 9 along with study 8 demonstrate that there is a subgroup of patients who will remain in prolonged remission after biological therapy is withdrawn. Therefore, it is possible that Patient A belongs to this subgroup. However, while this subgroup appears to have a significant number of patients, the percentage of Crohn's disease patients belonging to this subgroup is small, suggesting that some other factor may be responsible, at least in part, for Patient A's prolonged remission. One plausible factor is ingestion of bLF. However, it is also possible that an environmental trigger for his disease is more prevalent in the USA, where he developed the disease, than in Japan, where his disease remains in remission.

Is it credible that ingestion of bLF could affect Crohn's disease of the colon? As noted in the introduction, ingestion of bLF does appear to have an effect in the colon. We previously suggested one possible means by which ingested bLF could affect the colon (Alexander et al. 2014: Supplementary Text S2): Briefly, gastric digestion of bLF results in the generation of antimicrobial peptides (Furlund et al. 2013; Kuwata et al. 2001; Kuwata et al. 1998a; Kuwata et al. 1998b) that have the potential to kill a small fraction of intestinal microbes and produce microbial antigens such as lipopolysaccharide, lipoproteins, flagellin, and unmethylated CpG-containing DNA that are able to interact with pattern recognition receptors (PRRs) expressed by intestinal epithelial and immune cells (Abreu et al. 2005; Artis 2008; Rakoff-Nahoum et al. 2004). Interaction between microbial antigens and intestinal epithelial PRRs increases expression of IgA (Wells et al. 2011) and antimicrobial peptides (Froy 2005; Vora et al. 2004), thereby enhancing the barrier function of the overlying mucosal

layer of the intestinal epithelium (see Johansson et al. 2011), protecting the epithelium from the intestinal microbiota and helping to maintain remission in Crohn's disease patients. This proposal assumes (1) that microbial killing by bLF derived peptides results in an elevation of microbial antigens that reach the surface of the intestinal epithelium and (2) that the consequent increased production of antimicrobial factors is able to offset the deficiencies that can lead to development of Crohn's disease. Another proposal is that antimicrobial peptides can influence the composition of the intestinal microbiota and help to control Crohn's disease (Gunther et al. 2016). In support of this proposal, (1) a subset of Crohn's disease samples contain abnormal gastrointestinal microbiotas (Frank et al. 2007); (2) it has been shown that in mice, transfer of dysbiotic intestinal microbiota to genetically susceptible germ-free mice resulted in Crohn's disease-like inflammation (Schaubeck et al. 2016); and (3) that oral administration of a synthetic antimicrobial peptide to mice induced significant changes in the composition of the intestinal microbiota (Dupont et al. 2015).

This case study is the only physician-certified report of the status of Crohn's disease in a patient ingesting bLF. Is there any other evidence that ingested bLF is able to affect the status of Crohn's disease? There are a few studies using animal models of Crohn's disease that report on the effect of ingested LF. However, these animal models are of active Crohn's disease, and the results of the studies are mixed. For example, Togawa et al. (2002), Haversen et al. (2003), and Li et al. (2013) report suppression of active disease in trinitrobenzenesulfonic acid (TNBS)-induced colitis in rats and dextran sulphate (DSS)-induced colitis in mice, but Bodammer et al. (2013) report that administration of bLF did not affect the course of DSS-induced colitis in mice. Importantly, ingested lactoferrin (LF) is unlikely to directly affect the mucosa affected by active Crohn's colitis as (1) neither ingested LF nor peptides

derived from digested LF reach the cecum or colon in rats (Kuwata et al. 2001) or the colon in humans (Troost et al. 2002), and (2) abundant neutrophil-derived lactoferrin is present in the colons of patients with active Crohn's colitis (Walker et al. 2007). Therefore, the effects of ingested LF on Crohn's colitis is most likely indirect; for example, as suggested above, via interaction of peptides derived from gastric digestion of bLF with the intestinal microbiota. Notably, in the study by Haversen et al. (2003), administration of two peptides derived from the bactericidal region of hLF (hLF₁₆₋₄₀ and hLF₁₈₋₄₀) had similar results to hLF administration and Li et al. (2013) suggest (data not shown) that the effects of apo-bLF in their study were dependent on restoration of homeostasis of the intestinal bacteria. Finally, an indirect action of LF on chemically-induced colitis could also account for the different results obtained by the groups cited above: the results could depend on the digestion of the administered lactoferrin (Bodammer administered bLF by gavage to mice while Haverson et al. applied hLF to the back of the tongue of the mice) and the severity of the disease (Bodammer et al. used 5% DSS in the drinking water of mice for 7 days while Li et al. used 2.5% DSS in the drinking water of mice for 7 days and Togawa used TNBS to induce colitis in rats). Overall, there is some support from animal studies that ingestion of bLF could affect the status of Crohn's colitis.

Is ingestion by Crohn's disease patients in remission safe? One critically important factor is a patient's response to re-introduction of therapy. Studies 1, 4, 5, and 8 in Table 2 indicate that the response of a relapsing patient to restarting anti-TNF α antibody therapy is excellent. In addition, in study 27 in Table 1, 23 of 24 relapsing patients retreated with azathioprine entered remission. Another factor is the effect of prolonged exposure of the intestine to the antimicrobial peptides generated by gastric digestion of bLF. To date, no adverse effects have been reported in humans

ingesting lactoferrin. In trials with patients with chronic hepatitis C, 15 patients were given 3.6 g bLF daily for 6 months (Iwasa et al. 2002), 15 patients were given 7.2 g bLF daily for 8 weeks (Okada et al. 2002), and 97 patients were given 1.8 g bLF daily for 12 weeks (Ueno et al. 2006). In a clinical trial to determine whether ingestion of bLF had an effect on colorectal polyp growth, 34 patients ingested 3.0 g bLF daily for one year (Kozu et al. 2009). Four long term cancer survivors with metastatic renal cell carcinoma received recombinant human lactoferrin at a dose of 9 g per day given in cycles of 2 weeks on/2 weeks off for 30 months or longer (Lewis and Hayes 2011). Finally, the European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies (NDA) has concluded that intake of bLF up to 3.4 g per day by an adult is safe (EFSA 2012).

Concluding remarks: Crohn's colitis in Patient A has been in clinical remission for at least 3¹/₂ years and in symptomatic remission for 4¹/₂ years. It is plausible that ingestion of 1 g bLF daily has helped maintain this remissive state; although, environmental factors can not be ruled out. It has been suggested that stimulation of the protective innate immune system of the intestine could help protect against Crohn's disease (Gerseemann et al. 2008). Ingestion of bLF has the potential to stimulate the intestinal epithelium to produce antimicrobial agents, thus protecting the epithelium against the intestinal microbiota. As discussed above, it has also been suggested that the microbiota associated with active Crohn's disease can be abnormal and that ingestion of antimicrobial peptides may be able to induce changes in the composition of the intestinal microbiota and affect the course of the disease. Ingestion of bLF is safe and ingestion of bLF to maintain Crohn's disease in a remissive state should be further explored.

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Table 1. Relapse rates in various studies testing maintenance of remission of Crohn's disease after medication withdrawal

Arbitrary study No.	*Patients	Outcome vs location	Time in remission prior to medication withdrawal	First 9 months	10 to 12 months	18 months	24 months	36 to 40 months	48 months	5 years	
1	11	NS	NR	8 of 11							(Willoughby, et al., 1971)
2a	7	NS	NR	5 of 7							(Brignola, et al., 1988)
2b	4	NS	4 weeks or more	3 of 4							(Brignola, et al., 1988)
2c	5	NS	6 months or more	4 of 5							(Brignola, et al., 1988)
3a	13	IC & C	1 month or more	7 of 13							(Brignola, et al., 1992)
3b	9	I	1 month or more	6 of 9							(Brignola, et al., 1992)
4	#59	NS	20 - 3070 days	31%	52%						(de Franchis, et al., 1997)
5	19	NS	NR		17 of 19						(Candy, et al., 1995)
6	30	NS	NR		21 of 30						(Modigliani, et al., 1996)
7	15	NS	NR		8 of 15						(Vilien, et al., 2004)
8	18	IC & I	3 weeks or more		17 of 18						(Bresci, et al., 1991)
9a	#28	C	1 month or more		39%						(IMSG, 1990)
9b	#43	IC	1 month or more		36%						(IMSG, 1990)
9c	#34	I	1 month or more		31%						(IMSG, 1990)
10	#161	NS	1 month or more		45%						(Mahmud, et al., 2001)
11	20	NS	Less than 8 weeks		13 of 20						(Yamamoto, et al., 2007)
12	95	NS	8 weeks		62 of 95						(Gross, et al., 1998)
13	1	NS	Less than 1 year		0 of 1						(Lennard-Jones, 1977)
14a	11	C	3 months to 2 years		6 of 11						(Prantera, et al., 1992)
14b	18	IC	3 months to 2 years		9 of 18						(Prantera, et al., 1992)
14c	32	I	3 months to 2 years		17 of 32						(Prantera, et al., 1992)
15	36	NS	16 weeks or more		22 of 36						(Feagan, et al., 2000)
16	31	NS	6 months or more		15 of 27						(Arber, et al., 1995)
17a	50	IC & C	less than 18 months		28 of 50						(Thomson, et al., 1995)
17b	21	I	less than 18 months		10 of 21						(Thomson, et al., 1995)
18a	64	IC & C	less than 18 months		26 of 64						(Sutherland, et al., 1997)
18b	63	I	less than 18 months		21 of 63						(Sutherland, et al., 1997)
18c	93	NS	less than 18 months		39 of 93						(Sutherland, et al., 1997)

18d	35	NS	less than 18 months	8 of 35						(Sutherland, et al., 1997)
19	110	IC & C	NR		77 of 110					(Landi, et al., 1992)
20	40	NS	42 months or more		9 of 40					(Lemann, et al., 2005)
21a	#33	NS	Less than 3 months	68%		71%				(Gendre, et al., 1993)
21b	#48	NS	3 months to 2 years	22%		42%				(Gendre, et al., 1993)
22	101, 57	NS	1 - 2 years or less	36 of 101		34 of 57				(Summers, et al., 1979)
23a	7	C	12 months or more			0 of 7				(Wenzl, et al., 2015)
23b	14	IC	12 months or more			7 of 14				(Wenzl, et al., 2015)
23c	5	I	12 months or more			1 of 5				(Wenzl, et al., 2015)
24	§	§	§			§ ~ 65%				(Malchow, et al., 1984)
25	#10	NS	NR			50%				(Nyman, et al., 1985)
26	#222	DNI, NS	NR	†37%		†56%	†66%	†72%	†75%	(Fraser, et al., 2002)
27	#66	NS	42 months or more	14%		53%			63%	(Treton, et al., 2009)
28	#42	NS	6 to 93 months	38%			61%		75%	(Bouhnik, et al., 1996)

* , #: *Patients reaching the trial end-point, if given; otherwise, # the number of patients used for statistical analysis and calculation of relapse rates.

C: Colitis (Crohn's disease of the colon); IC: Ileocolitis (Crohn's disease of the ileum + Colon); I: Ileitis (Crohn's disease of the ileum);

NS: The outcome of patients with colitis, ileocolitis, and/or ileitis was not specified.

NR: These patients were in medically induced remission. The time spent in remission prior to medication withdrawal was not reported.

DNI: Data concerning the number of patients with colitis, ileocolitis, and/or ileitis was not included

Study 2: While the CDAIs of the patients enrolled in this study was below 150, other laboratory parameters suggested that these patients were at high risk of relapse.

Study 4: Steering committee terminated the study at the time of interim analysis: 50% of the planned patients had been enrolled and followed for 1 year.

Mean duration of remission prior to enrolment was 592 days.

Study 5: Data shown is from Phase 2 of this study, which compared azathioprine alone vs. placebo in the maintenance of remission induced in phase 1.

Study 7: These patients were not treated surgically.

Study 8: The single patient who did not experience a relapse during the first year of the study remained in remission until the end of the follow-up period of 3 years.

Study 10: Mean duration of remission prior to enrolment was 22 months.

Study 11: This patient was not treated surgically.

Study16: This study may have included patients in spontaneous remission.

Study 17a: Of 105 patients, 47 patients were withdrawn from the study due to adverse events or insufficient therapeutic effect. Relapses may be under reported.

Study 17b: Of 43 patients, 14 patients were withdrawn from the study due to adverse events or insufficient therapeutic effect. Relapses rate may be under reported.

Study 18a,b: The location of one CD case was unknown.

Study 18c: Patients stratified by type of remission: Remission in these patients was medically induced.

Study 18d: Patients stratified by type of remission: Remission in these patients was surgically induced.

Study 19: This study had 3 groups of patients. (A) Patients in clinical but not endoscopic remission started steroid tapering at the beginning of the study. (B) Patients in clinical but not endoscopic remission continued prednisolone treatment for 5 more weeks before starting steroid tapering. (C) Patients in clinical and endoscopic remission. Group C patients started steroid tapering at the beginning of the study. The remission rates in the 3 groups was not significantly different.

Study 22: Data shown is from part II of this study, which was composed of patients who had had active disease within the previous 2 years but were in medically induced remission at the time of entry into the study and patients who were in surgically induced remission within 1 year of entry into the study .

Study 23: The patients in this study had continuous azathioprine therapy for 4 years or more.

Study 24: § Specific data for the parameters in Table 1 are not reported. The life-table analysis based on failure and relapse of patients in remission at trial entry is shown in Fig. 6 of the report, however, specific data is not reported.

Study 26: †This study reports the proportion of Crohn's disease and ulcerative colitis patients in remission at 12 months (0.63), 24 months (0.44), 36 months (0.34), 48 months (0.28), and 60 months (0.25) after stopping azathioprine, and that 63 of the 79 Crohn's disease patients experienced at least one relapse during the follow-up period. Cox regression analysis of the proportion of patients in remission was identical for Crohn's disease and ulcerative colitis patients. The study reports that for Crohn's disease, colonic disease favoured remission, however, data supporting this conclusion was not included in the report.

Table 2. Relapse rates in various studies testing maintenance of remission of Crohn's disease after discontinuation of anti-TNFα antibody therapy

Arbitrary study No.	Patients	Concomitant medication	Within 1 year	Within 1.5 years	2 years	3 years	4 years	5 years	7 years	10 years	Response rate after restarting anti-TNFα antibody therapy	
1	22	32 & 35	18 of 22								81% (8 weeks)	(Farkas, et al., 2014)
2	121	72 & 103	45%								NR	(Molnar, et al., 2013)
3	55	55	17 of 55								NR	(Ampuero, et al., 2016)
4	17	16		5 of 17							4 of 4 (12 months)	(Molander, et al., 2014)
5	115	115	44%		52%						38 & 42 of 43	(Louis, et al., 2012)
6	110	92					50%				NR	(Schnitzler, et al., 2009a)
7	48	35		50%					65%		NR	(Vaughn, et al., 2010)
8	53	46	39%		50%					88%	24 of 25	(Steenholdt, et al., 2012)
9	100	95	4%		7%	12%	20%	27%		48%	NR	(Papamichael, et al., 2015)

NR: The response of relapsed patients restarting TNFα antibody therapy was not reported.

Study 1: A total of 41 patients with Crohn's disease were followed. 32 of these patients were treated with steroids and 35 with azathioprine in addition to biological therapy. 22 patients followed were in clinical remission + mucosal remission.

Study 2: 72 patients were treated with steroids and 103 patients were treated with thiopurines in addition to biological therapy.

Study 3: Patients achieved remission within 6 months of receiving combined biological (IFX or adalimumab) + immunosuppressive therapy and remained in remission for at least 6 months. After at least 6 month of combined therapy, biological therapy was withdrawn and the patients continued with immunosuppressive therapy.

Study 4: Patients were in remission for 6 months or more. Follow-up time was 12-15 months

Study 5: Patients were in remission for 6 months or more. 38 of 43 patients were in remission and 42 of 43 had a clinical response after restarting TNFα antibody therapy.

Study 6: The median time in remission for the 110 patients was 47.3 months.

Study 7: 50% relapse rate at a median of 477 days.

Study 8: Concomitant immunosuppression therapy was not associated with relapse or prolonged remission.

Study 9: 100 patients were followed for a median of 9.7 years. At the end of the follow-up period 52 patients were in sustained clinical remission. The cumulative probabilities of sustained clinical remission (defined as maintaining disease remission until the end of the follow-up period) at 1, 2, 3, 4, and 5 years was 96%, 93%, 88%, 79.9%, and 72.8%, respectively.

Figure Legends

Figure 1. Colonoscopy on 20 Dec. 2011. Colonoscopic examination showed extensive erosive and ulcerative lesions from the colorectal region to the transverse colon. In addition, longitudinal ulceration was found in the descending and transverse colon. Blue arrows: Inflammatory polyps. White arrows: Erosion and ulceration.

Figure 2. Colonoscopy on 19 Jan. 2012. The second total colonoscopy showed mucosal healing from transverse colon to cecum, and improvement of ulcer lesions from sigmoid colon to transverse colon. The patient's CDAI was 415. Blue arrows: Inflammatory polyps. White arrows: Erosion.

Figure 3. Colonoscopy on 23 Oct. 2015. The third colonoscopic examination showed almost complete mucosal healing. Blue arrows: Inflammatory polyp.