Oral administration of Alequel, a mixture of autologous colon-extracted proteins for the treatment of Crohn's disease

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Abstract: The pathogenesis of Crohn's disease involves an immune-mediated damage to the gut mucosa. Current developed therapies are based on the use of immunosuppressive drugs that can lead to significant drug-related adverse responses. There is a need for a therapeutic strategy that is more specific and less global in its effect on the immune system. Oral tolerance is an active process wherein oral administration of antigens is associated with the induction of regulatory cells and the suppression of effector cells directed toward specific and nonspecific antigens. Studies in animal models of experimental colitis suggest that oral administration of proteins extracted from the gut can induce tolerance and alleviate the disease symptoms. Recent clinical trials showed that oral administration of Alequel, an autologous protein-containing colon extract, to patients with Crohn's disease is safe and may be effective as a therapeutic modality for treating the disease. This treatment was associated with disease-associated antigen alterations of the immune response in the patients. Oral administration of Alequel could provide a patient-tailored approach that is side-effect-free for the treatment of patients with Crohn's disease.

Keywords: Crohn's disease, oral tolerance

Introduction: The need for a new way of thinking in the development of drugs for Crohn's disease

Crohn's disease (CD) is an idiopathic immune-mediated disorder that results in chronic inflammation of the gut [Baumgart and Carding, 2007a; Baumgart and Sandborn, 2007b]. The current understanding of the pathogenesis of CD is that the transmural inflammation, the primary presentation of CD, is the result of a cascade of events initiated by one or more as yet unspecified antigens. This pathogenic immune response may be elicited by one or more unidentified epitopes, or it may simply be an inappropriate response to one or more otherwise innocuous epitopes that also act on a secondary target [Sands, 2000].

Although in recent years several new therapeutic strategies for the treatment of CD have been developed [Egan and Sandborn, 2004], all currently available medications are based on the systemic suppression of certain arms of the immune system, which can lead to significant drug-related

adverse responses. Thus, there continues to be a need for a therapeutic strategy that is more specific and less global in its effect on the immune system.

The gut-associated lymphoid tissue

The gut mucosal immune system is the largest lymphoid organ in the body. At this site, there is a continuous antigenic challenge in the form of food antigens, antigens of the abundant normal bacterial flora, and pathogenic antigens [Dahan *et al.* 2007]. Antigenic contact initiated in the intestinal mucosa has an important impact on the general activity of the immune system. The surface area of the mucosa of the small intestine is estimated to be 300 m² in humans [Miller *et al.* 2007]. Compared with the skin, gut mucosal surfaces are more permeable to antigens [Johansson-Lindbom and Agace, 2007]. The microbiota in the small intestine is also an additional source of natural antigenic stimulation [Marchesi and Shanahan, 2007; Thompson-Chagoyan *et al.* 2007]. Another important factor is the large number of lymphoid cells per

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square meter of human small intestine [Chin *et al.* 2003; Makala *et al.* 2002]. The number of immunoglobulin-secreting cells located in the human gut exceeds by several fold the number found in all other lymphoid organs combined [Okamoto *et al.* 2005; Cortes, 2007]. Intestinal dendritic cells (DCs) are key regulators of pathogenic immunity, oral tolerance, and intestinal inflammation [Niess and Reinecker, 2006; Kelsall and Leon, 2005]. Studies have suggested that the largest proportion of orally administered proteins is taken up by DCs in the lamina propria [Mowat, 2005]. DCs can act as 'temporal bridges' that relay information from orally immunized memory T cells to naive T cells [Mowat, 2005]. The gut immune system is challenged to respond to pathogens while remaining relatively unresponsive to food antigens and the commensal microflora.

Induction of oral tolerance

Oral tolerance is a natural immunologic process driven by oral administration of an exogenous antigen [Ilan, 2009; Faria and Weiner, 2006a; Weiner, 2004]. In the normal state, low-level physiological inflammation of the gut is kept in check via a process of immune tolerance [Elson, 2002]. Tolerance has been defined as a lack of response to self, but a more appropriate definition of tolerance is 'any mechanism by which a potentially injurious immune response is prevented, suppressed, or shifted to a non-injurious class of immune response' [Weiner, 2004]. Thus, tolerance is related to productive self-recognition, rather than blindness of the immune system to its own components [Ilan, 2009; Weiner, 2004; Shibolet *et al.* 2004a; Safadi *et al.* 2003].

Oral exposure to antigens in the bowel results in an active immune response and is an attractive physiologic approach for immunotherapy toward antigens presented in the gut mucosa. Oral antigen administration can activate specific subsets of cells, suppressing effector cells and alleviating unwanted autoimmunity [Shibolet *et al.* 2004b; Safadi *et al.* 2003]. Multiple mechanisms of tolerance are induced by oral antigen administration. Due to their privileged access to the internal milieu, commensal bacteria and dietary antigens that continuously contact the mucosa represent a frontier between foreign and self components. Low doses favor active suppression, whereas higher doses favor clonal anergy/deletion. Oral antigen (Ag) can promote regulatory T cells (Tregs) [Mizrahi and Ilan,

2009], including Th2 (IL-4+/IL-10+), Th3 transforming growth factor beta (TGF- β), CD4+/CD25+ regulatory, and LAP+ T cells [Wu *et al.* 2009; Ishikawa *et al.* 2007; Ochi *et al.* 2006].

Oral tolerance has been shown to be effective in preventing or managing immune-mediated disorders in various animal models. Examples include experimental allergic encephalomyelitis (EAE), arthritis, diabetes mellitus and uveitis [Faria *et al.* 2006]. The first studies to show that orally administered myelin Ag could suppress EAE were performed in the Lewis rat [Javed *et al.* 1995]. High doses of myelin basic protein (MBP) were shown to suppress EAE via the mechanism of T cell clonal anergy [Javed *et al.* 1995], whereas multiple lower doses prevented EAE by transferable active cellular suppression [Miller *et al.* 1993].

Oral administration of proteins has been attempted as a therapeutic modality in several human diseases: multiple sclerosis (MS) [Miller *et al.* 1993; Weiner *et al.* 1993], myasthenia gravis [Drachman *et al.* 1996], uveitis [Thurau *et al.* 1999; 1997; Nussenblatt *et al.* 1996], thyroid disease [Lee *et al.* 1998], rheumatoid arthritis [Bagchi *et al.* 2002; Weiner and Komataga, 1998; Trentham *et al.* 1993], Behcet's disease [Stanford *et al.* 2004], and type 1 diabetes [Barker *et al.* 2007; Maron *et al.* 2001; Pozzilli *et al.* 2000; Bergerot *et al.* 1997]. The results of these studies, although demonstrating an immunomodulatory effect, in most cases did not lead to a profound suppression of disease activity [Faria *et al.* 2005].

Induction of tolerance in animal models of colitis

The efficacy of mucosal tolerance has been demonstrated in animal models of inflammatory bowel disease [Kolker *et al.* 2003; Dasgupta *et al.* 2001a,b; Ilan *et al.* 2000]. In one system, experimental colitis (EC) was induced in rodent populations treated with the chemical 2,4,6-trinitrobenzene sulfonic acid (TNBS). This autoimmune response resembles CD in human patients [Kolker *et al.* 2003; Dasgupta *et al.* 2001a,b; Ilan *et al.* 2000]. It has been shown that TNBS colitis can be prevented by oral administration of of haptenized colonic extracts, which act via the induction of TNBS-specific TGF- β responses [Neurath *et al.* 1996]. Oral administration of low doses of colon-extracted antigens to EC mice significantly

decreased the inflammatory response [Ilan *et al.* 2000; Neurath *et al.* 1996]. Oral administration of human fibroblasts or rat small intestine extracts was not effective in abrogating the inflammatory response, thus indicating that tolerance is organ specific. Oral administration of autologous colon-derived proteins from syngeneic mice proved to be more effective than oral administration of proteins derived from another animal species and even from other strains of mice [Gotsman *et al.* 2001; Shlomai *et al.* 2001]. Protected animals displayed low interferon-gamma (IFN- γ) and high TGF- β levels, and tolerance could be transferred by mesenteric lymph node cells [Dasgupta *et al.* 2001a,b].

Induction of tolerance in patients with Crohn's disease

Evidence in humans with CD points to an over-responsiveness and loss of tolerance of mucosal T cells [Himmel *et al.* 2008]. Epidemiologic data reveal the importance of priming of the immune regulatory system at an early age in preventing CD. A population study in young adults demonstrated a strong reverse correlation between surrogate markers of childhood hygiene and the risk of CD [Klement *et al.* 2008]. In multivariate analysis, variables significantly associated with CD were living in an urban environment, a small number of siblings in the family and higher birth order. The 'hygiene theory' for CD (termed the 'old friends' hypothesis) postulates that increased exposure in childhood to relatively harmless enteric microorganisms (including helminths, saprophytic mycobacteria and lactobacilli) may cause priming of immunoregulation. These nonpathogenic microorganisms have the ability to induce maturation of DCs such that they retain the ability to drive the T-reg system and thus afford protection against CD [Rook and Brunet, 2005].

Induction of tolerance towards keyhole limpet hemocyanin (KLH) was evaluated in patients with ulcerative colitis or CD in comparison with normal individuals. Prior oral administration of KLH decreased the magnitude of T cell proliferative and skin test responses to KLH in normal individuals who were immunized with KLH. In individuals with CD or ulcerative colitis, prior oral administration of KLH led to an augmentation of the T cell proliferative response. Although that study suggested an inability to induce oral tolerance in the patients, no *in vivo* parameters were measured and the interval between

administration of the tolerizing agent and the challenge by KLH was only 2 weeks. In fact, the results of that study support the concept that oral administration of antigens can alter the systemic immune balance in patients with CD. The alteration in patients with CD may not necessarily occur in the same direction as in normal subjects [Margalit *et al.* 2006].

Oral administration of Alequel, an autologous protein-containing colon extract, in Crohn's disease

Based on the findings in animal models of colitis, colon-derived proteins manufactured from autologous mucosal extract would be the most appropriate source of study drug in the management of CD by oral immune regulation. In a recently conducted phase I clinical trial, we examined the safety and efficacy of oral administration of subject-derived intestinal antigens for the treatment of moderate to severe CD in ten subjects [Israeli *et al.* 2005]. The study drug was individually prepared from mucosal tissue taken from each of ten subjects by colon biopsy. In this study, biopsies were obtained preferentially from inflamed areas in the colon or the terminal ileum. In some of the patients, however, biopsies were obtained from mucosa exhibiting a normal endoscopic appearance.

The protein extract was divided into 50 doses in 5 ml of normal saline and stored at -70°C . All subjects were given the antigen extracted from their own intestinal mucosa. A 2 week supply was given to each subject, who at each visit was instructed to maintain the vials in a refrigerator at home. Each subject received an oral regimen of three doses per week of the study drug for 16 weeks, for a total of 48 doses.

At the conclusion of this phase I trial, oral administration of the autologous study drug (Alequel) was found to be safe within the limits of the number of subjects tested. Furthermore, during the course of the trial, remission, defined as a decrease in the Crohn's Disease Activity Index (CDAI) score to equal to or less than 150, was achieved in seven of ten subjects. A significant increase in the mean inflammatory bowel disease questionnaire (IBDQ) score was noted at week 16 as compared with baseline (134 ± 9 versus 164 ± 12 , $p < 0.05$) [Israeli *et al.* 2005].

We then conducted a randomized, double-blind placebo-controlled phase II study to further

evaluate the safety and efficacy of oral administration of Alequel in subjects with CD. [Margalit *et al.* 2006]. We enrolled 31 patients with moderate to severe CD in a 27-week trial. Patients were randomized to receive either a placebo or the study drug.

Enrolled CD patients displayed clinical evidence of active (symptomatic) disease based on clinical history, blood tests, and/or histology, X-ray or endoscopy. Subjects were required to have a CDAI score between 220 and 400. Subjects who were receiving oral steroid therapy at the time of enrolment were required to be on a dose regimen of ≤ 25 mg prednisone per day. Patients who were on a regimen of immunosuppressive drugs, such as azathioprine/6-mercaptopurine (6-MP), methotrexate, cyclosporine, or anti-tumor necrosis factor-alpha (anti-TNF α), were excluded from the study. Each subject followed a regimen of three doses of autologous study drug per week for 15 weeks, for a total of 45 doses [Margalit *et al.* 2006].

Clinical remission, defined as a decrease to a CDAI of 150 or lower in two consecutive visits during the study period, was used as the primary measure of efficacy. Of the 12 evaluable subjects who received the study drug, seven (58%) achieved a CDAI of 150 or lower, while four of the 14 (29%) evaluable subjects in the placebo group achieved remission. Using an intention to treat analysis, seven of the 15 (46.6%) subjects who received the study drug and four of the 15 (26.6%) subjects in the placebo group achieved remission. The IBDQ score improved by an average of 43% in subjects who received the study drug [Margalit *et al.* 2006].

Treatment was well tolerated by all patients. No major treatment-related adverse events were reported or observed in any of the treated patients during the feeding and follow-up periods. No major changes in any of the extraintestinal systems monitored were reported in any of the patients.

Effect of oral administration of Alequel on the immune system

The data from the above trials support the concept that there was a distinct difference in the immune profile at the beginning of the study between subjects who were and were not able to respond to the study drug; this may have important implications in the ability to predict a beneficial response to the study drug. Several immunological parameters

were monitored during the course of the trials [Margalit *et al.* 2006]. A novel difference was found within the drug-treated group between those subjects who achieved remission (DR) and those who did not (DNOR).

Administration of Alequel induced a significant increase in the CD4+/CD8+ lymphocyte ratio in the peripheral blood in 7/10 subjects [Israeli *et al.* 2005]. Differences in the percentage of peripheral CD4+ cells, the percentage of peripheral CD8+ cells, and the CD4+/CD8+ cell ratio were observed between the DR and DNOR groups at initiation of treatment.

IFN- γ spot-forming cells (SFCs) were determined using a colitis-extracted protein (CEP)-specific ELISPOT assay. In five patients, positive T cell clones were detected prior to oral protein administration. In all five, a significant decrease in the number of IFN-positive SFC was noted (from 0.2–3.6 to 0–0.2 SFCs). A significant increase in IL-4 and IL-10 serum levels was observed in seven out of ten subjects during the treatment period. No significant changes in serum IFN- γ levels were observed [Israeli *et al.* 2005]. The results of the ELISPOT assay for SFCs, which indicate IFN- γ -producing colonies, showed that before the initiation of treatment there were more such colonies in the DR group than in the DNOR group. The number of SFCs decreased throughout the treatment period in the DR group, but increased in the DNOR group as well as in the placebo group [Margalit *et al.* 2006].

Natural killer T (NKT) cells are a unique lineage of T cells that share properties with both NK cells and memory T cells [Bendelac *et al.* 2007]. Their ability to generate both Th1 and Th2 responses indicates their importance as immunoregulatory cells [Zigmond *et al.* 2007; Godfrey and Kronenberg, 2004]. NKT cells play a role in the immune regulation of colitis [Singh *et al.* 2008; El Haj *et al.* 2007; Van Dieren *et al.* 2007] and have been suggested to be essential for oral tolerance induction [El Haj *et al.* 2007]. Oral tolerance is associated with the promotion of NKT cells in both animal models and in humans [Zigmond *et al.* 2007; Shibolet *et al.* 2004a,b; Gotsman *et al.* 2001; Shlomai *et al.* 2001; Ilan *et al.* 2000; Trop *et al.* 1999]. In a model of experimental colitis, induction of oral tolerance was associated with an increased number and altered function of NKT cells [Ilan, 2002; Shibolet *et al.* 2002; Trop and Ilan, 2002; Trop *et al.* 1999].

In a murine model of hepatocellular carcinoma (HCC), NKT cells were shown to play a role in oral immune regulation using HCC lysates and hepatitis B virus (HBV) envelope proteins, as well as a role in the adoptive transfer of DCs pulsed *ex vivo* with these antigens [Margalit *et al.* 2005; Shibolet *et al.* 2003].

The data from the clinical trials suggest that before the initiation of treatment the proportion of peripheral T cells that were NKT cells was significantly lower in the DR group (2.3%) than in the DNOR group (14.8%; $p = 0.0051$). The proportion of peripheral NKT cells in the DR group increased to 10.5% between weeks 0 and 9. The DNOR group demonstrated a significant decrease in the percentage of NKT cells, from 14.8% at the initiation of treatment to 4.3% after 15 weeks of treatment ($p = 0.04$). The peripheral NKT cell number increased significantly in five out of ten subjects.

The disease-associated antigen in Crohn's disease: bystander suppression

There has been much debate regarding the nature of the orally administered antigen and its effect on the efficacy of oral tolerance. Bystander suppression is the concept that regulatory cells induced by a fed Ag can suppress immune responses stimulated by a different Ag, as long as the fed Ag is present in the anatomic vicinity [Faria *et al.* 2005; Gotsman *et al.* 2001; Shlomai *et al.* 2001]. As regulatory cells induced by oral Ag secrete nonspecific cytokines after being triggered by the fed Ag, they suppress inflammation in the microenvironment in which the fed Ag is localized. During the course of chronic inflammatory autoimmune processes in animals, there is intra- and interantigenic spread of autoreactivity at the target organ [Tisch *et al.* 1993; Lehmann *et al.* 1992].

While the bystander effect is a fundamental mechanism in oral tolerance, its relevance to humans is less clear. In humans with autoimmune diseases, such as MS and type I diabetes, reactivities to multiple autoantigens occur in the target tissue [Zhang *et al.* 1994; Zhang and Raus, 1994]. In humans, the lack of success in clinical trials investigating oral tolerance in patients with MS or rheumatoid arthritis was partially attributed to the lack of specificity of the fed antigens [Weiner, 1997; Weiner *et al.* 1993; Trentham, 1998]. We used HBV- and hepatitis C virus-specific viral proteins to promote effective antiviral

immunity in humans. In contrast, nonspecific but autologous proteins were used for the induction of patient-tailored oral tolerance in humans with CD.

Improving the response rate to oral tolerance in humans

Various factors have been proposed as explanations for the variable response observed in humans as compared to animals. These include mode of administration, dose, frequency of administration, antigen specificity, immunogenicity of the ingested antigen, use of adjuvants, the immunogenetic background of the patient, and inter-individual variability [Wu and Weiner, 2003]. Additionally, it will be important to target the right patient population, wherein early therapy is an important factor since oral tolerance is most effective before or shortly after disease onset [Faria and Weiner, 2005]. Successful application of oral tolerance for the treatment of human diseases also depends on the strategies used to target the correct cells in the gut–liver axis and to improve antigen presentation. The development of immune biomarkers to assess immunologic effects may also assist in the development of this type of immunotherapy [Ilan, 2009; Faria and Weiner, 2005].

Conclusion

Recent progress in mucosal immunology provides new insights for the potential use of oral tolerance in clinical practice as a means for induction of regulatory T cells, which may play a role in the suppression of inflammation [Mizrahi and Ilan, 2009; Faria and Weiner, 2006b; Zhang *et al.* 2001]. The phase I and II clinical trials suggest that oral administration of Alequel is safe and may be an effective treatment for patients with CD [Margalit *et al.* 2006; Israeli *et al.* 2005]. The beneficial clinical effect of feeding this mixture of autologous proteins may involve tolerance induction towards bystander proteins, or it may be associated with presentation of the relevant antigens along with some mucosal adjuvants. This method of antigen-specific therapy is nontoxic and can be administered on a chronic basis without any deleterious effects on the general immune system [Ilan, 2009; Faria and Weiner, 2005]. Future large controlled trials are required for further evaluation of this mode of therapy and for assessment of valid biomarkers that can determine potential responders to the therapy and follow-up of response.

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Conflict of interest statement

Y. Ilan is a consultant for ENZO Biochem. Studies were supported in part by ENZO Biochem NYC NY.

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