The Effect of Acute Psychologic Stress on Systemic and Rectal Mucosal Measures of Inflammation in Ulcerative Colitis

JOEL E. MAWDSLEY,* MARION G. MACEY,† ROGER M. FEAKINS,§ LOUISE LANGMEAD,¶ and DAVID S. RAMPTON*

*Centre for Gastroenterology, Institute of Cell and Molecular Science, Barts and the London, Queen Mary School of Medicine and Dentistry, London; †Department of Haematology, and §Department of Histopathology, Barts and London NHS Trust, London; and ¶Department of Gastroenterology, University College London Hospital NHS Foundation Trust, London, United Kingdom

Background & Aims: **Recent studies suggest that life events and chronic stress increase the risk of relapse in inflammatory bowel disease. Our aim was to study the effects of acute psychologic stress on systemic and rectal mucosal inflammatory responses in patients with inactive ulcerative colitis (UC).** ***Methods:*** **Twenty-five patients with inactive UC and 11 healthy volunteers (HV) underwent an experimental stress test. Ten patients with UC and 11 HV underwent a control procedure. Before and after each procedure, systemic inflammatory response was assessed by serum interleukin (IL)-6 and IL-13 concentrations, tumor necrosis factor (TNF)-α and IL-6 production by lipopolysaccharide (LPS)-stimulated whole blood, leukocyte count, natural killer (NK) cell numbers, platelet activation, and platelet-leukocyte aggregate (PLA) formation. In patients with UC, rectal mucosal inflammation was assessed by TNF-α, IL-13, histamine and substance P release, reactive oxygen metabolite (ROM) production, mucosal blood flow (RMBF) and histology.** ***Results:*** **Stress increased pulse ($P < .0001$) and systolic BP ($P < .0001$). In UC, stress increased LPS-stimulated TNF-α and IL-6 production by 54% ($P = .004$) and 11% ($P = .04$), respectively, leukocyte count by 16% ($P = .01$), NK cell count by 18% ($P = .0008$), platelet activation by 65% ($P < .0001$), PLA formation by 25% ($P = .004$), mucosal TNF-α release by 102% ($P = .03$), and ROM production by 475% ($P = .001$) and reduced rectal mucosal blood flow by 22% ($P = .05$). The control protocol did not change any of the variables measured. There were no differences between the responses of the patients with UC and HV.** ***Conclusions:*** **Acute psychologic stress induces systemic and mucosal proinflammatory responses, which could contribute to exacerbations of UC in ordinary life.**

Psychologic stress has been cited anecdotally by patients and doctors as worsening disease activity in inflammatory bowel disease (IBD). Indeed, in the 1950s, ulcerative colitis (UC) was regarded as a model of psychosomatic disease.[1]

Although prospective analyses of the relationship between psychologic stress and disease activity in IBD are methodologically difficult, recent well-designed studies have suggested that adverse life events; high, chronic, perceived stress; and acute daily stress can increase the incidence of subsequent disease relapse and symptoms in patients with IBD.[2] Animal models of colitis confirm that stress can contribute to both the initiation and the reactivation of colonic inflammation. Rats subjected to restraint stress prior to induction of colitis by 2,4,6-trinitrobenzenesulfonic acid (TNBS) developed an increased mucosal inflammatory response.[3] Restraint stress also lowered the dose of TNBS required to reactivate healed TNBS colitis.[4]

There are little data on the effects of acute, experimental stress on the mucosal immune and inflammatory system in humans. Immersion of the hand in ice-cold water (physical stress) increased jejunal concentrations of the mast cell mediators, tryptase and histamine, in healthy volunteers and patients with food allergy.[5] Repeated sessions of this stress over 5 days increased the numbers of mast cells showing activation and degranulation at electron microscopy in volunteers and more so in patients with quiescent IBD.[6]

In this study, we have examined the effects of acute experimental stress on a variety of measures of the systemic and rectal mucosal inflammatory response in patients with inactive UC. The measures assessed had either been previously implicated in the stress response (histamine,[5] substance P [SP],[7] natural killer [NK] cells,[8] and recta mucosal blood flow [RMBF][9]), are thought to play a pathogenic role in IBD (interleukin [IL]-13,[10] tumor necrosis factor [TNF]-α[11]), or appear to be involved in both the stress response and IBD (IL-6,[12]

Abbreviations used in this paper: HV, healthy volunteers; IL, interleukin; IQR, interquartile range; LPS, lipopolysaccharide; NK, natural killer; PLA, platelet-leukocyte aggregate; ROM, reactive oxygen metabolite; RMBF, rectal mucosal blood flow; TNF, tumor necrosis factor; UC, ulcerative colitis.

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platelet activation,[13] platelet-leucocyte aggregate [PLA] formation,[14] reactive oxygen metabolite [ROM] production[15]). The inflammatory response to stress was subsequently related to psychometric assessments of chronic perceived stress levels of each of the participants.

Materials and Methods

Patients and Controls

Twenty-five patients with quiescent UC (13 males; median age, 44 years [range, 28–64]; 32% distal, 20% left-sided, 48% total UC; 76% on 5-ASA, 12% on thiopurines, 4% on methotrexate, none on steroids; median disease duration 10 years [range, 3–31]) and 11 healthy volunteers (HV) (4 males; median age, 27 years [range, 23–56]) underwent the stress protocol. Ten patients with quiescent UC (3 males; median age, 52 years [range, 23–65]; 40% distal, 30% left-sided, 30% total UC; 90% on 5-ASA, 10% on thiopurines, none on steroids; median disease duration, 11.5 years [range, 5–38]) and 11 HV (6 males; median age, 36 years [range, 27–57]) underwent the control protocol. UC was confirmed by standard clinical, radiologic, endoscopic, and/or histologic criteria. Disease activity was assessed using the Simple Colitis Activity Index (SCCAI)[16] and Baron's score of mucosal appearance.[17] Inactive disease was defined as Baron's score <2. HVs were university staff and students. Written consent was obtained before participation in the study, for which ethical approval was given by North-East London Strategic Health Authority Ethics Committee.

Stress/Control Protocol

All chemicals were supplied by Sigma Chemical Co. (Poole, United Kingdom) unless otherwise stated. Stress and control protocols were performed between 9 AM and 12 noon. Patients had a light breakfast but abstained from coffee, tea, or cigarettes on the morning of the test. In premenopausal females, RMBF were made only during the follicular phase of the menstrual cycle.[9]

Experiments were performed in a quiet room with the subjects reclined on a couch. First, patients completed the Hospital Anxiety Depression Score (HADS),[18] the State Trait Anxiety Inventory (STAI),[19] the Perceived Stress Questionnaire (PSQ),[20] and the Bradford Somatic Inventory (BSI).[21] Subjects were then given 10 minutes to acclimatize before baseline pulse and blood pressure (BP) were measured with a dynamap blood pressure monitor (Critikon, Tampa, FL).

Baseline blood test. A 20-mL blood sample was taken, and 6 mL was transferred to a vacutainer tube containing lithium heparin; 6 mL to an empty vacutainer; 2 mL to a vacutainer containing potassium ethylenediaminetetraacetic acid (KEDTA); and 4 mL to a vacutainer containing KEDTA and then 1 containing citrate, theophylline, adenosine, and dipyridamole (CTAD). The CTAD/EDTA sample was stored on ice because previous work has shown minimal ex vivo activation of platelets anticoagulated with this combination and stored at 2°C–8°C.[22]

Baseline sigmoidoscopy. In patients with UC, a rigid sigmoidoscopy was performed and mucosal appearance assessed using Baron's score.[17] RMBF was measured using a MoorLAB laser Doppler flowmeter with an MP6a endoscopic probe (Moorlab, Axminster, United Kingdom).[9] Four quadrantic, 30-second readings of RMBF were taken 10 cm from the anal margin by applying the Doppler probe to the rectal mucosa through the sigmoidoscope. Next, a sample of perimucosal fluid was collected using the filter paper technique.[23] In brief, a 30- by 7-mm strip of filter paper (Whatmann No. 42) was placed via the sigmoidoscope against the rectal mucosa until visibly wet or for about 30 seconds. The paper was then removed and placed in 1 mL preprepared buffer of bovine serum albumin (BSA) (0.3%), 1 KU/mL aprotinin, sodium azide (0.01%), and Tween 20 (0.002%) in phosphate-buffered saline (PBS) for later processing. Finally, a rectal biopsy was taken and placed in 1 mL preoxygenated Tyrode's buffer for measurement of ROM production[15] and histologic assessment.[24]

Stress protocol. After the initial assessment, a modified dichotomous listening test was used to induce stress. Subjects were first told that the aim of the study was to assess the relationship between their intelligence (measured using an IQ test), response to stress, and the inflammatory response. Subjects were then given 50 minutes to complete an IQ test, which normally requires an hour. As a distraction, contrasting types of music were played into each ear (folk and rock). Subjects were reminded, at increasingly frequent intervals, to increase their effort to finish the test. Pulse and BP readings were taken every 15 minutes, and the mean of the 3 values was calculated. The subjective level of stress induced was assessed by asking patients to mark a 10-point visual analogue scale (VAS). The control protocol was identical except that, rather than undergoing the IQ and dichotomous listening test, subjects listened to relaxing music of their choice for 50 minutes.

Immediately after the test, a second blood sample was taken and processed as above. RMBF measurement, collection of perimucosal fluid, and rectal biopsy were repeated. Thirty minutes after the end of the test, a third blood sample was taken, followed by pulse and BP reading. HVs underwent the same stress and control protocols except that sigmoidoscopy was omitted.

Assays

Serum cytokines and lipopolysaccharide-stimulated cytokine production. Serum samples were kept in frozen aliquots at −80°C until later analysis of IL-6 and IL-13 concentrations by ELISA (R&D Systems, Oxford, United Kingdom). Samples were measured in duplicate or triplicate using manufacturer's recommendations. The coefficients of variation (CV) for duplicate estimates of serum IL-6 and IL-13 measurement were 3.1% and 8.9%, respectively. Serum TNF-α concentrations were below minimum sensitivity of the ELISA (R&D Systems) in most subjects and are not reported here.

For LPS-stimulated cytokine production,[25] 1 mL blood anticoagulated with lithium-heparin from the pre- and post-stress samples was cultured in 24-well plates for 24 hours in the presence of LPS (25 μg/mL) in a humidified atmosphere of 95% O_2 /5% CO_2 at 37°C. TNF-α and IL-6 concentrations in cell-free supernatants were determined by ELISA. The CVs for duplicate assays of TNF-α and IL-6 production were 9.7% and 10.4%, respectively.

Flow cytometry. All samples were analyzed using a FACScan equipped with CellQuest software (BD Biosciences, Cowley, Oxford, UK). The flow cytometer was calibrated and standardized before use with fluorochrome-labelled beads (Fluorospheres; Dako, Ely, United Kingdom). Fluorescin isothiocyanate (FITC)-conjugated mouse IgG1, FITC-IgG2a, phycoerythrin (PE)-conjugated IgG1, FITC-CD42a, PE-CD45, FITC-CD62P, PE-CD56, and FITC-CD16 were from Immunotech (Beckman Coulter, High Wycombe, United Kingdom).

NK cell numbers. The number of NK cells was determined by labelling anticoagulated blood (CTAD/EDTA) (100 μL) for 5 minutes on ice with FITC-CD16 (10 μL), PE-CD56 (10 μL), or both.[26] Red cells were then lysed by processing through a Coulter TQ-Prep machine (Beckman Coulter, High Wycombe, UK). After centrifugation at 2200 rpm for 5 minutes, the pellet was resuspended in 1 mL cold Tryode's solution. When assessing NK cells as a proportion of lymphocytes and monocytes, CD16- and CD56-positive events were recorded as percentage of 10,000 gated lymphocytes and monocytes. Background nonspecific antibody binding was assessed by measuring the proportion of positive IgG1-FITC and IgG1-PE positive events. The CV for duplicate measurements of NK cells was 1.5%.

Platelet activation (expression of p-selectin [CD62P]). Anticoagulated blood (CTAD/KEDTA) (5 μL) was labelled for 5 minutes on ice with FITC-CD62P (5 μL) or FITC-IgG1 isotype control (5 μL) in 90 μL cold Tyrode's solution.[13] Samples were then diluted to 1 mL with Tyrode's solution and analyzed by flow cytometry. Data were acquired with a primary gate set on a histogram of forward light scatter vs side light scatter. Background fluorescence was assessed using platelets labelled with FITC-conjugated isotype control antibody. Cursors were set in a single parameter histogram of frequency and FITC fluorescence intensity so that <1% of platelets stained positively with control antibody. Changes in P-selectin expression and in forward and side light scatter were then recorded on the gated platelets. The CV for duplicate measurements of P-selectin expression was 7.2%.

PLA formation. Anticoagulated blood (CTAD/EDTA) (5 μL) was labelled for 5 minutes on ice with PE-CD45 (5 μL) and either FITC-IgG2a isotype control (5 μL) or FITC-CD42a (5 μL) in 90 μL cold Tyrode's solution.[14] Samples were then diluted to 1 mL with Tyrode's solution and analyzed. When assessing the proportion of leucocytes involved in PLAs, CD42a- and CD45-positive events were recorded as percentage total of 1000 gated leukocytes. Background nonspecific antibody binding was assessed by measuring the proportion of IgG2a- and CD45-positive events. The CV for duplicate assays of PLAs was 9.6%.

Cytokines in perimucosal fluid (filter paper technique). A sample of perimucosal fluid was collected as described above.[23] Filter paper samples were incubated in 1 mL preprepared filter paper buffer with gentle agitation for 24 hours at 4°C. The filter paper was then removed and the remaining buffer centrifuged at 8000 rpm for 5 minutes. The supernatant was aliquoted and stored at −80°C until analysis by ELISA for IL-13, substance P, and TNF-α. IL-6 did not dissociate from the filter paper into the buffer, and, therefore, its mucosal release could not be measured with this technique.[23]

Mucosal ROM production. Luminol was dissolved in dimethyl sulfoxide (DMSO) at 50 mg/mL on the day of use and then diluted to 300 μmol/L in Tyrode's solution.[15] Biopsy specimens were transferred from the preoxygenated Tyrode's solution to precounted scintillation vials containing 1 mL 300 μmol/L luminol. Luminescence from each sample was immediately counted for 4 minutes in a Bertholdt LB953 luminometer. Samples were then blot dried and weighed. Chemiluminescence was expressed as counts/min/mg tissue weight after subtraction of background. The CV for ROM production by paired biopsies assessed by this method is 47%.[15]

RMBF. Rectal blood flow measurements were taken as above. The Moorlab software was used to calculate mean blood flow for each 30-second quadrantic reading. The mean of these 4 values was calculated to give a mean blood flow. The CV for quadrantic sets of RMBF measurements was 17%.

Histologic assessment. Pre- and postprocedural rectal biopsy specimens were stained with a standard H&E protocol. The degree of inflammation present in each biopsy specimen was scored by a single histologist (R.M.F.), blinded to the origin of the samples.[24]

Calculations and Statistics

Results are expressed as median and interquartile range (IQR) unless otherwise stated. A Friedman test for paired nonparametric data was initially performed to compare all 3 time points. If the Friedman test was significant, individual time points were compared by Wilcoxon signed rank test. The Mann–Whitney *U* test for nonpaired nonparametric data was used to compare the changes recorded in the variables in response to stress between patients with quiescent UC and healthy controls. Spearman nonparametric rank test was used to assess whether levels of chronic perceived stress correlated with changes seen in response to the stress test. In each instance, 2-tailed $P < .05$ was taken as statistically significant.

Power and Sample Size

At the time of the design of the experimental protocol, there were no data available on the effects of acute psychologic stress on the autonomic and inflammatory response in patients with UC. Therefore, it was difficult to perform formal power calculations. However, to detect an autonomic response to the stress test, we calculated that, with a study sample size of 36

Table 1. Pulse, Systolic and Diastolic BP, Serum IL-6 and IL-13 Levels, and LPS-Stimulated IL-6 and TNF Production by Whole Blood, in Response to Stress and Control Protocols in Patients With UC and Healthy Volunteers

Protocol	N		Before	During/after	30 min after
Stress					
UC	25	Pulse	70 (65–74)	77 (70–85)[a]	70 (66–76)
		Systolic BP	118 (113–133)	130 (119–148)[a]	122 (116–137)[a]
		Diastolic BP	72 (68–79)	79 (75–88)[a]	76 (72–82)[a]
		Serum IL-6	1.3 (1.0–2.2)	1.4 (1.0–2.2)	1.5 (1.0–1.9)
		Serum IL-13	1.9 (.0–4.4)	1.7 (.0–4.5)	1.7 (.1–4.8)
		LPS TNF-α	28 (17–57)	43 (17–69)	43 (20–62)[a]
		LPS IL-6	255 (204–387)	232 (179–306)	284 (214–411)[a]
HV	11	Pulse	65 (62–82)	76 (74–82)[a]	69 (64–81)
		Systolic BP	114 (109–122)	123 (112–134)[a]	122 (108–129)
		Diastolic BP	74 (66–83)	75 (73–82)	78 (73–84)[a]
		Serum IL-6	1.1 (.8–1.5)	.84 (.5–1.3)	1.2 (.6–1.4)
		Serum IL-13	.2 (0–2.3)	.4 (0–3.4)	.8 (0–2.5)
		LPS TNF-α	19 (12–35)	27 (19–60)	37 (14–60)[a]
		LPS IL-6	212 (156–293)	220 (187–305)	245 (211–382)
Control					
UC	10	Pulse	67 (55–82)	66 (56–79)	67 (57–78)
		Systolic BP	139 (111–158)	130 (108–150)	133 (113–150)
		Diastolic BP	77 (60–89)	75 (61–88)	76 (60–87)
		Serum IL-6	2.8 (2.4–4.4)	2.6 (2.4–3.4)	2.7 (2.1–3.7)
		Serum IL-13	1.3 (.8–2.2)	1.2 (.9–2.3)	1.6 (.9–2.1)
		LPS TNF-α	18 (13–59)	19 (12–60)	20 (16–72)
		LPS IL-6	223 (169–537)	218 (143–427)	254 (173–527)
HV	11	Pulse	71 (56–74)	66 (61–74)	68 (60–73)
		Systolic BP	114 (102–121)	110 (102–122)	112 (104–124)
		Diastolic BP	70 (63–75)	69 (62–75)	72 (63–77)
		Serum IL-6	.6 (.5–.9)	.6 (.4–1.3)	.7 (.4–1.5)
		Serum IL-13	.8 (0–2.1)	1.0 (0–2.4)	.7 (0–1.8)
		LPS TNF-α	18 (8–30)	19 (11–26)	20 (11–32)
		LPS IL-6	223 (164–302)	245 (203–345)	232 (195–315)

NOTE. Values are median (interquartile range).
Pulse in bpm, systolic and diastolic BP in mm Hg, serum IL-6 and IL-13 in pg/mL, LPS-stimulated and TNF production in ng/mL by whole blood.
[a]$P < .05$ from baseline value.

patients with a 2:1 randomization to the stress and control protocols, we would be able to detect a change in pulse rate of 5 bpm with a power of 80% and a significance level of $P = .05$ (2 tailed) (the standard deviation of baseline heart rate was 5.8 bpm).[27] Similarly, a study sample size of 24 patients with UC and 12 healthy controls would allow detection of a difference in pulse rate of 5 bpm in response to the stress protocol between groups with a power of 80% and a significance level of $P = .05$ (2 tailed).

Results

Autonomic Response to Stress Protocol

The mean of the 3 pulse rate readings measured during the stress protocol was increased compared with pretest values by 7 bpm (median) in patients with quiescent UC ($P < .0001$) and by 11 bpm in HV ($P = .02$). Thirty minutes after the end of the stress protocol, the mean pulse rate had returned to baseline in both groups (Table 1).

Mean systolic blood pressure increased during the stress protocol by a median of 12 mm Hg in patients with UC ($P < .0001$) and 9 mm Hg in HV ($P = .03$). In patients with UC, the systolic BP fell, but remained elevated compared to pretest levels, by a median of 4 mm Hg when measured 30 minutes after the stress protocol ($P < .0001$).

In patients with UC, mean diastolic BP increased by 7 mm Hg during stress ($P < .0001$). As with systolic BP, in patients with UC the diastolic BP fell when measured 30 minutes later but remained elevated by a median of 4 mm Hg compared with baseline ($P = .01$). Diastolic BP was also elevated in HV 30 minutes after the stress protocol ($P = .006$).

Subjective Response to Stress Protocol

As assessed by visual analogue scale (VAS), patients with UC and HV rated the stress protocol as stressful with an increase in the median VAS score of 2.5 units in UC (3 [interquartile range (IQR), 1–5] units vs 5.5 [IQR, 4–8] units, $P < .0001$) and 2.5 units in HV (2 [IQR, 2–3] units vs 4.5 [IQR, 3.5–6.0] units, $P = .008$). The 1 patient with UC and 2 HV who scored the

Table 2. Total Leukocyte Count, Natural Killer Cell Number, Platelet Activation, and Platelet-Leukocyte Aggregate Formation in Response to Stress and Control Protocols in Patients With UC and Healthy Volunteers

Protocol	N		Before	Immediately after	30 min after
Stress					
UC	25	WBC	6.3 (5.3–8.1)	7.0 (5.5–7.9)	7.3 (5.8–8.4)[a]
		NK count	6.4 (4.8–8.7)	7.6 (5.4–9.3)[a]	6.7 (4.0–8.8)
		P-selectin	2.2 (1.3–4.5)	3.5 (2.2–5.2)[a]	2.8 (2.1–4.6)[a]
		PLA	2.4 (1.7–3.3)	3.0 (2.4–3.9)[a]	3.0 (2.4–4.1)[a]
HV	11	WBC	5.7 (5–7.5)	6.2 (5.6–8.7)	6.7 (5.8–8.3)[a]
		NK count	6.2 (5.1–8.6)	7.4 (5.8–9.1)	5.8 (5.1–7.0)
		P-selectin	3.1 (2.4–3.7)	5.0 (2.5–6.3)[a]	3.8 (3.0–6.7)[a]
		PLA	3.4 (2.2–4.0)	3.6 (2.7–4.8)[a]	4.1 (2.8–5.4)
Control					
UC	10	WBC	7.4 (5.2–9.1)	7.7 (5.5–8.6)	7.8 (5.7–9.2)
		NK count	7.1 (3.2–9.4)	5.9 (3.7–9.0)	6.9 (4.0–8.0)
		P-selectin	3.1 (2.2–4.1)	3.5 (2.4–4.2)	3.0 (2.1–3.7)
		PLA	2.3 (1.7–3.2)	2.4 (1.8–3.3)	2.1 (1.8–2.9)
HV	11	WBC	5.4 (4.6–8)	5 (4.4–6.6)	4.9 (4.7–6.5)
		NK count	7.3 (6.1–10.1)	8.8 (6.0–10.6)	8.2 (5.6–9.9)
		P-selectin	3.1 (2.3–3.5)	2.9 (2.5–4.0)	3.2 (2.5–4.8)
		PLA	2.5 (2.2–3.8)	2.4 (2.0–3.4)	2.5 (2.0–4.2)

NOTE. Values are median and interquartile range.
Leukocyte count in white blood cells (WBC) as (cells $\times$ 10^4/mm^3), NK cells as percentage, platelet activation as percentage P-selectin expression, and PLA formation as percentage.
[a]$P < .05$ from baseline value.

stress procedure nonstressful on VAS showed no autonomic response or inflammatory response in the majority of variables measured.

Serum Cytokine Concentrations

Serum IL-6 and IL-13 concentrations were unaffected by stress in either UC or in HV (Table 1).

LPS-Stimulated Cytokine Production

The production of TNF-α by LPS-stimulated whole blood was increased in the sample taken 30 minutes after stress by 54% in patients with UC ($P = .004$) and by 94% in HV ($P = .03$). Median production of IL-6 by LPS-stimulated whole blood rose in the sample taken 30 minutes after the stress protocol by 11% in patients with UC ($P = .04$) (Table 1).

Leukocyte Count

Total white cell count (WBC) increased compared with baseline in the sample taken 30 minutes after stress by 16% (median) in patients with UC ($P = .01$) and 17% in HV ($P = .04$) (Table 2).

NK Cell Numbers

In patients with quiescent UC, median NK cell numbers, expressed as a percentage of lymphocytes and monocytes, were increased by 18% in the sample taken immediately after the stress protocol ($P = .0008$) but had returned to baseline in the sample taken 30 minutes later (Table 2).

Platelet Activation

The median percentage of platelets expressing P-selectin was increased by 65% in patients with UC ($P < .0001$) and by 61% in HV ($P = .04$) in the blood sample taken immediately after the stress protocol compared with the pretest sample. This percentage fell in the sample taken 30 minutes later but remained elevated compared with baseline (29% in patients with UC [$P = .001$] and 22% in HV [$P = .001$]) (Table 2).

PLA Formation

The median percentage of leukocytes forming PLA was increased by 25% in patients with quiescent UC ($P = .004$) and by 6% in HV ($P = .03$) in the blood sample taken immediately after the stress protocol compared with baseline. In patients with quiescent UC, PLA formation remained elevated by 25% in the sample taken 30 minutes later ($P = .002$) (Table 2).

Rectal Perimucosal Fluid Cytokine Levels

In patients with UC, the median TNF-α concentration in rectal perimucosal fluid was increased by 102% after the stress protocol ($P = .03$); the concentrations of IL-13, histamine, and substance P in the perimucosal fluid did not change (Table 3).

Mucosal ROM Production

The stress protocol increased median mucosal production of ROM by 475% ($P = .001$) (Table 3).

Table 3. Perimucosal Fluid Cytokine Levels, Reactive Oxygen Metabolite Production by Mucosal Biopsies, Rectal Blood Flow, and Histologic Score in Response to Stress and Control Protocol in Patients With Quiescent UC

Protocol	N	Before	After
Stress			
Perimucosal fluid cytokines			
TNF-α	25	12.8 (8.6–20.2)	25.8 (12.7–41.5)[a]
IL-13	25	10.1 (4.5–16.8)	10 (4.3–28.5)
Histamine	25	3.8 (.7–14.5)	3.1 (1.4–21.9)
Substance P	25	21 (0–96)	15 (0–24)
ROM production	17	64 (24–833)	368 (123–1569)[a]
Blood flow	25	122 (87–158)	95 (72–153)[a]
Histologic score	17	0 (0–.5)	0 (0–2)
Control			
Perimucosal fluid cytokines			
TNF-α	9	8.9 (0–15.5)	5.8 (.6–12.2)
IL-13	9	14.5 (8.5–20.1)	16.8 (9.2–25.2)
Histamine	9	2.0 (.1–12.2)	2.1 (.6–14.7)
Substance P	9	32 (0–94)	0 (0–5.2)
ROM production	9	31 (5–56)	25 (4–158)
Blood flow	9	111 (106–141)	111 (71–142)
Histologic score	9	0 (0–1)	0 (0–1)

NOTE. Values are median and interquartile range.
Perimucosal fluid cytokine levels are as pg/mL, ROM production by mucosal biopsies as photons/mcg/min, and rectal blood flow as arbitrary units.
[a]$P < .05$ from prestress value.

RMBF

RMBF fell by 22% (median) after stress ($P = .05$) (Table 3).

Histologic Assessment

Seventeen pairs of pre- and poststress biopsy specimens were available for assessment. There was no overall change in histologic score,[24] with 12 pairs scoring zero both before and after the stress protocol. However, in all 5 patients from whom there was a degree of inflammation present in the prestress biopsy specimen, there was an increase in histologic score in the poststress sample. The control protocol caused no change in histologic score in any of the pairs of biopsy specimens assessed (Table 3).

Comparison of Patients With Quiescent UC and HV

There were no differences between baseline pretest values in any of the variables measured between patients with quiescent UC and HV (Tables 1 and 2). There were also no differences in the changes elicited by the stress protocol in any variables between patients with inactive UC and HV (Tables 1 and 2).

Relationship Between Age and Responses to Stress Protocol

Although the patients with UC had a higher median age than the HV, age did not correlate with any of the changes seen in response to the stress protocol in either group.

Effect of Immunosuppressant Medication on Response to Stress Protocol

Four of the patients with UC undergoing the stress protocol were taking either thiopurines or methotrexate. This is too small a number to allow comparison with the response to stress by patients who were not taking immunosuppressants, but removal of these 4 patients from the analysis did not significantly alter any of the results reported above.

Psychometric Questionnaires

Patients with inactive UC scored higher than HV on the Hospital Anxiety Depression anxiety scale (HADS-A) (median, 7 [IQR, 5–10 vs 4 [IQR, 3–8], $P = .02$), the Hospital Anxiety Depression depression scale (HADS-D) (median, 3 [IQR, 2–4] vs 0 [IQR, 0–1], $P = .02$), the Perceived Stress Questionnaire (median, 62 [IQR, 53–70] vs 50 [IQR, 40–61], $P = .03$), and the Bradford Somatic Inventory (median, 9 [IQR, 5–15] vs 4 [IQR, 2–8], $P = .03$). Scores were similar for patients with UC and HV on the State Trait Anxiety Inventory State scale (STAI-S) (median, 33 [IQR, 25–40] vs 31 [IQR, 25–39]), a measure of anxiety at that moment, and the State Trait Anxiety Inventory Trait scale (STAI-T) (median, 36 [IQR, 33–43] vs 32 [IQR, 28–44]), a measure of anxiety over the long term. The differences

observed in scores on the HADS-D scale between patients with UC and HV may partly be explained by the higher median age of patients with UC because HADS-D scores were found to correlate with age within the UC group ($R = +0.35$, $P = .01$). No correlation was found between age and scores on any of the other psychometric questionnaires used. None of the measures of long-term stress (STAI-T, HADS, PSQ, or BSI) correlated with the changes in any of the variables measured in response to the stress protocol. However, scores on the STAI-S scale did correlate with changes in NK cells levels ($R = +0.56$, $P = .004$) and PLA formation ($R = +0.43$, $P = .01$). There were no differences in scores of any of the psychometric questionnaires between patients with UC undergoing the stress or control protocols.

Effects of Control Protocol

The control protocol caused no changes in any of the variables assessed in either patients with inactive UC or HV (Tables 1–3).

Discussion

In this study, experimental stress caused increases in a range of inflammatory variables, each of which could contribute to stress-induced relapses in UC.

LPS-Stimulated Cytokine Production

Other studies have shown that stress alters cytokine production by whole blood in healthy volunteers. Blood taken from students before an examination produced more TNF-α and IL-6 when stimulated with LPS than blood taken after an examination.[28] Although the mechanism by which this occurs is unknown, adrenaline infusion has been shown to increase LPS-stimulated production of IL-8 and IL-10 by whole blood, and it is likely that sympathetic activation is important in mediating this effect.[29,30] TNF-α is a pivotal cytokine in the pathogenesis of IBD,[11] and the therapeutic benefit of anti-TNF-α antibodies has been shown in both Crohn's disease[31] and UC.[32] IL-6 is also a potentially important inflammatory cytokine in IBD[33] and is the stimulus for C-reactive protein production.[34] It is at least conceivable that psychologic stress could worsen UC by increasing the production of TNF-α and IL-6 by leucocytes subsequently stimulated by exposure to bacterial products such as LPS.

NK Cell Numbers

Increases in circulating NK cell number are consistently found in association with acute stress.[8] Although traditionally considered a component of the innate immune system, more recently, NK cells have also been shown to affect adaptive immunity via interactions with dendritic cells.[35] NK cells can localize to areas of inflammation and interact with immature dendritic cells to stimulate their maturation and proliferation, which in turn influences their interactions with T cells. However, the increases in NK cell number in this study were short-lived, having resolved within 30 minutes of the stress test. It therefore seems unlikely that changes in NK cell number play a major role in mediating stress-induced increases in IBD activity.

Platelet Activation and PLA Formation

Platelet activation and PLA formation have been shown to be increased by experimental stress in healthy subjects.[36,37] We have found stress to have similar effects in quiescent UC. Beta-adrenergic stimulation may underlie this effect because exercise-induced platelet activation can be prevented by β-blockers.[36] Platelets circulate in a more activated state in patients with IBD, and platelet activation may contribute to pathogenesis through direct proinflammatory effects and by causing mucosal thrombosis and microinfarction as a result of microvascular ischemia.[13] PLA formation is also increased in IBD and may facilitate extravasation of leukocytes to sites of mucosal inflammation.[14] Increases in both platelet activation and PLA formation had not resolved in blood taken 30 minutes after stress, and it is possible that increases in these variables could be partly responsible for stress-induced increases in IBD activity.

Rectal Perimucosal TNF-α, Histamine, and Substance P

It has been proposed that stress-induced increases in inflammation are mediated through increases in intestinal permeability, allowing exposure of the mucosal immune system to bacterial flora. Restraint stress in rodents increased epithelial permeability to inert marker molecules such as EDTA and to antigenic proteins such as horseradish peroxidase[38] and increased the phagocytic uptake of *Escherichia coli* into follicular-associated epithelium.[39]

The effects of restraint stress in these experiments did not occur in mast cell–depleted animals, and it is likely that mucosal mast cell degranulation is an important step in mediating the proinflammatory effects of stress on the gastrointestinal tract.[40,41] Physical stress, caused by immersion of the hand in iced water, has been shown to increase the proportion of activated and degranulating mast cells seen on electron microscopy in healthy controls and even more so in patients with IBD. Mast cell granules contain a range of inflammatory cytokines in-

cluding histamine and TNF-α.[42] In vitro studies have shown that increases in ileal permeability are dependent on the production and release of TNF-α.[43] In this study, we have shown an increase in the concentration of TNF-α, but not of histamine, in rectal perimucosal fluid after stress, and this could conceivably lead to an increase in mucosal permeability and consequently inflammation.

The stimulus for mast cell degranulation after stress is likely to involve the release of specific neuropeptides from the enteric and autonomic nervous systems. One candidate neuropeptide, substance P (SP), has been shown to increase the release of mast cell mediators from colonic biopsy specimens in patients with UC.[7] SP also has direct proinflammatory effects, stimulating IL-8 secretion from epithelial cells.[44] In our study, stress did not increase the concentration of SP in rectal perimucosal fluid. This result may partly reflect the sensitivity of our method for assessing SP release because, in many samples, the SP concentrations were below the limit of detection.

Rectal Mucosal ROM Production

Mucosal ROM production is increased in active compared with inactive UC[15] and may play a pathogenic role in IBD. Short-term mental stress has been shown to increase the oxidative activity of neutrophils in peripheral blood from HV.[45] In this study, we have shown that psychologic stress increases rectal mucosal ROM production, an effect that could contribute to mucosal damage.

RMBF

Psychologic stress reduced RMBF in patients with quiescent UC as it does in patients with irritable bowel syndrome and in HV.[9] This is thought to be due to alterations in autonomic tone, with stress increasing sympathetic and reducing parasympathetic activation. It is conceivable that stress-induced reductions in RMBF could trigger relapse by causing mucosal ischaemia.

Histologic Assessment

The failure to find an overall increase in inflammation in pre- and poststress rectal biopsy specimens may be partly due to the short time course of the protocol and the relatively mild nature of the stressor. However, we did observe an increase in histologic score in all 5 pairs of biopsy specimens in patients in whom there was a degree of inflammation[24] already present prior to the protocol. This observation might suggest that, in individuals who are "primed," with a degree of inflammation already present, acute stress is able to worsen microscopic inflammation.

Comparison of Patients With UC and Healthy Controls

Previous work has suggested that patients with IBD may have a relative imbalance of hypothalamic-pituitary-adrenal (HPA) axis and autonomic function, with autonomic hypereflexia, and that this may be important in driving mucosal inflammation.[46,47] However, in our study, there were no differences observed between patients with UC and HV in both their autonomic and systemic inflammatory responses to the stress protocol. This would suggest that, whereas stress may act as a trigger for exacerbations of UC, an exaggerated autonomic and inflammatory response to stress is not a primary etiologic factor in UC.

Psychometric Questionnaires

In animal studies, maternal deprivation in infancy, a model of chronic stress, rendered the adult rat more susceptible to the effects of acute restraint stress in augmenting dextran sulphate-induced colitis.[48] Levenstein et al found high scores on the Perceived Stress Questionnaire to be predictive of relapse in patients with inactive UC.[20] In our study, acute anxiety scores in patients with UC, as measured by the STAI-S scale, directly correlated with stress-induced NK cell numbers and PLA formation. However, whereas patients with inactive UC reported higher chronic stress levels (PSQ score), were more anxious (HADS-A score) and somatized (BSI score) more than healthy volunteers, scores in these indices did not correlate with the changes observed in any of the inflammatory variables in response to the stress protocol.

Study Limitations

For ethical reasons, and because its inclusion would have likely reduced recruitment of HV, we did not consider it appropriate to perform sigmoidoscopy with assessment of cytokine concentrations in perimucosal fluid, ROM production by mucosal biopsies, and RMBF in this group. Therefore, it is not possible to conclude from this study whether the stress-induced mucosal inflammatory responses observed in patients with UC also occur in healthy controls.

The study sample size of both the UC and HV groups was small, limiting the generalizability of the findings. Because, however, stress-induced changes were observed in the majority of variables measured, we believe that there were few type II errors.

In conclusion, we have shown that stress has a range of proinflammatory effects in patients with quiescent UC at both systemic and mucosal levels. These observations may help to explain how acute stress could cause relapse

in patients with inactive UC. The therapeutic benefits of stress reduction therapy in UC remain largely unexplored, in part because of the methodologic difficulties of such studies: the results to date of such studies are conflicting.[2] However, the data presented here add weight to the proposal that stress is a risk factor for relapse in UC, and we suggest that investigation of the therapeutic potential of stress reduction should be undertaken.

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Address requests for reprints to: D. S. Rampton, MD, Prof, Endoscopy Unit, Royal London Hospital, London E1 1BB, United Kingdom. e-mail d.rampton@qmul.ac.uk; fax: (44) 207 377 7441.

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