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Azathioprine in Refractory Sprue: Results From a Prospective, Open-Label Study

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OBJECTIVE: Refractory sprue is a rare and severe malabsorptive disorder that mimics celiac disease but is refractory to a gluten-free diet and is without initial evidence of overt lymphoma. Treatment is largely empiric and often ineffective, with steroids and immunosuppression being the mainstream therapeutic options. The aim of this study was to evaluate prospectively the effect of azathioprine on a group of patients diagnosed with refractory sprue.

METHODS: We studied seven consecutive patients (five women and two men) with a well-defined diagnosis of refractory sprue and a lack of response to oral or parenteral steroids. At diagnosis, five patients had endoscopic evidence of ulcerative jejunitis, and five underwent exploratory laparotomy for exclusion of malignancies. The characteristic monoclonal TCRγ gene rearrangement was shown in five of six patients studied. Patients were treated for a mean of 11 months (range 8–12 months), and clinical, biochemical, molecular, and histological parameters were reassessed at the end of the trial. The study was a prospective, open-label, non–placebo-controlled study using azathioprine (2 mg/kg/day) plus oral prednisone (1 mg/kg/day). A gluten-free diet (n = 7) as well as enteral (n = 6) and parenteral nutrition (n = 5) were administered during the trial.

RESULTS: After treatment, five patients had a complete clinical remission, and biochemical and nutritional parameters were significantly improved. Steroids were tapered after the onset of azathioprine, and no patient was on steroids at the end of the trial. Intestinal histology improved significantly in all cases (normal histology in three cases and minor infiltration in the lamina propria in two). Two patients did not respond to treatment at any time and died in months 10 and 9, of an irreversible ventricular fibrillation and sepsis, respectively. No overt lymphoma was demonstrated during the follow-up.

CONCLUSIONS: The present study confirms earlier anecdotal reports on the efficacy of azathioprine in refractory sprue, with clear clinical and histological improvement shown in most patients. However, monoclonality persisted after treatment. We consider that a larger number of patients should be evaluated before a definitive recommendation is adopted for use of this drug in refractory sprue. (Am J Gastroenterol 2002;97:2595–2602. © 2002 by Am. Coll. of Gastroenterology)

INTRODUCTION

Celiac disease is a T-cell–mediated disorder produced by a permanent intolerance to gluten in genetically susceptible individuals (1). Refractory, or unclassified, sprue is a rare heterogeneous malabsorption syndrome of a still obscure etiology in which patients have symptoms and an enteropathy similar to or indistinguishable from those of celiac disease (2–12). Furthermore, both celiac disease and refractory sprue may present similar complications such as ulcers, stenosis, or intestinal lymphoma (3, 6, 9, 13–17). However, patients with refractory sprue do not respond to treatment with a gluten-free diet. Although most patients show a refractory course that can be established from the time of diagnosis, others may show suboptimal response for very long periods, becoming refractory with no overt causes. Finally, few cases can initially respond to a gluten-free diet and then evolve toward a refractory status (8, 9, 12). Recently, Biaggi and Corazza (18) defined refractory enteropathy on the basis of a review in the literature and proposed a categorization in three groups: 1) refractory celiac disease, 2) nonceliac refractory sprue, and 3) undefined sprue.

Very recent studies have shown that a subgroup of patients with refractory sprue present with mucosal infiltration by a clonal subset of intestinal intraepithelial lymphocytes with an abnormal phenotype (intracytoplasmic CD3ε+, CD8-, CD4-) and a monoclonal TCR gene rearrangement (9, 19–21). From a clinical point of view, patients with refractory sprue exhibit a progressive clinical deterioration and represent a difficult therapeutical problem, with very variable response and a potential fatal course (4, 8–9). Current therapeutic options include the combination of di-

Table 1. Epidemiological, Clinical, and Biochemical Data of Patients at Time of Inclusion in Trial

Age (yr), Sex	Age at CD Diagnosis (yr)	Body Weight (kg)	BMI (kg/m^2)	Hemoglobin (g/dl)	Albumin (g/dl)	Fecal α_1 ATC1 (ml/day)	IgA AGA (UA/ml)	IgG AGA (UA/ml)	IgA EmA	tTG (UA/ml)	Nutritional Support
29 F	29	39.6	15.6	9.5	1.5	170	4	38	+	98	EN TPN
46 F	46	36.1	15.4	9.0	3.3	80	7	33	−	3	EN TPN
44 M	44	50.1	19.3	9.5	0.9	460	7	48	−	7	EN TPN
58 F	56	45.0	18.9	9.3	1.0	71	2	17	−	3	EN TPN
38 F	38	35.1	13.9	12.0	2.9	97	22	54	−	2	EN
29 M	28	52.2	17.2	9.8	2.4	132	30	35	+	36	EN TPN
71 F	50	51.2	11.6	12.4	3.0	30	147	105	−	15	−

AGA = antigliadin antibodies; BMI = body mass index; CD = celiac disease; EN = enteral nutrition; EmA = endomysial antibodies; Fecal α_1ATC1 = fecal α_1 antitrypsin clearance; F = female; M = male; TPN = total parenteral nutrition.

etary manipulations (gluten and other protein restrictions), nutritional support (enteral and parenteral nutrition), oral and/or parenteral glucocorticoids, and immunosuppressive therapy (8, 12, 22). All of these options have shown a wide range of efficacy, with improvement in some cases, and progressive deterioration and death, with or without evidence of malignancy, in others.

Among immune-modifying agents, cyclosporine, cyclophosphamide, methotrexate, and azathioprine have been reported to be useful in some patients but not in others (23–27). The collective therapeutic experience has been variable and largely based on anecdotal reports; therefore, no definitive conclusions have emerged. In this context, azathioprine has been reported to induce and to sustain remission, when very high doses of prednisone either are required (steroid-sparing effect) or have failed to improve refractory sprue. In contrast, a retrospective study by Cellier *et al.* (9) showed that azathioprine did not improve most steroid-refractory cases collected from French gastroenterology referral centers. Therefore, it seems necessary to have prospective studies addressing the value of this therapy. Our aim in this study was to evaluate prospectively the efficacy of azathioprine in a homogeneous group of patients diagnosed with refractory sprue in which the presence of lymphoma was excluded.

MATERIALS AND METHODS

Between October 1998 and July 2000, seven patients diagnosed with refractory sprue (five women and 2 men; median age 41 yr, range 29–71 yr) were included in a prospective trial on an inpatient (n = 5) and outpatient (n = 2) basis. Refractory sprue was defined based on the presence of a severe enteropathy refractory to conventional therapeutic measures, after excluding other causes of villous atrophy and the presence of overt intestinal lymphoma at the time of diagnosis (18). All patients had severe malabsorption and required intensive treatment as inpatients before being included in the protocol. None of them successfully responded to a strictly monitored gluten-free diet, and required oral and/or parenteral steroids and dietary support because of the profound nutritional compromise. Despite all these measurements, a lack of response and a progressive deterioration was evident in all patients. At the onset of azathioprine use, all patients had been considered refractory to the use of steroids. Exclusion of intestinal lymphoma or other malignancies was established based on evidence provided by small bowel double-contrast radiological examinations (n = 7), push enteroscopy and multiple biopsies (n = 4), CT scans (n = 7), and laparotomy (n = 6).

Clinical and biochemical data for each patient at baseline are shown in Table 1. Patients had had symptoms for a median of 48 months (range 3–54 months) before diagnosis. The most relevant symptoms at diagnosis were chronic diarrhea (n = 6), fat malabsorption (n = 6), weight loss (n = 7), abdominal pain (n = 6) of suboclussive type (n = 3), and fever (n = 4). IgA and/or IgG antigliadin antibodies were positive in six patients (only IgG positive in three cases, and both types in other three patients), and one patient was seronegative for both antibody types. Only two patients were seropositive for the endomysial antibody test, and both were the sole positive cases for anti–tissue transglutaminase antibodies. Two other patients had IgA anti–smooth muscle antibody. Antienterocyte autoantibodies tests were negative in the seven patients.

HLA class II (DQB1) typing was done following protocols from a commercial DQB1* "high resolution" polymerase chain reaction (PCR)–sequence specific primers typing kit (Fastype System, Bio Synthesis) (28). All patients had a DQB1 allele of either the DQ2 or DQ8 molecules primarily associated with celiac disease susceptibility (Table 2).

The small bowel radiological examination showed multiple ulcers in five patients (ulcerative jejunitis), a jejunal stenosis in one (patient 5), and a nonspecific pattern in one. On the other hand, duodenoscopy and push enteroscopy confirmed ulcers in those patients whose suspected diagnoses were based on radiology, and surgical macroscopic examination detected ulcers in the patient with jejunal strictures. Histological analysis of intestinal biopsy specimens showed mucosal atrophy in all cases (total atrophy [Marsh IIIa] in five patients and subtotal atrophy [Marsh IIIb] in two). Macroscopic evaluation of surgical specimens determined the presence of jejunal ulcers in three of five patients (one of whom also had a fibrous stricture in the jejunum).

Table 2. HLA Typification (DQβ1) and Immunophenotypic and Genotypic Analyses of Mononuclear Infiltrating Cells in Biopsy Samples, and Histological Characteristics of Small Intestinal Mucosa (According to Marsh's Criteria) Determined at Time of Diagnosis

		Phenotypical Analysis													
		Epithelium						Lamina Propria							
Patients	HLA DQ β1*	CD3	CD4	CD8	CD20	CD56	CD45	CD3	CD4	CD8	CD20	CD56	CD45	TCRγ-PCR Analysis	Histological Classification (Marsh)
	B1201														
1	B1201	+++	−	+	−	−	+++	+	++	+	++	−	+++	Monoclonal	IIIc
	B1604														
2	B1201	+++	−	+	−	−	+++	+++	+	++	++	−	+++	Monoclonal	IIIc
	B1201														
3	B1201	ND	−	+	−	+++	+++	++	+	++	++	+++	+++	Policlonal	IIIb
	B1201														
4	B1201						ND							Monoclonal	IIIb
	B1303														
5	B1302						ND							Monoclonal	IIIc
	B1201														
6	B1201	+++	−	+	−	−	++	+++	+	++	++	−	+++	Monoclonal	IIIc

Patient 7 had a policlonal infiltration and a histological classification type III. HLA typification and phenotypical analysis were not performed. ND = not done. +++ = 60–100% of intraepithelial or mononuclear cells are positive for the marker. ++ = 30–60% of cells are positive for the marker. + = 5–30% cells are positive. − = no positive cells were detected. Histological classification according to Marsh in type 0, I, II, IIIb, and IIIc (1).

One patient (patient 2) had unexpected mesenteric lymph node cavitations.

Protocol Design

The study was a prospective, 1-yr, non–placebo-controlled, open-label trial to determine the efficacy of azathioprine on patients with well-established refractory sprue. Patients were included in this study if they had a previous diagnosis of refractory sprue, or if they were diagnosed with celiac-like enteropathy and had a proven lack of clinical and/or histological response to a gluten-free diet and steroids, and/or if they had required high doses of steroids to maintain the clinical status. The clinical response was monitored by the presence of diarrhea and malabsorption, biochemical parameters (Hb, total serum proteins, serum albumin and fecal α_1 antitrypsin clearance), requirements of nutritional support, and nutritional status. Patients were excluded if they presented with recent or current infections, high suspicion or diagnosis of lymphoma, pregnancy, or low white blood cell count ($<$3,000 cells/mm^3).

At entry the trial, patients received a standardized protocol consisting of a gluten-free diet and oral or parenteral steroids (oral prednisone, 1 mg/kg body weight/day [n = 3] or *i.v.* hydrocortisone, 4.5 mg/kg body weight/day [n = 4] for a median of 24 days, followed by oral prednisone at equivalent doses). Patients were monitored weekly by clinical, nutritional, and laboratory parameters. Based on a demonstrated lack of response on these therapeutic measures after 2 months, patients started on azathioprine (2 mg/kg of body weight/day *p.o.*, administered *b.i.d*). Patients were clinically evaluated each week during the first 2 months and then each month until the end of the trial. Laboratory tests were performed at study entry and every 3 months after starting azathioprine. Blood cell count were determined weekly during month 1 and then every 3 months afterward. In the six patients on steroids, the drug was tapered after 2 months of azathioprine administration, based either on the theoretical onset of effect of the study drug or when we detected clinical and laboratory response to the treatment. In patient 7, steroids were tapered as soon as possible because of the clear side effects (osteoporotic fractures) demonstrated. At the end of the trial (after 1 yr on azathioprine and 14 months after the onset of steroid use), a complete clinical, laboratory, radiological (including small bowel double-contrast study, ultrasound, and CT scan), endoscopic, and histological assessment was performed (Table 2). We established that a lack of improvement of the parameters referred, worsening of the clinical condition, or requirement of withdrawal because of adverse effects were criteria for treatment failure.

Specialized Determinations and Procedures

Determinations of antigliadin (IgA and IgG subtypes) and anti–tissue transglutaminase antibodies were performed by an ELISA method using commercial kits (INOVA Diagnostics, San Diego, CA) as previously reported (29). Endomysial antibodies were determined by indirect immunofluorescence on monkey esophagus substrate (INOVA Diagnostics) (29). Antienterocyte antibodies were determined by a homemade indirect immunofluorescence method using human small intestinal substrate. Fecal α_1-antitrypsin clearance was determined using radial immunodiffusion as previously reported (30).

Duodenoscopy and push enteroscopies were performed using commercial videoendoscopes (Pentax, Tokyo, Japan), and several small intestinal biopsy samples (n $>$6) were obtained with endoscopic forceps during the same procedures. Samples were either fixed in 10% formalin for conventional histological and immunophenotypic assessment or snap-frozen in liquid nitrogen for molecular studies. Formalin-fixed, paraffin-embedded blocks of duodenal or jejunal biopsies and resection specimens were retrieved from

the Pathology Service files. In two cases, fresh frozen tissue was available for the study. Tissue sections (3 μm) were cut on positive charge–coated slides (BioGenex, San Ramón, CA) and studied by routine morphological and immunophenotypical analyses. The antibodies used and their specificity were as follows: CD3-12 (CD3ε) and CD4-1F6 (CD4) (Novocastra Laboratories, Newcastle, UK); C8/144B (CD8), T199 (CD56 and UCHL1 (CD45Ro) (Dako, Carpinteria, CA); and L26 (CD20) (BioGenex, San Ramón, CA). Alterations of the small intestinal mucosal morphology were graded as type 0 (normal), type I (intraepithelial lymphocytosis), type II (lymphocytosis and moderate crypt hyperplasia), or type III (IIIb subtotal atrophy, IIIc total villous atrophy) on the basis of the Marsh's classification (1). Intraepithelial lymphocyte (IEL) count was expressed as percentage of lymphocytes per 100 epithelial cells.

DNA was extracted from paraffin-embedded or frozen samples by proteinase K digestion and phenol-chloroform purification, precipitated with ethanol, and resuspended in 10 mmol/L of Tris and 1 mmol/L of ethylenediaminetetraacetate. All samples gave a detectable product of 274 pb from the DRβ gene after amplification with primers PSP-49 and Amp-A (31). We amplified rearranged TCR genes by PCR as a useful strategy to establish clonality in biopsy material (31). Amplification reactions were performed with 250 ng of DNA in a 50-μl reaction that contained 16.6 mmol/L of ammonium sulfate, 67 nmol/L of Tris-HCl, pH 8.8, 10 mmol/L of β-mercaptoethanol, 2.0 mmol/L of $MgCl_2$, 200 μg/ml of gelatin, 200 μmol/L of each deoxyneucleotide triphosphate, 25 pmol of each primer, and 1 U Taq polimerase. Temperature conditions were 90 s at 94°C, and 120 s at 72°C for mixes I and III for 45 cycles. Staining was performed with ethidum bromide and was visualized under ultraviolet light. Pooled primers in mixes I and III and the respective sequences were as previously published (32). Genomic DNA was also extracted from whole blood and amplified with the allele specific primer sets and an internal control primer set specific for human G3PDH gene using a PT100 thermal cycler (MJ Research, Watertown, MA), followed by the high resolution PCR-SSP typing procedure described above.

Study Approval

The study was approved by the Research Council of the "Dr. C. Bonorino Udaondo" Gastroenterology Hospital. Patients gave an expressed consent to be included in this trial and were clearly informed about the experimental nature of the trial, as well as eventual complications of the use of azathioprine and steroids.

Statistical Analysis

Data were processed using the software Statistica for Windows 5.1 (Stat-Soft Co., Tulsa, OK). Results are expressed as median values and ranges. Baseline parameters were considered to be those determined at the time of starting on azathioprine. Baseline and final data from a per-protocol analysis were compared using Wilcoxon's matched pair test or *t* test as appropriate.

RESULTS

Baseline Characteristics

Five patients were included in the trial as inpatients because of their severe clinical condition and the impossibility of maintaining their nutritional status as outpatients. At the time of inclusion in the trial, laboratory tests of the seven patients recruited showed low Hb levels in six (9.5 g/dl, range 9.0–12.4), low serum albumin in all seven (2.4 g/dl, range 0.9–4.1), and increased fecal α_1 antitrypsin clearance (97.2 ml/day, range 30.5–460.0). Their body weight averaged 46.0 kg (range 30.5–53.7) and their body mass index 17.2 kg/m^2 (range 11.9–21.2). Baseline data showed that six patients had hypoalbuminemia (in one case $<$1.0 g/dl, in two others 1.1–2.0, and in three 2.1–3.0), whereas one patient was normal. According to Marsh's classification, baseline assessment of intestinal mucosa morphology showed that five patients had type IIIc enteropathy and two patients a type IIIb lesion. Most patients had intraepithelial lymphocytosis (median 38% IEL, range 12–55%).

Immunohistochemical analysis (n = 4) of samples obtained at diagnosis showed that the majority of the IEL (70–100%) were positively stained with an anti-CD3ε; however, only 5–30% of them were CD8+, whereas in the lamina propria, 30–60% of the mononuclear cells were CD8+ (Table 3). This immunophenotype allowed us to make the distinction between refractory sprue and pure celiac disease. In four celiac patients, we showed that 60–100% of IEL were CD3+ and CD8+, and that 60–100% of lamina propria mononuclear cells were CD8+ (data not shown) (Table 2). A monoclonal TCRγ gene rearrangement was seen in five of the six patients tested. In some of them, both TCRγ alleles were rearranged, representing a clonospecific molecular marker (Fig. 1). This genotypic feature was clearly different from that observed in a group of classical celiac disease patients (n = 6) and healthy subjects (n = 7), in which a polyclonal population was evident (33).

Outcome

Overall, five patients completed the 1-yr trial. All of these patients experienced improvement of their clinical condition. Diarrhea, abdominal pain, and fever (the major presenting symptoms) subsided. Table 3 shows the comparison of baseline and final parameters. Body weight and body mass index improved in all of them, and posttreatment values were significantly higher than at baseline ($p < 0.04$ for both parameters). Concomitantly, Hb level ($p < 0.05$), serum albumin ($p < 0.03$), and fecal α_1-antitrypsin clearance ($p < 0.05$) improved significantly. Figure 1A–D shows the outcome of some biochemical parameters at diagnosis, baseline, and follow-up. Histological assessment of intestinal biopsies at the end of the trial demonstrated complete mucosal normalization (type 0) in three patients and type II

Table 3. Baseline (at Time of Starting on Azathioprine) and Final (After 1 Yr of Treatment) Clinical, Biochemical, and Histological Data of Refractory Sprue Patients Who Completed the 1-Yr Trial

Parameter	Baseline (n = 5)	Final (n = 5)	
Diarrhea (n)	4/5	0/5	
Abdominal pain (n)	5/5	0/5	
Fever (n)	2/5	0/5	
Body weight (kg)			
Median	46	60	
Range	42–54	49–77	$p < 0.03$
BMI (kg/m^2)			
Median	17	26	
Range	12–21	19–30	$p < 0.05$
Hemoglobin (g/dl)			
Median	10	13	
Range	8–12	12–14	$p < 0.01$
Serum albumin (g/dl)			
Median	2	4	
Range	1–3	3–4	$p < 0.002$
Fecal α_1 AT Cl (ml/day)			
Median	190	21	
Range	91–247	2–83	$p < 0.01$
EmA positive (n)	2/5	0/5	
Anti-tTG antibodies negative (n)	2/5	0/5	
Histological classification (Marsh)	Marsh IIIc, 3/5 Marsh IIIb, 2/5	Marsh II, 2/5 Marsh 0, 3/5	
IELs (%)			
Median	48	12	
Range	12–55	7–16	
Monoclonal TCR γ (n)	4/5	4/5	

IELs = proportion of intraepithelial lymphocytes per 100 epithelial cells.

histology (mild lamina propria infiltration) in the remaining two. Intraepithelial lymphocyte count was normal in all five patients.

Two of the seven patients originally included did not finish the protocol and died during the follow-up period. At month 4 on azathioprine, patient 7 developed pneumonia. She was successfully treated with antibiotics at the same time as immunosuppressive treatment was continued. She eventually died of ventricular fibrillation in month 10 on azathioprine. Her clinical outcome and biochemical parameters were considered to be stable, but a functional deterioration was evident. In month 7 on azathioprine, patient 2 was withdrawn from the trial because of the detection of leukopenia and of maxillary and ethmoidal sinus infection by *Staphyloccocus aureus*. At that time, the evaluation did not show any improvement of clinical and biochemical parameters. Discontinuation of azathioprine and specific treatment for the infection was recommended by the study personnel. She died of sepsis 2 months later. Both cases were considered to be treatment failures. In addition, patient 3 had sepsis secondary to a small intestinal perforation after laparotomy. Afterward, he was started on steroids and azathioprine according to the present protocol.

Adverse Effects

During the trial, two patients experienced adverse effects as a consequence of the study drug. Patient 2 developed leukopenia and an opportunistic infection that was the cause of death. As previously reported, patient 7 had pneumonia during the trial, which was controlled without the necessity of an anticipated withdrawal. No other minor or major adverse events were reported by patients or detected by the physicians in charge.

Outcome After the End of the Trial

Of the five patients who completed the trial, one patient (patient 4) died of a superior mesenteric artery infarction 3 months after completion of the 1-yr trial. At that time, the clinical, nutritional, and histological assessment had demonstrated complete remission, and the patient continued on a gluten-free diet only. In addition, radiological and CT final examinations showed no abnormalities. The remaining four patients continue to be in good health on a gluten-free diet only. The last assessment was a mean of 11 months (range 4–16 months) after the end of the trial. To date, no evidence of lymphoma has been seen, either in responder or nonresponder patients.

DISCUSSION

Refractory sprue is a very interesting, heterogeneous, and rare syndrome that presents several points of controversy. One of the most relevant aspects is whether it represents an evolutionary state of a preexisting celiac disease, or whether it is a celiac-like, non–gluten dependent condition produced by some still unknown cause (10). In general, the prognosis of these patients is severe, with a progressive clinical and nutritional deterioration and, in most cases, even if a suc-

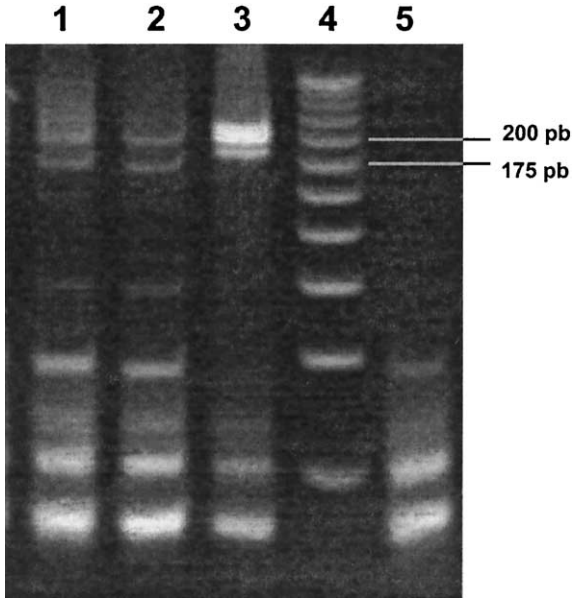

Figure 1. Polyacrylamide gel of TCRγ PCR amplification products from duodenal biopsy specimens from patient 2, obtained before (lane 1) and after (lane 2) azathioprine treatment, showing two discrete bands representing the rearrangement of both TCRγ alleles. DNA from bone marrow aspirate of a T-cell acute lymphoblastic leukemia is a positive control of T-cell clonality (lane 3). Molecular weight DNA markers, 25 bp DNA step-ladder (lane 4). Negative control without DNA (lane 5).

cessful response to treatment is achieved, deterioration can culminate in death (7–10). Therefore, identification of patients with refractory sprue may allow one to start an intensive treatment, with a potential life-saving objective. Treatment strategies comprise general and nutritional support measures (enteral and/or parenteral nutrition) and a pharmacological approach. Oral and parenteral steroids are used as first-line treatment in most cases (22). Although some patients respond with a successful outcome, others are steroid dependent; these patients require very high doses to maintain remission, and they may relapse when a reduction of the dose is initiated (8, 9). Finally, other patients are unable to maintain their clinical condition despite use of very high doses of steroids. In every circumstance, several alternative drugs were essayed with variable success (7–9). Thus, azathioprine has been one of the immunomodulator drugs used for its steroid-sparing effect, and for its ability to induce and to maintain remission in some cases (8).

To our knowledge, this is the first prospective, open-label trial using azathioprine in very ill patients with a well-documented diagnosis of refractory sprue. In contrast to a recent retrospective French analysis in which azathioprine had a limited clinical and histological effect (9), five of seven patients in our series had an almost complete clinical and histological remission after a 1-yr trial. Two patients did not respond to treatment and died during the trial, one of them likely as a consequence of immunosuppression and the other of a concomitant disease not related to the use of azathioprine, but both in the context of a severe clinical deterioration. No other adverse effects were detected. Four of the five patients who did respond to the study drug remain in good health after discontinuation of azathioprine with a gluten-free diet as the only therapeutic measure. One of the responder patients died of an unrelated disorder 4 months after the end of the trial.

Several aspects of this study deal with very interesting controversial features of refractory sprue that deserve further comment. A correct diagnosis of refractory sprue is key when including patients in trials exploring drugs with potentially harmful effects. In this study, we included patients that fulfilled the recently developed and more comprehensive criteria for diagnosis of refractory sprue (18). They presented with a severe clinical status with a typical histological intestinal lesion, a characteristic immunophenotype in the majority of cases, and a celiac-like genetic predisposition in all. In addition, all patients recruited in this study were shown to be completely or partially refractory to the use of oral and/or parenteral steroids. Finally, the possibility of an autoimmune enteropathy was ruled out by the lack of antienterocyte antibodies. However, we are aware that some aspects of these topics will require further elucidation.

One of the controversial topics is the relation between celiac disease and refractory sprue. Based on our present data, we suggest that our patients diagnosed with refractory sprue seem to be true celiac disease patients who evolved toward a refractory condition. Thus, all patients had the characteristic celiac HLA DQB*2 or DQB*8 alleles, and some of them presented with celiac disease specific antibodies. However, only two of them had previously been diagnosed with celiac disease, and only one formerly had a clear clinical and histological response to a gluten-free diet. In our opinion, and according to Ryan and Kelleher (8), the majority of our cases must be considered as refractory to the initial institution of a gluten-free diet. These findings are concordant with those recently reported by two retrospective studies (9).

The rationale for using azathioprine in refractory sprue is based on its potent action in preventing the clonal expansion of both B and T lymphocytes (34, 35). Through interference in the purine synthesis, the drug may lead to DNA damage and hence to the therapeutic effect (36). However, azathioprine can produce toxicity affecting rapidly growing cells, including bone marrow and GI cells. Leukopenia, thrombocytopenia, hepatotoxicity, and GI toxicity can be recognized during treatment (34, 35). However, the most severe side effects are the increased risk of infections and the possible carcinogenicity in special lymphoma (36). Interestingly, in most reports on azathioprine use for refractory patients, the drug was associated with the administration of glucocorticoids. Sometimes, it is impossible to differentiate the effect of prednisone from that of azathioprine. However, we attributed to the study drug an important and key role in the significant clinical, biochemical, histological, and nutritional improvement in the patients. We based this statement

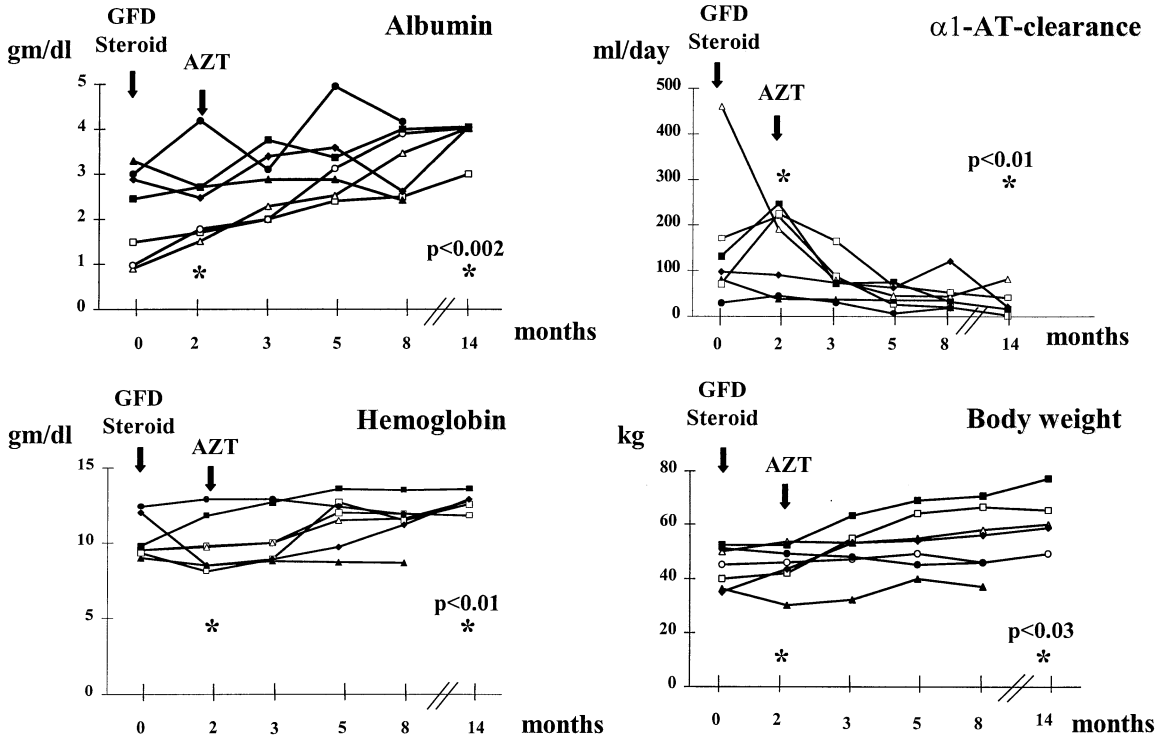

Figure 2. Schematic representation of the follow-up of selected parameters (serum albumin, fecal α_1 antitrypsin clearance, Hb, and body weight) in patients with refractory sprue. Determinations were performed at the time of starting on steroids (month 0), when azathioprine (AZT) was initiated (month 2), and at months 3, 5, 8, and 14 on azathioprine. Statistical analysis was applied to the five patients who completed the trial (paired *t* test).

on the fact that in this study, steroids had been administered for 2 months before the onset of azathioprine, and the clinical, nutritional, and laboratory parameters of patients did not show any significant change during that period (Fig. 2).

At least two other very interesting aspects require further elucidation. On the one hand, it is necessary to better estimate the most appropriate scheme for administration of both pharmacological agents. On the other hand, it is necessary to determine the best treatment strategy after successful use of azathioprine. Both alternatives were arbitrarily determined for our study protocol. The dose of azathioprine administered in our study (2 mg/kg body weight per day) was similar to that for other autoimmune digestive disorders and to that used in the French study (9). The duration of treatment for this trial was arbitrarily established as 1 yr and, therefore, further studies should estimate the most appropriate time. Finally, our data confirm previous reports on the high prevalence of deaths from this condition. However, deaths in the present study were not related to malignancies, as it was very common in the available retrospective studies (8, 9, 17, 19, 20, 37). However, taking into consideration that the clonal infiltration persisted after successful treatment, the further development of malignancy should not be excluded.

In conclusion, our present data show that azathioprine may be a valid and effective alternative drug for patients with refractory sprue. This effectiveness was shown for patients who were refractory to a gluten-free diet and to high doses of steroids. We also showed evidence that some patients cannot respond to this type of immunosuppressive therapy, and that the use of the drug must be meticulously monitored for potential complications. We confirm a high mortality rate for refractory sprue that, in the present experience, was not related to malignancy. Further studies are necessary to expand the number of patients treated with this drug to obtain definitive conclusions.

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