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Jacobs Journal of Gastroenterology and Hepatology

Review Article

Long-Term Efect of Short-Term Oral Administration of A Mixture of Autologous Proteins Extracted From The Colon of Patients with Crohn's Disease: A Memory Effect of Oral Tolerance Induction

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Received: 11-12-2014

Accepted: 12-31-2014

Published: 02-24-2015

Abstract

Background: The long-term learning ability of the gut immune system has never been studied before. Oral administration of the autologous colonic extract, Alequel™, was shown to be a safe and effective treatment for patients with Crohn's disease. 92 patients were enrolled in three randomized, placebo-controlled, double-blind trials.

Aims: To evaluate the long-term safety and efficacy of the oral administration of Alequel™.

Study: Clinical and laboratory parameters were followed retrospectively to determine the long-term effects of Alequel™.

Results: Patients in group A (treated and responded to Alequel™), had a mean flare-free interval of 7.3±3.96 months. Patients in group C (treated and responded to placebo) had a flare-free interval of 3.2±5.4 months. Conclusions: Short-term oral administration of autologous colonic extracts exerts long-term memory and beneficial effects in patients with moderate to severe Crohn's disease.

Keywords: Oral tolerance; CD- Crohn's disease; NKT- Natural Killer T Cells

Abbreviations

CD: Crohn's Disease;
GALT: Gut Associated Lymphoid Tissue;
Tregs: Regulatory T Cells;
MDSCs: Myeloid-Derived Suppressor Cells;
IBDQ: Inflammatory Bowel Disease Questionnaire Score;
APCs: Antigen-Presenting Cells;
DCs: Dendritic Cells;
TLR: Toll-Like Receptor

Introduction

The gut-associated lymphoid tissue (GALT) plays a role in the induction of oral tolerance toward self and non-self-antigens [1]. Oral tolerance is a natural immunologic process driven by the oral administration of an exogenous antigen [2-4]. The mechanism of tolerance induction is associated with activation of subsets of immune cells at the level of the gut associated lymphoid tissue [5], followed by systemic promotion of suppressor cells [6]. Oral antigen administration can promote regulatory T cells (Tregs) [7], including Th2 (interleukin (IL)-4+/IL-10+), Th3 (transforming growth factor, TGF)- beta) cells, $CD4^{+}CD25^{+}$ regulatory cells, and LAP^{+}T cells8-10. Induction of oral tolerance in animal models of various immune mediated disorders was shown to be beneficial in suppressing autoimmunity and inflammation [7,11,12]. Progress in the field of mucosal immunology provides new insights into the potential use of oral tolerance in humans via systemic promotion of regulatory T lymphocytes to suppress inflammation [3, 7, 13, 14].

Exposure of the GALT to antigens derived from the bowel mucosa is an attractive physiologic approach for immunotherapy toward antigens presented in the gut mucosa [15]. This method of antigen-specific/ bystander therapy is non-toxic and was shown to be effective in animal models of inflammatory bowel disease [15,25-27]. Phase I and Phase II clinical trials have suggested that oral administration of a mixture of autologous proteins extracted from colon, AlequelTM, is safe and effective in patients with moderate to severe Crohn's disease (CD) [16-18]. The results of these studies showed induction of remission in 58% vs. 29% and in 50% versus 33%, respectively, in the two trials, for patients receiving AlequelTM versus placebo [16-18]. These data suggest that the beneficial clinical effect noted by oral administration of this mixture of autologous proteins may involve the induction of tolerance toward bystander proteins or may be associated with the presentation of disease-associated antigens along with mucosal adjuvants [15]. The beneficial clinical effects noted in these studies were associated with the alteration of several subsets of lymphocytes.

Oral tolerance via activation of the GALT was suggested to underlie the long-term memory of suppression toward foreign environmental epitopes, including food epitopes. However, the ability to actively induce long-term memory effects in humans has not been shown. The aim of the present study was to determine the long-term effect of induction of oral tolerance toward a mixture of autologous proteins extracted from colon in patients with Crohn's disease (CD). The results of the study suggest a trend for long-term memory effects of short-term oral administration of AlequelTM.

Materials and Methods

Patient Population

The medical records of a total of 92 (31, 18 and 43 in each of the three trials) patients with CD who were enrolled in three randomized, placebo-controlled, double-blind trials [17,18] were retrospectively evaluated. The study was carried out with the approval of the Hebrew University-Hadassah Institutional Committee for Human Clinical Trials according to its guidelines.

Patients were divided into four groups

Group A: Patients treated with AlequelTM who were responders (see below); Group B: patients treated with AlequelTM who were non-responders; Group C: patients treated with placebo who were responders; Group D: patients treated with placebo who were non-responders.

Inclusion and exclusion criteria in the trials were similar [17,18]. Participants (men and women older than 18 years of age) were evaluated for eligibility after they had signed a written informed consent form. The diagnosis of CD with clinical evidence of active (symptomatic) disease was based on clinical history, blood tests and/or histology, x-ray, or endoscopy. Subjects were required to have a Crohn's disease activity index (CDAI) score between 220 and 400 as a condition for enrollment. Subjects receiving oral steroid therapy at the time of enrollment were required to be on a stable dose regimen of 10 mg or less of prednisone per day for four weeks before enrollment. Patients falling into the following categories were ineligible for entry into the study: subjects who underwent bowel surgery within three months before the commencement of the trial; those who had experienced a prior colostomy, ileostomy, or colectomy with ileorectal anastamosis; subjects whose symptoms were believed to be due to the presence of fibrotic strictures; and individuals who were likely to require emergency surgery for persistent intestinal obstruction, bowel perforation, toxic megacolon, uncontrolled bleeding, or abdominal abscess or infection. Subjects with an infectious or neoplastic disease were also ineligible. Potential subjects on a dose regimen of oral steroid therapy greater than 10 mg of prednisone per day and those who were receiving an elemental diet or parenteral nutrition were also ineligible. In addition, subjects who had been treated with methotrexate, cyclosporine, or anti-tumor necrosis factor (TNF)-α or who had participated in another clinical trial within three months before enrollment were ineligible. However, patients on 6-mercaptopurine/azathioprine were included.

Study Drug Preparation and Administration

The subjects who fulfilled the inclusion criteria for participation in the study were scheduled for a colonoscopy. During the colonoscopy, colon biopsies were removed for preparation of the colon-specific antigen-containing extract (the study drug, AlequelTM). Each subject received a regimen of three doses of autologous study drug per week for 15 weeks, for a total of 45 doses in the three trials [17,18]. The subjects were randomized by a computer-generated randomization program to receive either the study drug or the placebo. All subjects and investigators were blinded to the treatment allocation. Confidentiality of the blinding code was ensured by using an independent statistician.

Clinical and Laboratory Follow-Up

The medical records of all patients in the three trials were retrospectively reviewed. The files were reviewed to determine the time from completion of the study to clinical and/or laboratory exacerbation. Response was defined as a decrease of ≥ 100 points of the CDAI score at the end of the trial. The Flare-Free Interval (FFI) – the length of time (in months) between the end of the trial and the first flare of the disease after the trial ended – was determined for each patient. Examples of flare events included, e.g., lack of response (LOR)/ relapse;

start of treatment with a new drug or increase in the dose of current drug(s); an emergency-room visit due to symptoms of CD or admission to hospital due to exacerbation of CD; and elective or emergency surgery due to a complication of CD.

Statistical Analysis

Summary statistics were calculated, and the statistical significance of differences from baseline was assessed by Student's t-test.

Results

Study population

The medical records of 92 patients enrolled in three double-blind trials were reviewed. Figure 1 shows the data that was available for evaluation. Records of a total of 24 (26%) patients were available for the study.

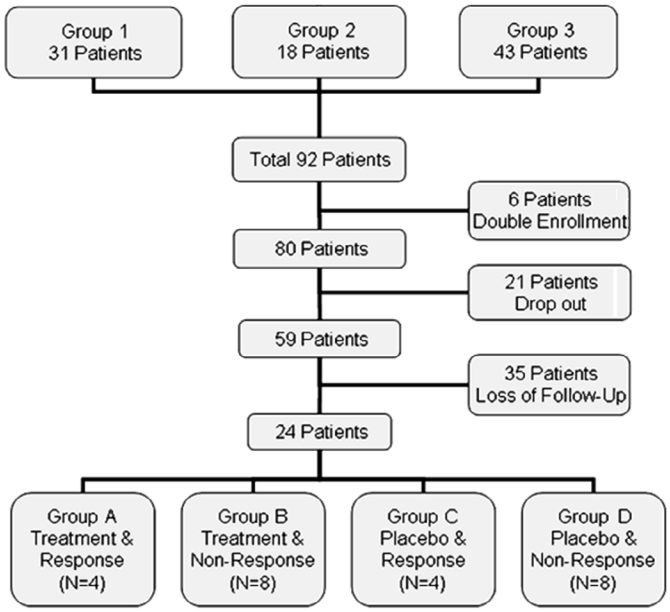

Figure 1: Patients from the three cohorts whose medical records were available for analysis in the present study.

Follow up period

Medical records of patients who were defined as responders (groups A and C) were reviewed until the time of first flare up. Records were reviewed for the time in months since completion of the study to clinical and/or laboratory exacerbation. Medical records were divided into four groups: Group A: patients treated with AlequelTM who were responders (4 out of 14 were available); Group B: patients treated with AlequelTM who were non-responders (8 out of 15 were available); Group C: patients treated with placebo who were responders (4 out of 14 were available); Group D: patients treated with placebo who were non-responders (8 out of 16 were available). Table 1 shows the characteristics of the patients available for the present analysis. (A total of 24 patients had available medical records, out of the 59 patients who completed the treatment.)

Gender	Age[1]	Intervention	Start CDAI	End CDAI	CDAI Change[2]	Group[3][4]	Flare	Flare-Free Interval[5]
M	47.5	Treatment	390	257	-133	A	Surgery (Unknown)	2.4
F	37.7	Treatment	335	228	-107	A	Relapse & Surgery (Ileectomy)	6.9
F	52.4	Treatment	308	181	-127	A	Surgery (Total Colectomy)	8.0
M	25.8	Treatment	359	164	-195	A	Surgery (Ileo-Colic Resection)	12.0
F	27.3	Treatment	239	147	-92	B	Start New Drug (Imuran)	0.9
F	34.5	Treatment	305	289	-16	B	Start New Drug (Prednison)	0.0
F	26.1	Treatment	249	208	-41	B	Relapse/LOR (Diarrhea)	0.7
F	37.1	Treatment	233	154	-79	B	Start New Drug (ABx)	8.2
F	27.3	Treatment	301	220	-81	B	Admission & Start New Drug (Steroids)	10.0
F	31.2	Treatment	290	520	230	B	ER visit	0.0
F	37.6	Treatment	382	430	48	B	Relapse/LOR	1.2
M	31.1	Treatment	230	153	-77	B	Start New Drug (Prednosi	0.2
F	58.1	Placebo	320	56	-264	C	Relapse/LOR	1.0
M	33.2	Placebo	235	31	-204	C	Surgery (Ileectomy)	0.5
M	29.2	Placebo	269	116	-153	C	Start New Drug	11.3
F	19.1	Placebo	394	242	-152	C	Start New Drug (Remicade)	0.0
M	56.4	Placebo	230	203	-27	D	Start New Drug (6-MP)	2.8
F	29.0	Placebo	236	142	-94	D	Start New Drug (Steroids)	1.9
M	18.8	Placebo	297	455	158	D	Admission & Start New Drug (Steroids, ABx)	14.0
F	24.9	Placebo	373	333	-40	D	Increase Drug (Imuran)	2.8
F	26.9	Placebo	397	354	-43	D	Surgery (Total Colectomy)	16.9
M	47.8	Placebo	278	239	-39	D	Relapse	13.9
M	20.8	Placebo	221	135	-86	D	Start New Drug (ABx)	2.8
F	22.9	Placebo	231	138	-93	D	Increase Drug (Steroids),Pregnancy	0.9

Remarks:

(1) Age at Enrollment (Years).

(2) CDAI Change – The difference between the CDAI score at the beginning of the trial to the CDAI score at the end of the trial (15 weeks of intervention).

(3) Group – A – Treatment & Response (at the end of trial)
B – Treatment & No Response (at the end of trial)
C – Placebo & Response (at the end of trial)
D – Placebo & No Response (at the end of trial)

(4) Response – Decrease of > 100 points at CDAI score at the end of the trial.

(5) Flare-Free Interval (FFI) – The length of time (in months) between the end of the trial and the first flare of the disease after the trial ended (e.g., lack of response/relapse, start of treatment with a new drug or increase in the dose of current drug(s), an ER visit or admission due to exacerbation of Crohn's disease, or elective or emergency surgery due to exacerbation or complication of Crohn's disease).

Short-term oral administration of AlequelTM was associated with a trend for long-term remission

The number of patients available for the present analysis was too small to obtain statistically significant results. Table 2 and Figure 2 show the effect of the response to short-term oral administration of AlequelTM on the time to disease flare up. For patients in group A (treated with and responding to Alequel), the disease-free interval was 7.3±3.96 months, in contrast with group C (treated with and responding to placebo), which had a flare-free interval of 3.2±5.4 months (p=0.13). Overall, the data suggests a trend toward a memory effect of short-term oral administration of autologous bowel proteins in patients with CD.

Table 2

Group		N	Flare-Free Interval (Average, Months)	SD	CDAI (Average, Week 15)	SD
A	Treatment & Response	4	7.3	3.96	208	43
C	Placebo & Response	4	3.2	5.43	111	94

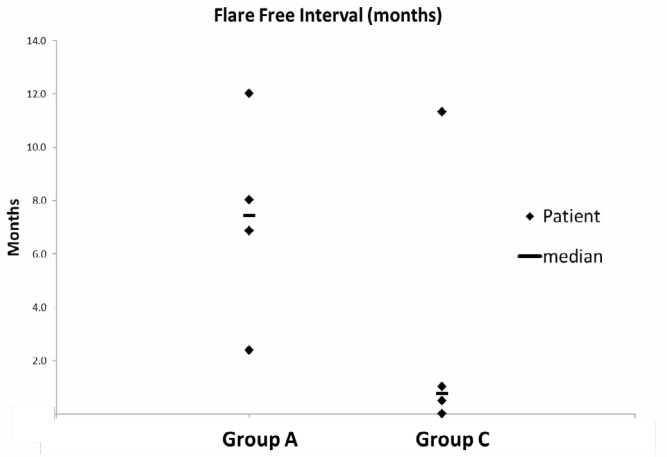

Figure 2: Flare-free interval in months in patients treated with Alequel.

Discussion

Presentation of exogenous antigens in the GALT promotes long-term tolerance, thereby enabling the body to safely interact with the environment including with food. Oral tolerance was shown to be effective in multiple animal models1, [19]. Promotion of various types of mucosal and systemic regulatory T cells mediates the effect1. Studies have suggested that oral tolerance may be effective in humans [11,20] with multiple sclerosis (MS) [21,22], myasthenia gravis [23], uveitis [24-27], thyroid disease [28], rheumatoid arthritis [29-31], Behcet's disease [32], hepatitis [6,33], and type 1 diabetes [34]. Induction of long-term tolerance toward self and non-self antigens is a major task in immunotherapy. Long-term tolerance depends on the promotion of various subsets of "memory regulatory cells". The ability to induce long-term oral tolerance has never been studied before in humans. The present study examined the long-term efficacy of short-term oral administration of AlequelTM, an autologous protein-containing extract of colon mucosal tissue. Three cohorts of patients were retrospectively studied. All patients were enrolled in double-blind placebo-controlled studies that examined the effect of oral administration of AlequelTM.. Although the number of patients on whom long-term data were available was too small to reach statistical significance, the data suggest that short-term oral administration of AlequelTM may be associated with memory induction and a trend for long-term remission.

Over-responsiveness and loss of tolerance of mucosal T-cells toward one or more unspecified antigens may underlie the chronic inflammation that is symptomatic of CD [35-37]. Induction of oral tolerance was previously shown to be successful in alleviating the clinical and laboratory parameters of the disease. The beneficial effect was associated with alteration of CD4+, CD8+, and NKT lymphocytes [17,18]. In the first cohort of patients, oral administration of a protein-containing extract of autologous colon mucosal tissue resulted in clinical remission (58% vs. 29% of evaluable subjects; 47% vs. 27% using an intent-to-treat analysis), clinical response (67% vs. 43% of evaluable subjects; 53.3% vs. 40% using an intent-to-treat analysis) and improved quality of life (IBDQ score improvement of 43% vs. 12%) in the drug study group compared to the placebo group [17]. In the second cohort, 9 patients were enrolled: 2 in group A, 3 in group B, 1 in group C and 3 in group D (at week 15). In the third cohort of patients, oral administration of Alequel™ resulted in an improved clinical remission rate (43% compared to 33%) at week 6 to week 9 in the drug-treated group compared to the placebo group. From week 8 to week 12, the clinical remission rates were 50% and 33% for the drug-treated and placebo-treated groups, respectively [18].

In the present study, the medical records of 24 of the patients were analyzed for the time to disease flare (Flare-Free Interval, FFI). A comparison was performed between those patients receiving placebo and those receiving the drug. A longer time to flare was found in patients treated with and responding to the drug than in those not responding and with those treated with and responding to placebo. These results, although they are based on a small cohort, may suggest a long-term induction of memory cells in those responding to short-term oral administration of autologous proteins.

The mechanism of oral tolerance in the gut is associated with DCs that continuously take up and transport antigens from the gut lumen to the draining lymph nodes, where they interact with antigen-specific T cells [38]. This interaction leads to unresponsiveness of effector T cells. NKT cells are a unique lineage of T cells that share properties with both NK cells and memory T cells [39]. This subset of cells has a potent immunoregulatory effect and is autoreactive to self-antigens [40-42]. NKT cells play a role in mucosal immunology and in tolerance induction [38]. NKT cell interact with myeloid antigen-presenting cells (APCs) such as monocytes, dendritic cells (DCs) and myeloid-derived suppressor cells (MDSCs) which constitutively express CD1d [42]. NKT cells can convert MDSCs into immunity-promoting

antigen-presenting cells [43].

The potential role of NKT cells in oral tolerance induction is still controversial [38,44]. NKT lymphocytes were suggested to be essential for oral tolerance induction [34,45]. Oral tolerance was associated with promotion of NKT cells in both animal models and in humans [33,40,45-48]. NKT-deficient mice have impaired tolerance induction [49]. Activated NKT cells were suggested to contribute to the promotion of tolerogenic DCs [38,50]. Mucosal NKT cells are involved in both protection and pathogenesis of the inflammatory response in the gut [40,51]. Different regulatory mechanisms may be involved with their phenotype [51]. Both intraepithelial cells and DCs express CD1d on their cell surface [40]. In mouse models of inflammatory bowel disease, a CD1d-dependent production of cytokines was shown to mediate bowel inflammation [52]. CD1d-deficient mice are protected from the development of colitis. Other studies suggested that NKT cells, under certain conditions, can be involved in protection against colitis [40,53]. Aberrant interactions between mucosal immune cells and microbiota have been implicated in the pathogenesis of IBD [38]. To maintain homeostasis, the mucosal immune system must remain tolerant to a high number of commensal microbiota [38]. Alequel™ contains a mixture of bacterial antigens that can serve as targets to GALT-mediated tolerance induction in these patients.

The long-term effect of oral tolerance induction may be determined by the type of cell(s) being promoted, and/or different interactions between different subsets of cells. Promotion of regulatory/suppressor cells with memory phenotypes may underlie the noted effects.

Tregs are promoted after self-antigen recognition during the maturation of cells in the thymus, or after self-antigen recognition in the periphery [54]. Human Tregs are functionally and phenotypically diverse [55]. Some of these subsets have memory phenotypes [55]. A subset of Tregs survive even in the absence of antigen expression54. These "memory" Tregs continue to control inflammatory reactions in the tissues independent of antigen presentation. The mechanisms of suppression mediated by these cells, and the factors that regulate their commitment, are not yet fully determined [55]. Memory T cells generate effector functions upon re-exposure to antigen56. Both continuous antigen exposure and the presence of IL-2 were suggested to be imperative for promotion of memory Tregs [54]. In addition, the memory capacity was suggested to be associated with reduced activation thresholds and with high-level expression of trafficking and adhesion molecules compared with naive T cells [56]. An interaction between memory T cells and "memory Tregs" may underlie some of the effects noted in the present study. The data from the present study does not rule out the possibility that Alequel™ may promote "memory Tregs"; however, the phenotype of such "memory Tregs" cannot be determined.

FoxP3-expressing CD4(+) Tregs control autoimmunity by supporting self-tolerance [57]. Deficiencies in either naturally occurring Tregs (nTregs) themselves and/or their ability to control pathogenic effector T cells have been associated with autoimmunity. It was suggested that nTregs can be replaced by FoxP3(+) adaptive Tregs (aTregs), which are uniquely equipped to combat autoreactivity in autoimmunity. Unlike nTregs, aTregs are stable and provide long-lived protection [57]. Memory Treg cells may be important in controlling and treating autoimmune disorders. Defects in the generation and maintenance of these cells was suggested to underlie chronic, relapsing inflammatory diseases [54]. The development and maintenance of memory Tregs is important in multiple clinical settings to prevent recurrence of immune-mediated disorders [54]. Both IL-2 and the antigen itself were explored as potential candidates for promoting memory Tregs [54]. Studies suggested that persistent antigen expression that mimics self-antigen can eliminate effector cells while maintaining Tregs. On the other hand, transient antigen exposure has the opposite effect [54,58,59]. DCs regulate both innate and adaptive immune responses and therefore can direct the immune response toward tolerance [60]. The mechanisms by which DCs promote central and peripheral tolerance include clonal deletion, induction of Tregs, and inhibition of memory T cell responses.

A diverse repertoire of memory T cells derived from heterologous immunity or from before exposure to alloantigen was previously described [56]. Memory T cells that respond to antigens in an accelerated fashion are relevant for chronic inflammatory disorders [61]. They are a major threat to any attempt to keep long-term tolerance [61]. These cells participate in transplant rejection and resist interventions that usually contain naive T cells. Thus, the means to prevent memory T cells from attacking self- and allo-antigens (in the transplant setting) are important for maintaining tolerance [61]. Effector T cells, both freshly activated T cells and memory T cells, can acquire an 'exhausted' phenotype and progressively lose their effector phenotype [61]. T-cell exhaustion may be an alternative way of preventing memory development.

Oral administration of the autologous colonic extract Alequel™ is a patient-tailored approach that may be an effective method for the treatment of patients with moderate to severe CD. The results of the present study suggest that it may induce long-term tolerance and suggest that it may be a valid method for immune modulation. The different effects noted between patients in the present study may be explained by a diversity of factors associated with "memory T cell promotion". Future trials will be required to determine the type of cells responsible for the induction of memory for the antigen-specific- or bystander antigen-mediated long-term tolerance described herein.

Grant support: This work was supported in part by the Roaman-Epstein Liver Research Foundation (to Y.I).

Disclosure: *YI is a consultant for ENZO Biochem; The studies were supported by ENZO Biochem, NYC, NY.

The first two authors contributed equally.

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