^{123}I-Interleukin-2 Scintigraphy for In Vivo Assessment of Intestinal Mononuclear Cell Infiltration in Crohn's Disease

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Activated mononuclear cells expressing interleukin-2 (IL2) receptors (IL2-Rs) heavily infiltrate the Crohn's disease (CD) gut wall. A new technique for the in vivo detection of tissue infiltrating IL2-R positive (IL2R+ve) cells was developed based on ^{123}I-IL2 scintigraphy. The aim of this study was to investigate whether ^{123}I-IL2 accumulates in the CD gut wall in different phases of the disease and to evaluate the specificity of ^{123}I-IL2 binding to activated IL2R+ve cells infiltrating the gut wall. **Methods:** Fifteen patients with ileal CD (10 active and 5 inactive) and 10 healthy volunteers were studied by ^{123}I-IL2 scintigraphy. Six patients with active CD were studied before and after 12 wk of steroid treatment. After scintigraphy, patients were followed up for 29–54 mo. Ex vivo autoradiography was performed to determine specificity of ^{125}I-IL2 binding to IL2R+ve cells. For bowel scintigraphy, ^{123}I-IL2 (75 MBq) was injected intravenously and γ camera images were acquired after 1 h. Bowel radioactivity was quantified in 64 regions of interest (ROIs). **Results:** Autoradiography showed specific binding of ^{125}I-IL2 to IL2R+ve mononuclear cells infiltrating the CD gut wall. Intestinal ^{123}I-IL2 uptake assessed by the number of positive ROIs was higher in patients with active or inactive CD than in healthy volunteers ($P < 0.0001$ and $P = 0.03$, respectively) and positively correlated with the CD activity index ($P = 0.01$). ^{123}I-IL2 intestinal uptake significantly decreased in patients with CD in steroid-induced remission ($P = 0.03$). A significant correlation was observed between the number of positive ROIs and time to disease relapse. **Conclusion:** ^{123}I-IL2 accumulates in the diseased CD gut wall by specific binding to IL2R+ve cells, infiltrating the involved tissues. ^{123}I-IL2 scintigraphy may be an objective tool for the in vivo assessment of intestinal activated mononuclear cell infiltration.

Key Words: Crohn's disease; mucosal lymphocytes; interleukin-2; scintigraphy

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T-cell activation and macrophage activation are features of Crohn's disease (CD) (*1,2*). In particular, activated mononuclear cells expressing interleukin-2 (IL2) receptors (IL2-Rs) and capable of binding IL2 represent a major component of gut inflammation in CD (*3,4*). No imaging techniques are currently available for the in vivo detection and quantitation of mononuclear cells infiltrating the human gut wall. Soluble IL2-R levels have been reported to reflect accurately disease activity in CD, thus suggesting that the assessment of intestinal activated mononuclear cell infiltration may help to define the lesional activity of the disease (*5,6*). A technique capable of detecting and quantitating in vivo activated mononuclear cells within the diseased gut may be useful in identifying complete remission in patients with CD and in assessing the need for specific treatment to be continued or modified. Such a technique should be first validated for its capacity to specifically detect activated mononuclear cells and then to correctly assess the clinical and/or lesional activity of CD.

The use of ^{123}I-IL2 scintigraphy for the in vivo detection of activated mononuclear cells expressing IL2-R infiltrating the pancreas has been described in autoimmune diabetes (*7–9*).

In this study we showed by ex vivo microautoradiography the specificity of ^{125}I-IL2 binding to activated mononuclear cells infiltrating the gut wall in patients with CD. We also measured in vivo the intestinal ^{123}I-IL2 uptake in patients with both active and inactive CD and compared it with healthy volunteers.

MATERIALS AND METHODS

Patients

We studied 15 patients (8 men, 7 women; mean age, 38.4 ± 12 y) with ileal CD diagnosed by conventional clinical, endoscopic, radiologic, and histological criteria (10 as primary and 5 as postoperative recurrences of the disease) (Table 1).

Inclusion criteria were: presence of distal ileal involvement assessed by colonoscopy and small bowel follow-up examination performed within the last 3 mo; regular follow-up at the Gastroenterology Unit of the Department of Clinical Sciences of the University of Rome "La Sapienza"; and no treatment with corticosteroids or immunosuppressive drugs for 6 mo preceding the scan.

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TABLE 1
Clinical and Scintigraphic Data of Patients with CD

Patient no.	Age (y)	Sex	Disease localization	Disease activity				IL2 uptake (no. of ROI)*		Follow-up status (time elapsed)
				CDAI	BSI	D	Activity	Upper	Lower	
1	34	M	Ileum	0	0	3	Inactive	4	7	R (54 mo)
2	42	M	PA-ileum	36	3	3	Inactive	0	0	R (51 mo)
3	44	F	Ileum	34	2	4	Inactive	4	4	S (13 mo)
4	52	M	Ileum	43	0	3	Inactive	0	1	R (29 mo)
5	39	M	Ileum	16	0	3	Inactive	0	0	R (29 mo)
6	24	M	PA-ileum	295	7	7	Active	3	2	S (8 mo)
7	30	F	PA-ileum	310	9	8	Active	5	9	S (6 mo)†
8	27	F	Ileum	278	8	8	Active	10	14	S (1 mo)
9	24	M	Ileum	204	4	7	Active	8	4	S (1 mo)
10										
Pre‡	34	F	PA-ileum	207	6	8	Active	6	6	
Post‡				24	0	3	Inactive	1	8	S (2 mo)
11										
Pre	30	M	Ileum	210	5	7	Active	4	5	
Post				172	3	4	Active	3	3	S (2 mo)
12										
Pre	44	F	Ileum	210	6	8	Active	5	1	
Post				98	4	3	Inactive	0	0	R (37 mo)
13										
Pre	70	F	Ileum	220	7	8	Active	6	7	
Post				102	4	3	Inactive	3	1	RR (6 mo)
14										
Pre	49	M	Ileum	274	7	8	Active	1	3	
Post				72	2	3	Inactive	0	4	RR (3 mo)
15										
Pre	34	F	PA-ileum	266	8	8	Active	4	12	
Post				105	4	3	Inactive	1	2	R (34 mo)

*ROI = positive regions of interest (tumor-to-background ratio > normal mean + 3 SD in 32 upper and 32 lower abdominal ROIs).
†2 mo before surgery, CDAI was 24.
‡Pre and Post = pre- and post-12-wk steroids.
R = complete remission; PA-ileum = preanastomotic ileum; S = surgery; RR = relapsing/remitting disease requiring steroid therapy.

The mean disease duration was 9 ± 4 y. The day of ^{123}I-IL2 scintigraphy, the clinical activity of the disease was evaluated according to the CD activity index (CDAI) (*10*) and to the Bristol simple index (BSI) (*11*), supplemented by laboratory measurements (*12*). At the time of the study, 10 patients had active disease (CDAI > 150), and 5 were in remission (CDAI ≤ 150) (Table 1).

Six patients with active CD were also studied after 12-wk treatment with prednisone (tapered from 1 to 0.1 mg/kg/d in 12 wk).

Routine hematological parameters (white blood cell count, hematocrit, erythrocyte sedimentation rate, C-reactive protein, orosomucoids, total proteins, α1-globulins) were regularly evaluated in each patient. Serum-soluble IL2-R levels were also evaluated in patients with CD and healthy volunteers by enzyme-linked immunosorbent assay (Genzyme Diagnostic, Cambridge, MA). Fecal α1-antitrypsin clearance was measured in the 5 CD patients studied longitudinally from the active phase to steroid-induced remission (*13*).

After IL2 scintigraphy, patients were followed up for 29–54 mo. As a control group, we studied 10 age-matched healthy subjects. This study was approved by the local ethics committee and all patients gave their written consent.

Labeling

Human recombinant IL2 (Chiron Corp., Berkeley, CA) was labeled as previously described (*7*), with slight modifications. Briefly, 40 µL ^{123}I (370 MBq; Cygne, The Netherlands) were incubated with 50 µL IL2 (100 µg), 5 µL lactoperoxidase (Sigma, UK; 200 µg/mL phosphate-buffered saline [PBS]), 5 µL glucoseoxidase (Sigma; 20 µg/mL PBS), and 10 µL D-glucose (10 mg/mL PBS). IL2-to-^{123}I ratio was about 5:1 to minimize overiodination of the protein. After 30 min, unbound iodine and unlabeled IL2 were removed by high-performance liquid chromatography (HPLC). HPLC-purified ^{123}I-IL2 was then purged for 20 min at 30°C with N_2 to remove acetonitrile, diluted to 5 mL with 5% glucose, and sterilized by filtration through a 0.22-µm low-protein-binding filter.

For ex vivo autoradiography, IL2 (10 µg) was labeled with ^{125}I (10 MBq, Nycomed-Amersham, Amersham, UK) using the method described earlier. IL2-to-^{125}I ratio was about 5:1.

γ Camera Imaging and Data Analysis

Patients and healthy subjects fasted for at least 8 h and received 400 mg $KClO_4$ orally 20 min before the study to prevent stomach and thyroid uptake of free ^{123}I. An average of 75 MBq ^{123}I-IL2 (<10 µg IL2) were administered intravenously, and images were

acquired with a single-head Elscint SP4 γ camera (Elscint, Haifa, Israel) fitted with a low-energy and medium-resolution collimator. Planar anterior images (1000 kcts collected in a 256 × 256 pixel matrix) and tomographic (SPECT) images (collected in a 64 × 64 pixel matrix acquiring 60 frames of 20 s each during a 360° rotation) were acquired 1 h after injection.

Figure 1 schematically represents the method used to measure bowel radioactivity. As shown, we have reconstructed 2 consecutive transaxial sections (upper and lower) between the lower kidney poles and the bladder. Each transaxial section (10 pixels thick) was divided in 32 regions of interest (ROIs) of 5 × 5 pixels drawn over the bowel. One circular ROI was drawn over the spine for calculation of background radioactivity. Target-to-background radioactivity ratio (T/B) was calculated for each ROI, after normalization of counts per ROI area, in patients and in healthy volunteers. An ROI was defined as positive if the T/B was higher than the mean of T/Bs in the same ROI from healthy volunteers + 3 SDs.

Results have been expressed as mean T/B of the 32 upper and 32 lower ROIs and number of positive ROIs in each section.

Autoradiography and Isolation of Intestinal Lamina Propria Mononuclear Cells

Ex vivo microautoradiography and isolation of intestinal mononuclear cells were performed in 2 patients with CD undergoing elective ileocecal resection. Patients were investigated by ^{123}I-IL2 scintigraphy and 7–10 d later, during surgery, were injected intravenously with 2–6 MBq ^{125}I-labeled-IL2. From 30 to 45 min later, the terminal ileum and cecum were dissected and several samples of both involved and uninvolved mucosa were frozen in liquid N_2 to perform autoradiography as previously described (*7*). Briefly, cryostat sections were fixed and stained with a mouse antihuman CD25 (Becton-Dickinson, San Jose, CA), followed by a biotin-conjugated rat antimouse IgG (Dako, Hamburg, Germany) and a peroxidase-conjugated streptavidin (Dako). After diaminobenzidine and H_2O_2 reaction with peroxidase, sections were dipped into K5 liquid emulsion (Ilford, Ilford, UK) and incubated for 60 d at 4°C. After development and fixation, slides were counterstained with hematoxylin–eosin (*7*).

Isolation of intestinal mononuclear cells from surgical specimens was performed by the dithiothreitol–ethylenediamine tetracetic acid–collagenase sequence and Percoll gradient, as previously described (*4,14*). Isolated mononuclear cells were stained with a fluorescein isothiocyanate-conjugated anti-CD25 monoclonal antibody (Becton-Dickinson) (*7*). A total of 180 × 10^6 cells was sorted by FACScan flow cytometer (Becton-Dickinson). Radioactivity associated with sorted CD25-positive (CD25+ve) cells and CD25-negative (CD25−ve) cells was then counted for 600 min in a highly sensitive γ counter (LKB Wallac, Turku, Finland). Results (counts per minute [cpm]) were expressed as cpm/10^7 cells − cpm background.

To estimate the number of IL2 molecules bound per activated lymphocyte, we assessed a standard curve with several known amounts of ^{125}I-IL2 (from 1 to 0.001 pmol).

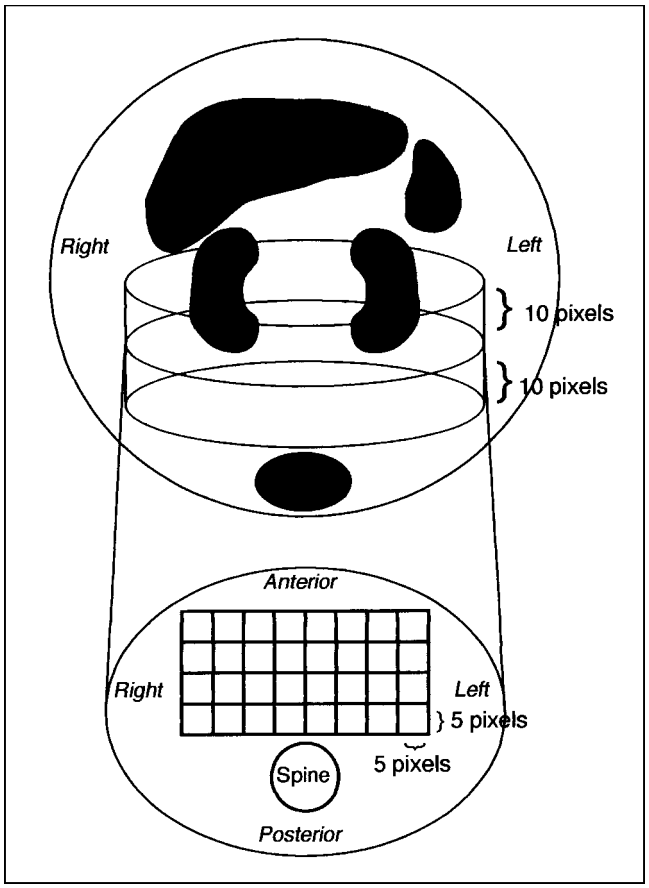

FIGURE 1. Schematic representation of method used to quantitate bowel radioactivity. Two consecutive transaxial intestinal sections (upper and lower) of 10 pixels, and ROIs of 5 × 5 pixels, drawn over bowel, are shown.

Statistical Analysis

Results were expressed as mean ± SD. Differences in T/Bs and in number of positive ROIs were evaluated using ANOVA (StatWorks [Heyden & Son, Ltd., London, UK] program for Macintosh PC). The *t* test for paired data was used in the longitudinal study. Regression analysis was applied between the T/B or number of positive ROIs and clinical as well as hematological parameters.

RESULTS

Labeling, Autoradiography, and Isolation of Intestinal Lamina Propria Mononuclear Cells

HPLC-purified ^{123}I-IL2 had a specific activity ranging from 7–15 MBq/μg and showed high stability in vitro over 4 h (trichloracetic acid precipitable > 96%). Immunoreactivity of ^{123}I-IL2 in vitro has been previously shown by binding assay (*7,8*).

Ex vivo autoradiography performed in the diseased gut of 2 patients with active CD shows that radiolabeled IL2 specifically associates only to IL2-R positive (IL2R+ve) cells as detected by immunoperoxidase staining (Fig. 2). In the same tissue sections, no significant binding of ^{125}I-IL2 was found to be associated to the IL2-R negative (IL2R−ve) mononuclear cells nor to other cell types. Tissue background was comparable with the background observed outside the section, indicating undetectable non–cell-associated ^{125}I-IL2 within the CD ileal mucosa. This finding was also confirmed by counting the radioactivity associated with the CD25+ve and CD25−ve lamina propria mononuclear cells isolated from resected diseased CD ileal mucosa (Fig. 3). As shown in Figure 3, the percentage of CD25+ve cells was 27%, and radioactivity was associated with only the CD25+ve cell

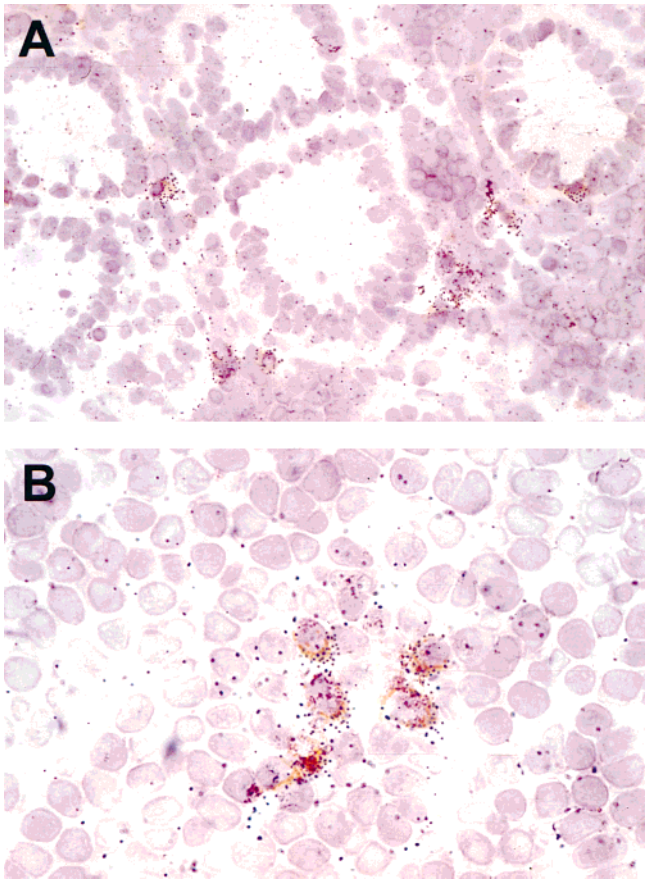

FIGURE 2. Ex vivo microautoradiography of criostat section of diseased ileal mucosa from patient with CD after intraoperatory intravenous injection of ^{125}I-IL2. Sections were stained using anti-CD25 monoclonal antibody to reveal IL2R+ve cells (stained in brown). Radioactivity (black granules) is associated with CD25+ve cells. (A) Magnification = ×100. (B) Magnification = ×400.

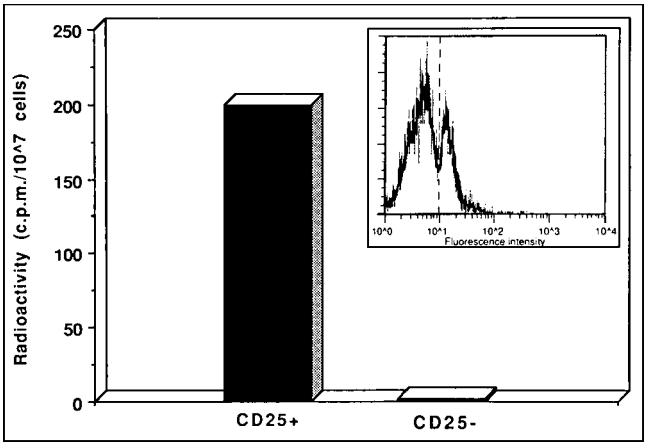

FIGURE 3. Radioactivity (mean cpm) associated with CD25+ve and CD25−ve intestinal mononuclear cells isolated from ileal mucosa of 2 patients with CD who had undergone ileocecal resection and intraoperatory intravenous injection of ^{125}I-IL2. CD25+ve cells were 27% of total infiltrating lamina propria cells in both patients. Insert shows fluorescence intensity histogram of CD25-stained intestinal mononuclear cells obtained by fluorescence-activated cell sorter analysis.

population (200 versus 5 cpm/10^7 cells). Thus, as calculated from the standard curve, 0.008 pmol of IL2 was associated with 10^7 cells, corresponding to approximately 480 IL2 molecules bound per CD25+ve cell.

γ Camera Imaging and Data Analysis

After the intravenous injection of ^{123}I-IL2, no adverse reactions or side effects were observed in all patients and healthy volunteers studied. The average injected dose of IL2 was <20,000 IU, thus being less than 1/100 of a therapeutic dose. This tracer dose, given intravenously as a bolus, had no detectable biological effects in humans (*15*). In healthy subjects, ^{123}I-IL2 rapidly cleared from plasma by renal metabolism, as described for unlabeled IL2 (*15*). Thus, 30 min after injection, only a small amount of circulating radioactivity was detectable in the blood.

Table 1 shows the clinical characteristics and scintigraphic results as assessed by the number of positive ROIs in each of the 15 patients with CD. The time to relapse of disease from IL2 scintigraphy is also shown. During the follow-up period, the 5 patients who had active disease at time of IL2 scintigraphy required surgery within 8 mo (3 for recurrent subobstructions and 2 for steroid dependence), whereas among the 10 patients with inactive disease, 6 are still in clinical remission (after 29–54 mo), 2 required surgery (both for recurrent subobstructions and steroid dependence), and 2 had relapsing/remitting disease. In all patients but 1, IL2 scintigraphy indicated that more than 3 positive ROIs could predict an early clinical relapse of the disease.

As shown in Figure 4, the degree of intestinal ^{123}I-IL2

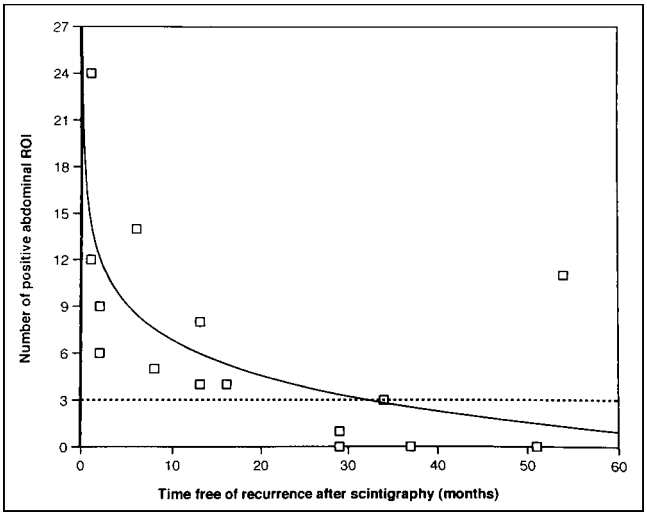

FIGURE 4. Correlation between degree of intestinal ^{123}I-IL2 uptake (as number of positive ROIs) and time to CD relapse (months after IL2 scintigraphy). All patients with >3 positive ROIs had clinical relapse or worsening of symptoms, requiring surgery within 16 mo of scintigraphy. Only 1 patient with >3 positive ROIs had no disease relapse (false-positive) ($P = 0.03$; $r^2 = 0.486$ by polynomial regression analysis).

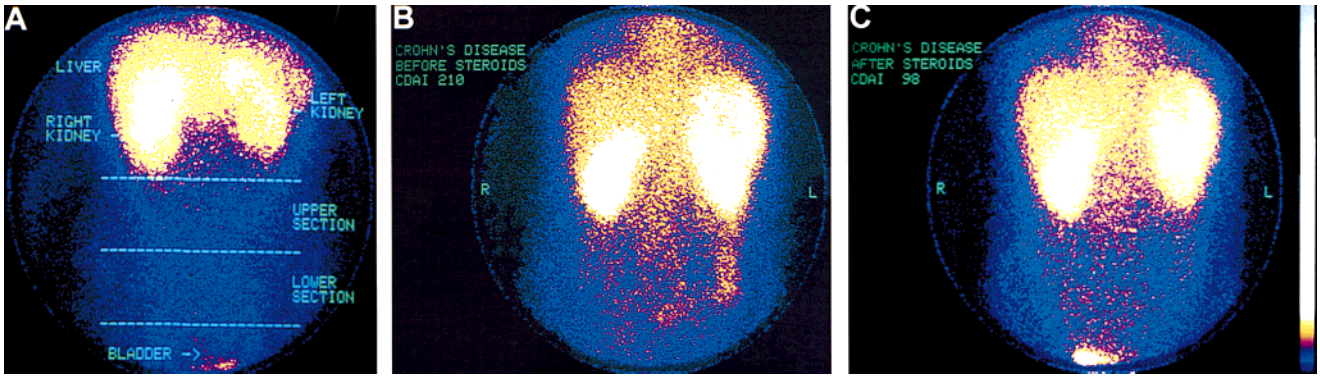

FIGURE 5. (A) Anterior view of abdomen of healthy volunteer, 1 h after injection of ^{123}I-IL2. Figure shows position of 2 transaxial sections reconstructed to quantitate bowel ^{123}I-IL2 uptake. There is physiological uptake of labeled IL2 in kidneys and bladder related to IL2 metabolism. However, there is almost undetectable intestinal uptake of ^{123}I-IL2. (B and C) Anterior views of abdomen of patient with active CD, studied from active phase (B) to steroid-induced remission (C), 1 h after injection of labeled IL2. As shown, besides physiological accumulation of ^{123}I-IL2 in kidneys and bladder, there is also significantly higher uptake in bowel compared with healthy volunteer. Intestinal uptake during active phase (B) significantly decreased during steroid-induced remission (C) .

uptake, as assessed by the number of positive ROIs, was correlated with the severity of the disease ($P = 0.03$). Figure 5 shows the anterior view of the abdomen from a healthy subject (A) compared with a patient with active CD studied before (B) and after steroid-induced remission (C), 1 h after injection of ^{123}I-IL2. As shown, gastrointestinal uptake in the healthy volunteer was homogeneous and almost undetectable. Radioactivity in kidneys and bladder reflects the physiological metabolism of IL2 (Fig. 5A). The variable degree of spleen IL2 uptake detected in healthy volunteers may be explained by the physiological presence of IL2R+ve mononuclear cells in this organ. Unlike its behavior in healthy volunteers, ^{123}I-IL2 accumulated extensively into the gut to a variable degree and extent in all patients with active CD (Fig. 5B; Table 2). Intestinal uptake of labeled IL2 significantly decreased during the steroid-induced remission (Fig. 5C; Table 3). The observed differences in terms of intestinal uptake between healthy subjects and patients with active and inactive CD was detectable to an even greater extent when we analyzed the 2 transaxial sections for quantitative purposes (Fig. 6).

The results of ^{123}I-IL2 scintigraphy in patients with active and off-steroid inactive CD, in comparison with healthy volunteers, are summarized in Table 2. As shown, the intestinal uptake, as assessed by the mean T/B of the 32 upper and 32 lower abdominal ROIs, was significantly higher in patients with active CD than in healthy volunteers ($P = 0.001$, upper section; $P < 0.0001$, lower section). Likewise, the number of positive ROIs in the 2 transaxial sections was significantly higher in patients with active CD than in healthy volunteers ($P < 0.0001$ in both sections). In the off-steroid inactive CD group, the average T/B in both sections did not significantly differ from that in healthy volunteers, whereas the number of positive ROIs was significantly higher than that for healthy volunteers ($P = 0.03$ and $P = 0.02$ in the upper and lower sections, respectively). In this group of patients, intestinal uptake assessed by both the mean T/B and number of positive ROIs was higher in patients with active CD than in those with inactive CD, but these differences reached statistical significance only for the number of positive ROIs in the upper section ($P = 0.01$) (Table 2). Among the 6 patients with active CD studied before and after 12-wk prednisone therapy, 5 achieved remission after treatment (CDAI < 150) (Table 1). Table 3 shows the ^{123}I-IL2 intestinal uptake and laboratory parameters (soluble IL2-R, fecal α1-antitrypsin clearance) in the 5 patients with CD studied from the active phase to steroid-induced remission. As shown, T/B significantly decreased during steroid-induced remission, both in the upper and lower transaxial section ($P = 0.02$ and $P = 0.03$ versus pretreatment, respectively). The number of

TABLE 2
Intestinal ^{123}I-IL2 Uptake in Patients with CD and Healthy Volunteers: Transversal Study

		Patients with CD*	
	Healthy volunteers* (n = 10)	Active (CDAI >150) (n = 10)	Off-steroid remission >3 mo (n = 5)
Upper section			
Mean T/B	1.35 ± 0.14	1.65 ± 0.17†	1.45 ± 0.24
Positive ROIs	0‡	5.20 ± 2.53§	1.60 ± 2.19
Lower section			
Mean T/B	1.14 ± 0.17	1.48 ± 0.12‖	1.30 ± 0.15
Positive ROIs	0¶	6.30 ± 4.27	2.41 ± 3.05

*Results in each group are expressed as average T/B of 32 ROIs in upper and lower transaxial sections and as number of positive ROIs in each section.

†$P = 0.001$ vs. healthy volunteers.

‡$P < 0.0001$ vs. active CD, and $P = 0.03$ vs. off-steroid remission CD.

§$P = 0.01$ vs. off-steroid remission CD.

‖$P < 0.0001$ vs. healthy volunteers.

¶$P < 0.0001$ vs. active CD, and $P = 0.02$ vs. off-steroid remission CD.

TABLE 3
Scintigraphic and Laboratory Parameters in Patients with CD Longitudinally Studied from Active Phase to Steroid-Induced Remission

	Patients with CD	
	Active (CDAI >150) (n = 5)	Steroid-induced remission (CDAI ≤ 150) (n = 5)
Upper section		
Mean T/B	1.60 ± 0.19	1.36 ± 0.17*
Positive ROIs	4.40 ± 2.07	1.00 ± 1.22†
Lower section		
Mean T/B	1.47 ± 0.11	1.30 ± 0.10‡
Positive ROIs	5.80 ± 4.21	3.00 ± 3.16
Soluble IL2-R		
Serum (pg/mL)	478 ± 107	411 ± 66
α1-antitripsin		
Fecal clearance (mL/d)	392 ± 171	277 ± 109§

*$P = 0.02$, remission vs. active CD.
†$P = 0.01$, remission vs. active CD.
‡$P = 0.03$, remission vs. active CD.
§$P = 0.01$, remission vs. active CD.

positive ROIs in the upper transaxial section also significantly decreased after treatment ($P = 0.01$), whereas in the lower section this difference was not significant (Table 3).

Within both inactive CD groups (off-steroid and steroid-induced remission) a wide range of intestinal ^{123}I-IL2 uptake was observed, and therefore both the mean T/Bs and the number of positive ROIs did not significantly differ between the 2 groups (Tables 2 and 3, respectively).

Serum IL2-R levels and fecal α1-antitrypsin clearance were not related to the intestinal ^{123}I-IL2 uptake as assessed by both the T/B and the number of positive ROIs both in patients with CD and in healthy volunteers. By contrast, the number of positive ROIs in the 2 transaxial sections was significantly related to the clinical activity of CD as assessed by either the CDAI ($r = 0.54$; $P = 0.01$) or BSI ($r = 0.53$; $P = 0.01$) scores.

DISCUSSION

No method is currently available for the in vivo detection and assessment of activated mononuclear cells within the gut wall. Increasing evidence suggests that new and more sophisticated drug regimens capable of modulating the immune-mediated inflammatory response within the gut wall will soon be available for treatment of CD (e.g., anti-tumor necrosis factor-α [TNFα]) (*16*). As these drugs exert their effects specifically on the cellular components of the intestinal inflammatory response, new and more objective techniques capable of detecting in vivo the mononuclear-cell–mediated inflammatory process in CD tissue may well be of use.

Scintigraphy that uses both ^{111}In-labeled leukocytes and ^{99m}Tc-hexamethyl propyleneamine oxime leukocytes, although useful in assessing the acute intestinal inflammatory process, does not address this specific question nor does it demonstrate a low specificity in this respect (*17*). Recently, other approaches have been proposed based on the use of radiolabeled anti-E-selectin antibodies (*18*) and radiolabeled interleukin-8 (*19*).

In vivo T-cell activation and macrophage activation are features of CD, and the diseased CD tissue is severely infiltrated by activated mononuclear cells expressing IL2-R and capable of binding free IL2 (*2–5*). In this study, we describe the use of radiolabeled IL2 for in vivo γ camera imaging of activated mononuclear cells infiltrating the CD gut wall. We also demonstrated, by ex vivo autoradiography and by in vitro counting of tissue infiltrating CD25+ve cells isolated from surgical ileal specimens, that ^{123}I-IL2 specifically bound only to activated IL2-R–expressing cells, thus confirming our previous results in vitro and in animals (*7–9*). From our calculations, the number of IL2 molecules bound

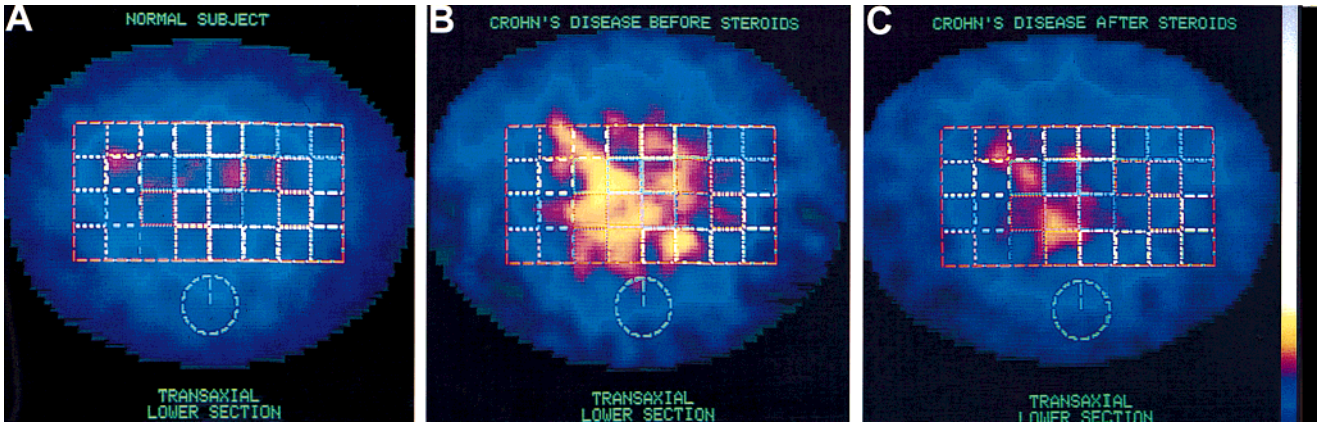

FIGURE 6. (A) Upper transaxial section obtained after reconstruction of SPECT images in healthy volunteer, 1 h after injection of ^{123}I-IL2. Thirty-two squared ROIs (5 × 5 pixels, over bowel) and 1 circular ROI (drawn over spine) are shown for calculation of T/Bs. (B and C) Upper transaxial sections obtained in patient with CD studied from active phase (B) to steroid-induced remission (C). During active phase, there is significantly higher intestinal accumulation of ^{123}I-IL2, compared with healthy volunteer, particularly in central regions, which decreased during steroid-induced remission.

per CD25+ve cell was 480; however, this figure may be underestimated because of the time required to isolate mononuclear cells from the intestinal lamina propria, with subsequent intracellular IL2 metabolism and loss of radioactivity from the cells, but it is on the order of magnitude of high-affinity IL2-Rs expressed on activated T-cells (*20*).

IL2 scintigraphy is easy to perform, does not involve handling of patient's blood, and delivers a low radiation absorbed dose (effective dose equivalent, 0.2 mSv/37 MBq; critical organ kidneys, 2 mGy/37 MBq). ^{99m}Tc-labeled IL2 will soon be available, thereby reducing the time and cost of the preparation (*21*). In this study, we also evaluated the extent of ^{123}I-IL2 accumulation into the CD gut wall. Results indicated that in all CD patients ^{123}I-IL2 accumulated into the gut wall with a patchy and focal distribution that is in agreement with the histopathological features of CD lesions (*22*). For this reason we quantitated ^{123}I-IL2 uptake in 64 small intestinal regions. Areas showing high ^{123}I-IL2 uptake close to areas with no uptake were indeed detected within each abdominal transaxial section. Because the aim of this study was to evaluate whether ^{123}I-IL2 scintigraphy allows the in vivo visualization and quantitation of CD25+ve cells infiltrating the CD gut wall, we did not compare the extent and site of ^{123}I-IL2 uptake with the localization of the disease as assessed by radiology and endoscopy, because these techniques provide different information.

In this study, ^{123}I-IL2 uptake was significantly higher in patients with active CD compared with healthy volunteers. Moreover, we also found a significantly greater number of positive ROIs in the upper and lower transaxial sections from the 2 groups of patients with inactive CD (off-steroids and in steroid-induced remission) when compared with healthy volunteers. The same findings were not observed when intestinal uptake was assessed by the mean T/Bs in both inactive CD groups. This observation most likely reflects the focal uptake detected by the number of positive ROIs when compared with the mean T/B in each transaxial section. Because of the patchy distribution of intestinal inflammation in CD tissue (*22*), it is not surprising that the number of positive ROIs, but not the mean T/Bs, detected a higher intestinal uptake in inactive CD groups when compared with healthy subjects.

Finally, the degree of intestinal IL2 uptake (as evaluated by the number of positive ROIs) did correlate with the duration of disease remission, suggesting a role of IL2 scintigraphy for predicting CD relapse. It is interesting that 4 of 10 patients clinically classified as having inactive disease showed 4 or more positive ROIs on IL2 scintigraphy and underwent clinical disease relapse within 13 mo.

The results in patients studied before and after steroid treatment showed that ^{123}I-IL2 uptake significantly decreased in both transaxial sections in patients in remission after treatment. However, it did not decrease to normal values, and the number of positive ROIs significantly decreased only in the upper section, suggesting the persistence of infiltrating activated T-cells in high numbers despite clinical remission (*21*). This finding most likely relates to the presence of a focal accumulation of activated mononuclear cells in CD-involved mucosa (distal ileum imaged in the lower transaxial section), even during the steroid-induced remission (*23*). Thus, we show that residual mucosal immune activation in CD tissue, as detected by IL2 scintigraphy, is associated with early clinical relapse of the disease. The concept that an increased mucosal immune activation in inactive CD is associated with an early relapse is also suggested by recent data showing higher TNFα and IL1β-release by intestinal lymphocytes isolated in vitro from patients with inactive CD undergoing early clinical relapse (*24*). A large prospective longitudinal study is in progress to investigate the possible usefulness of IL2 scintigraphy in the identification of subgroups of patients with inactive CD undergoing early relapse.

The extent of intestinal ^{123}I-IL2 uptake was significantly correlated to the CDAI and BSI scores, a finding that is in agreement with the reported higher percentage of mucosal CD25+ve cells in active CD (*4*). The absence of correlation between IL2 intestinal uptake and serum IL2-R levels is not surprising, as ^{123}I-IL2 uptake reflects the number of IL2R+ve cells in situ within the gut wall, whereas the same has not been shown for serum IL2-R levels.

CONCLUSION

We have tested the ^{123}I-IL2 scintigraphy in patients with CD to detect in vivo activated mononuclear cells infiltrating the gut wall. ^{123}I-IL2 accumulated in CD gut by specific binding to IL2R+ve cells. The degree of intestinal infiltration, as assessed by ^{123}I-IL2 scintigraphy, seems to parallel CD activity, and its measurement may offer an objective evaluation of the response to anti-inflammatory treatment in these patients. ^{123}I-IL2 scintigraphy, by providing an in vivo quantitation of activated mononuclear cells within the gut wall, may be a new and objective tool for assessing CD activity.

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