

Evidence for aberrant regulation of the p21Ras pathway in PBMCs of patients with chronic idiopathic urticaria

Ronit Confino-Cohen, MD,^a Dorit Aharoni, PhD,^{b,c} Arnon Goldberg, MD,^a
Irena Gurevitch, MD,^a Andreas Buchs, MD,^{b,c} Mordechai Weiss, MD,^b
Joshua Weissgarten, MD,^d and Micha J. Rapoport, MD^{b,c} Tel Aviv, Israel

Background: Recent data suggest that a subpopulation of patients with chronic urticaria has an autoimmune disorder. Aberrant expression and regulation of the p21Ras pathway has been reported in lymphoid cells in a variety of systemic autoimmune diseases but not in chronic idiopathic urticaria (CIU).

Objectives: The aim of this study was to examine the expression, regulation, and function of the p21Ras pathway in patients with CIU.

Methods: Twenty-four patients with CIU and 14 control subjects were enrolled. All patients and 9 control subjects were intradermally injected with autologous serum. PBMCs were isolated, and the p21Ras and its regulatory proteins were studied.

Results: We found increased expression of the p21ras proto-oncogene in patients with CIU. This was associated with a low expression of the p21Ras stimulatory element human son of sevenless (hSOS1) but normally expressed p21Ras inhibitory element p120GTPase-activating protein. The basal nonstimulated membrane/cytoplasmic ratio of hSOS1, which indicates the p21Ras pathway activity, was higher in patients compared with that seen in control subjects. Moreover, after stimulation, both patients and control subjects decreased their hSOS1 membrane/cytoplasmic ratio. The magnitude of this decrease was much higher in patients than in control subjects: 14- and approximately 2-fold, respectively. The basal and stimulated activities of the p21Ras downstream key regulatory enzyme mitogen-activated protein kinase were comparable in patients and control subjects, as was their *in vitro* mitogen-stimulated lymphocyte proliferation.

Conclusion: Our data demonstrate for the first time an aberrant signaling through the p21Ras pathway in lymphocytes of patients with CIU. This finding further supports the autoimmune basis of this disease. (*J Allergy Clin Immunol* 2002;109:349-56.)

Key words: Human, signal transduction, T lymphocytes, chronic idiopathic urticaria, autoimmunity, p21Ras, human son of sevenless, p120GTPase-activating protein

Abbreviations used

CIU:	Chronic idiopathic urticaria
ERK:	Extracellular signal-regulated kinase
GDP:	Guanine nucleotide diphosphate
GTP:	Guanine nucleotide triphosphate
hSOS1:	Human son of sevenless
M/C:	Membrane/cytoplasmic
MAP-kinase:	Mitogen-activated protein kinase
p120GAP:	p120GTPase-activating protein
SLE:	Systemic lupus erythematosus
T-PBS:	0.05% Tween-20/PBS

Chronic idiopathic urticaria (CIU) is a common form of chronic urticaria in which the cause is not detected after intensive clinical and laboratory investigation.^{1,2} In the past few years, it has been observed that intradermal injection of autologous serum elicits immediate wheal-and-flare responses in 60% of the patients with CIU.^{3,4} This skin reactivity is due to cutaneous mast-cell degranulation.^{5,6} Subsequent work demonstrated the presence of the IgG autoantibodies anti-IgE and IgG anti-FcεRI in sera from most patients with CIU.⁷⁻¹² In view of this data, it was suggested that at least part of the patients with CIU have an autoimmune process induced by the anti-IgE and anti-FcεRI antibodies targeted at basophils and mast cells.⁷⁻¹³ However, the mechanisms involved in the regulation and activation of the immune cells in patients with CIU have not yet been investigated. The p21Ras proto-oncogene product is a key regulatory protein that transduces a variety of extracellular signals, activating tyrosine kinase-associated and nonassociated membrane receptors and leading to cell growth and differentiation.¹⁴ The p21Ras protein is involved in both T-cell receptor activation and in IL-2 receptor activation, suggesting a pivotal role of this protein in mediating immunologic responses.¹⁵ The activity of p21Ras is determined by the ratio between its active guanine nucleotide triphosphate (GTP)-bound and inactive guanine nucleotide diphosphate (GDP)-bound forms. The p120GTPase-activating protein p120GAP and other GAP-like proteins downregulate the p21Ras activity by accelerating its relatively weak intrinsic GTPase activity, whereas the guanine nucleotide-releasing factors, such as son of sevenless (SOS), enhance its activity by accelerating the dissociation of prebound GDP.^{16,17} Migration of SOS from the cytosol to the cell membrane and its association with p21Ras through an adapter protein,

From ^athe Allergy and Clinical Immunology Unit, Sapir Medical Center, Kefar Saba, ^bthe Diabetes and Endocrinology Unit, ^cthe Department of Internal Medicine "C," and ^dthe Nephrology Service, Assaf Harofeh Medical Center, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel.

Received for publication July 24, 2001; revised October 19, 2001; accepted for publication October 23, 2001.

Reprint requests: Micha J. Rapoport, MD, Department of Internal Medicine "C," Assaf Harofeh Medical Center, Zerifin 70300, Israel.

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0091-6749/2002 \$35.00 + 0 1/85/121314

doi:10.1067/mai.2002.121314

TABLE I. Demographic and clinical characteristics of patients with CIU and control subjects

	Activity	Sex	Age (y)	Skin test (mm)*	Disease duration (mo)
Patients					
1	Active	F	25	—	42
2	Active	F	42	27	7
3	Active	F	47	29	9
4	Active	M	68	—	48
5	Active	F	38	24.5	360
6	Active	F	31	21	3
7	Active	F	80	24	6
8	Active	F	71	17	8
9	Active	F	36	—	6
10	Active	M	17	14	13
11	Active	F	37	20	80
12	Active	F	29	19	6
13	Nonactive	F	56	—	3
14	Nonactive	F	49	12	5
15	Nonactive	M	67	—	16
16	Active	M	78	16	36
17	Nonactive	F	63	22	40
18	Active	F	43	10	6
19	Active	M	40	16	72
20	Active	F	30	18	4
21	Active	F	43	—	9
22	Active	M	61	—	4
23	Active	F	56	20	5
24	Active	F	32	8.5	11
Mean $\pm$ SD			47.46 $\pm$ 17.04		33.29 $\pm$ 71.48
Control subjects					
1		F	50	ND	
2		F	29	ND	
3		M	28	ND	
4		F	40	ND	
5		M	37	—	
6		F	34	—	
7		M	29	—	
8		F	41	—	
9		F	50	—	
10		F	31	—	
11		F	42	—	
12		M	65	—	
13		F	59	—	
14		M	48	—	
Mean $\pm$ SD			41.64 $\pm$ 15.64		

ND, Not done.

*Skin test reactivity to autologous serum injection was calculated as described in the "Methods" section.

namely Grb2, is an early event in p21Ras pathway signaling.^{18,19} Thus the ratio of membrane and cytosolic fractions of SOS is an indirect indicator of p21Ras pathway signaling.²⁰ Generation of GTP-bound p21Ras results in activation of downstream regulatory key enzymes, including Raf-1 and the family of mitogen-activated protein kinases (MAP-kinases), which ultimately regulates the transcription of cellular growth and differentiation genes.²¹ We previously reported an aberrant signaling through the p21Ras pathway in patients with 2 other autoimmune diseases, namely type I dia-

betes mellitus^{22,23} and systemic lupus erythematosus (SLE)²⁴; a reduced stimulated GTP-bound p21Ras and decreased expression of the human son of sevenless (hSOS1) p21Ras stimulatory element, respectively. Expression of p21Ras itself and its inhibitory element, p120GAP, were normal. These data suggested that aberrant signaling of the p21Ras pathway may be a common finding in lymphocytes of patients with autoimmune diseases. We demonstrate for the first time that PBMCs of patients with CIU are characterized by a signaling defect along the p21Ras pathway.

METHODS

Patients

Twenty-four patients (mean age, 47.4 $\pm$ 17.0 years; 18 women and 6 men) and 14 healthy control subjects (mean age, 41.6 $\pm$ 15.6; 9 women and 5 men) participated in this study.

Inclusion criteria for the patients' group were the following:

1. history of frequent (>2/week) urticarial rash for at least 6 weeks;
2. normal physical examination, except for urticarial lesions;
3. normal laboratory work-up that included sedimentation rate, complete blood cell count, blood chemistry, complement levels (C3 and C4), thyroid-stimulating hormone, anti-nuclear antibodies, anti-microsomal antibodies, anti-thyroglobulin antibodies, normal urine examination, and no parasites in 3 stool samples;
4. no gingival inflammation or vaginal infection (as judged by a dentist and gynecologist, respectively).

All patients stopped treatment with H₁ blockers for at least 48 hours before blood sampling and autologous serum injection, except for one patient (patient 15). None of the patients was treated with corticosteroids or other immunosuppressive therapies at the time of venipuncture or had any other significant acute or chronic disease at the time of enrollment. At the time of examination, 4 of the 24 patients with CIU were in remission for at least 10 days and therefore were defined as having nonactive disease (Table I). The institutional committee for human experimentation approved this study.

Autologous serum injection

Serum was obtained from venous blood samples. For serum preparation, blood was clotted at room temperature for 30 minutes in silicone-coated vacutainer tubes, followed by centrifugation at 550g for 4 minutes. Fifty microliters of the freshly prepared serum was injected intradermally on the volar surface of the forearm. Normal saline and histamine (0.1 mg/mL) were also injected as negative and positive controls, respectively. All injection sites were scored 45 minutes after inoculation. The largest diameter (D1) of the wheal reaction and the diameter perpendicular to this (D2) were recorded. Results were expressed according to the following formula:

$$D1 + D2/2.$$

Reactions showing a mean diameter of more than 5 mm were considered positive.²⁵

Antibodies and reagents

Rabbit anti-GAP polyclonal antibody and rabbit anti-hSOS1 polyclonal antibody were purchased from Santa Cruz Biotechnology Inc. Monoclonal anti-activated MAP-kinase antibody (diphosphorylated extracellular signal-regulated kinase [ERK] 1 and ERK-2) was purchased from Sigma Israel Chemicals. Purified ascites containing Y13-259 anti-p21v-H-ras mAb was kindly donated by Dr G. B. Mills (Toronto General Hospital). PHA-P was purchased from Sigma, and Ficoll Hypaque was purchased from Pharmacia Biotech.

In vitro proliferation assay

Twenty milliliters of blood was drawn from an antecubital vein into heparinized tubes from each patient. PBMCs were isolated by means of Ficoll-Hypaque gradient centrifugation. Cells were plated in RPMI medium (2×10^5 cells/0.2 mL in round-bottom microtiter plates) supplemented with 5% heat-inactivated bovine calf serum, 2 mmol/L glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin (Biological Industries). Cells were cultured in the presence of increasing concentrations of PHA (2.5–20 μ g/mL) in an air-humidified 37°C incubator. After 72 hours in culture, the proliferative response to PHA was assessed in triplicates by means of tritiated thymidine uptake (20-hour pulse).

Preparation of cell lysates

Cells (10×10^6) were stimulated with 10 μ g/mL PHA for 15 minutes at 37°C. At the end of stimulation, the supernatant was discarded, and cells were incubated for 10 minutes on ice in the presence of cold lysis buffer containing 50 mmol/L HEPES (pH 7.2), 150 mmol/L NaCl, 1.5 mmol/L $MgCl_2$, 1 mmol/L EGTA, 1% Triton X-100, 10% glycerol, 1 mmol/L Na-orthovanadate, 30 mmol/L Na-pyrophosphate, 1 mmol/L phenylmethylsulfonyl fluoride, 10 μ g/mL leupeptin, and 10 μ g/mL aprotinin and centrifuged for 5 minutes to remove the DNA. A modified hypotonic lysis buffer (NaCl, Triton, and glycerol-free 20 mmol/L HEPES) was used for separation of membranes and cytoplasm fractions. Cell lysates were centrifuged at high-speed centrifugation for 30 minutes at 4°C. The supernatant containing the cytoplasmic fraction was separated, and the pellet containing the membranes was dissolved in hypotonic buffer with 1% Triton X-100 and centrifuged for an additional 5 minutes to remove the DNA.

Western blot analysis

Lysates from 10×10^6 cells were boiled for 7 minutes in the presence of sample buffer. Equal amounts of proteins (150 μ g/lane) were separated on SDS-PAGE by using a 4% polyacrylamide upper gel and a 10% polyacrylamide lower gel and transferred to nitrocellulose membrane. Nonspecific binding sites on the nitrocellulose membrane were blocked by means of 1-hour incubation with 1% BSA dissolved in 0.05% Tween-20/PBS (T-PBS). The nitrocellulose membrane was reacted for 1 hour at 24°C with Y13-259 anti-p21v-H-ras rat mAb (3 μ g/mL), rabbit anti-GAP polyclonal antibody (0.4 μ g/mL), rabbit anti-hSOS1 polyclonal antibody (4 μ g/mL), and mouse mAb (4 μ g/mL) against the phosphorylated form of the activated MAP kinase enzymes ERK1 and ERK2,²⁶ followed by 6 washings with T-PBS. Membrane was then incubated for 1 hour at 24°C with anti-rat IgG, anti-rabbit IgG, or anti-mouse IgG conjugated to horseradish peroxidase (1:10,000), followed by 6 washings with T-PBS. Detection was carried out by using the enhanced chemiluminescence method with a detection kit purchased from Pierce. Protein expression was determined by means of photodensitometry (Computing densitometer, Molecular Dynamics, Model 300A).

RESULTS

Autologous serum reactivity

All patients were intradermally injected with autologous serum, as well as with normal saline and histamine. In all patients skin tests with histamine elicited positive results, whereas saline elicited negative results. Positive skin reactivity to autologous serum was observed in 17 (71%) of 24 patients (Table I). Ten of 14 healthy control subjects were also injected with autologous serum, and all had negative results.

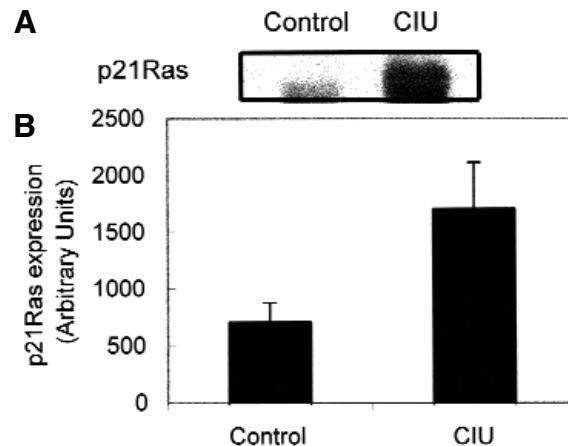


FIG 1. Increased p21Ras expression in patients with CIU. PBMCs were lysed, and Western blot analysis was performed with specific antibody against p21Ras. **A**, Representative Western blot demonstrating p21Ras expression in control subjects and patients with CIU. **B**, Expression of p21Ras was determined by means of photodensitometry in whole-cell lysate of normal control subjects and patients with CIU. Data are means $\pm$ SEM of each group and expressed in arbitrary units.

Expression of p21Ras in patients with CIU

Expression of p21Ras in whole-cell lysate was significantly increased in patients compared with in control subjects (1700 ± 410 and 706 ± 170 , respectively, $P = .02$; Fig 1). When analyzed according to their response to autologous serum injection, there was no difference in p21Ras expression between patients with positive and negative skin reactivity. No correlation was found between p21Ras expression and any of the clinical or demographic parameters of patients and control subjects (data not shown). These data suggest that p21Ras expression is increased in PBMCs of patients with CIU.

Expression of p21Ras regulatory elements hSOS1 and p120GAP in patients with CIU

To further examine the regulation on the p21Ras pathway in patients with CIU, we studied the expression of the p21Ras regulatory elements hSOS1 and p120GAP in whole-cell lysate PBMCs. Fig 2 demonstrates that levels of hSOS1 were decreased in patients compared with those seen in control subjects (140 ± 50 and 340 ± 85 , respectively; $P = .026$). In contrast, no difference was observed in the mean levels of p120GAP between the 2 groups (340 ± 115 and 540 ± 150 for patients and control subjects, respectively). When analyzed according to skin reactivity to autologous serum injection, patients with a negative response demonstrated an hSOS1 expression comparable with that of the control subjects (260 ± 120). In contrast, patients with positive skin test responses demonstrated a significant decrease in hSOS1 expression compared with that seen in control subjects (100 ± 45 and 340 ± 85 , respectively; $P = .02$). No difference in the levels of hSOS1 or p120GAP was found between

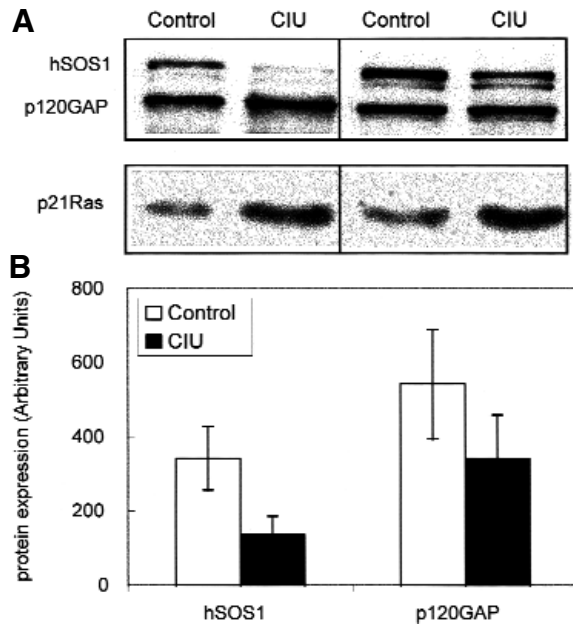


FIG 2. Decreased expression of hSOS1 with normal p120GAP expression in patients with CIU. **A**, Representative Western blot of hSOS1, p120GAP, and p21Ras expression of 2 different patients with CIU with positive skin reactions and their paired control subjects. Reduction in hSOS1 expression is associated with elevated levels of p21Ras within the same patients. **B**, Expression of hSOS1 and p120GAP in patients with CIU (filled bars) and control subjects (open bars) was determined by means of photodensitometry. Data are means $\pm$ SEM of each group and expressed in arbitrary units.

patients with active and nonactive disease (data not shown). These data demonstrate that the overall expression of the p21Ras stimulatory element hSOS1 is down-regulated in PBMCs of patients with CIU, whereas the expression of its inhibitory element is normal.

Increased membrane/cytoplasmic ratio of hSOS1 in patients with CIU

The membrane/cytoplasmic (M/C) ratio of hSOS1 serves as an indirect indicator of the activation of the p21Ras pathway.²⁷ Therefore we separately determined the levels of hSOS1 in isolated membrane and cytoplasmic fractions in stimulated and nonstimulated PBMCs from patients with CIU and calculated their mean M/C hSOS1 ratio. In the basal nonstimulated state the mean M/C hSOS1 ratio was approximately 4-fold higher in patients than in control subjects (14 ± 3.2 and 3.7 ± 0.9 , respectively; $P = .01$). After exposure to PHA (for 15 minutes), this ratio was significantly decreased in the patients but not in the control subjects (1 ± 0.35 and 2.3 ± 1.2 , respectively; $P = .003$). The magnitude of this decrease was much larger in the patients than in the control subjects (14-fold vs approximately 2-fold decrease, respectively; Fig 3). Analysis of patients according to their skin test reactivity revealed that there was no difference in the basal and stimulated M/C hSOS1 ratio between patients with negative and positive skin test results. Patients with active and non-

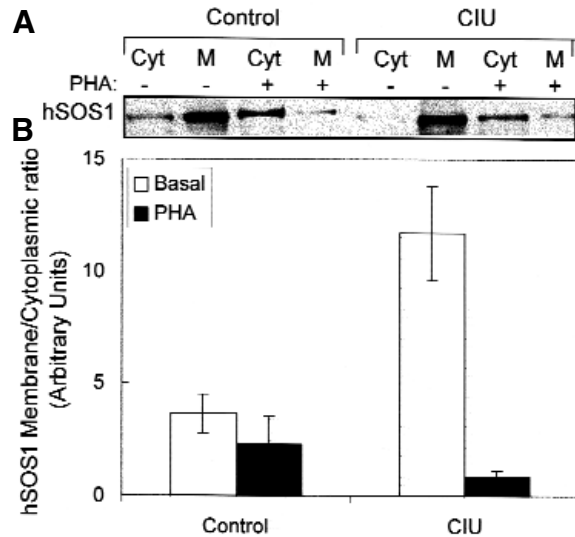


FIG 3. hSOS1 M/C distribution. **A**, Representative blot demonstrating hSOS1 expression in cytoplasm (Cyt) and membrane fractions (M) of nonstimulated and PHA-stimulated PBMCs of normal control subjects and patients with CIU. **B**, hSOS1 protein level in membrane and cytoplasmic fractions (means $\pm$ SEM of each group) were determined by means of photodensitometry, and the M/C ratio was calculated before (open bars) and after (filled bars) stimulation.

active disease also demonstrated a comparable decrease in their hSOS1 M/C ratio (data not shown). Taken together, these data demonstrate that the basal activity of the p21Ras pathway is upregulated in patients with CIU. Moreover, after cellular activation, the hSOS1 redistribution is different in patients with CIU and in normal control subjects.

Normal M/C ratio of p120GAP in patients with CIU

Membrane receptor-mediated p21Ras pathway signaling requires the presence of p120GAP at the cell membrane.²⁸ We examined the membrane-bound and cytoplasmic levels of p120GAP and calculated its M/C ratio in patients with CIU and control subjects. As can be seen in Fig 4, in the basal nonstimulated state this ratio was comparable in patients and control subjects (2 ± 0.6 and 2 ± 0.7 , respectively). After stimulation, this ratio was somewhat reduced in the control group (mean decrease of 0.9 ± 0.7) but not in the patients. No correlation was found between the p120GAP M/C ratio and skin reactivity to autologous serum or disease activity (data not shown). These data demonstrate that unlike hSOS1, the basal and stimulated cellular distribution of p120GAP remains unaltered in patients with CIU.

MAP-kinase activity in patients with CIU

To determine the effects of the upregulated M/C ratio and the different kinetics of hSOS1 on p21Ras signaling, we examined the basal and stimulated activities of the p21Ras downstream key regulatory enzyme MAP-kinase in patients with active and nonactive CIU. Fig 5, A, demonstrates that

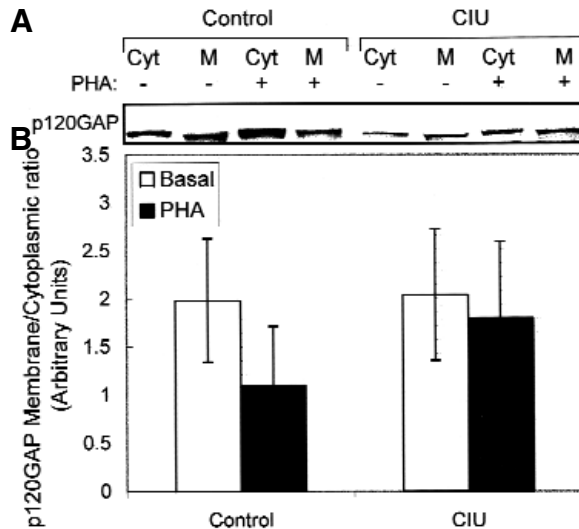


FIG 4. p120GAP M/C distribution. **A**, Representative Western blot demonstrating p120GAP expression in cytoplasm (Cyt) and membrane fractions (M) of nonstimulated and PHA-stimulated PBMCs of normal control subjects and patients with CIU with active disease. **B**, M/C ratio of p120GAP was determined before (open bars) and after (filled bars) stimulation. Protein levels (means $\pm$ SEM of each group) were determined by means of photodensitometry and expressed in arbitrary units.

the basal activity of MAP-kinase in whole-cell lysates, as indicated by its phosphorylated form, was comparable in patients and control subjects (3800 ± 1200 and 4400 ± 1500 , respectively). Exposure to PHA for 15 minutes resulted in a significant and comparable increase of MAP-kinase activity in the 2 groups (9800 ± 2400 and 9900 ± 2900 for patients and control subjects, respectively; Fig 5, B).

No correlation was found between MAP-kinase activity and the clinical parameters of the patients, including age, sex, disease activity, disease duration, or skin reactivity to autologous serum injection (data not shown). Taken together, these data indicate that the late signaling events of the p21Ras pathway remain unaltered in patients with CIU.

Proliferative response of patients with CIU is normal

To examine whether the aberrant expression and signaling of the p21Ras pathway in PBMCs of patients with CIU affected their cellular activation, we determined their proliferative response. Fig 6 demonstrates that the proliferative response was comparable in patients and control subjects (stimulation index of 62 ± 8 and 50 ± 14 , respectively). Thus the different expression of p21Ras and hSOS1, as well as the increased hSOS1 M/C ratio, in lymphocytes of patients with CIU did not modify the cellular activation sufficiently to affect the proliferative response.

DISCUSSION

This pilot study provides the first evidence for disturbed signaling through the p21Ras pathway in lympho-

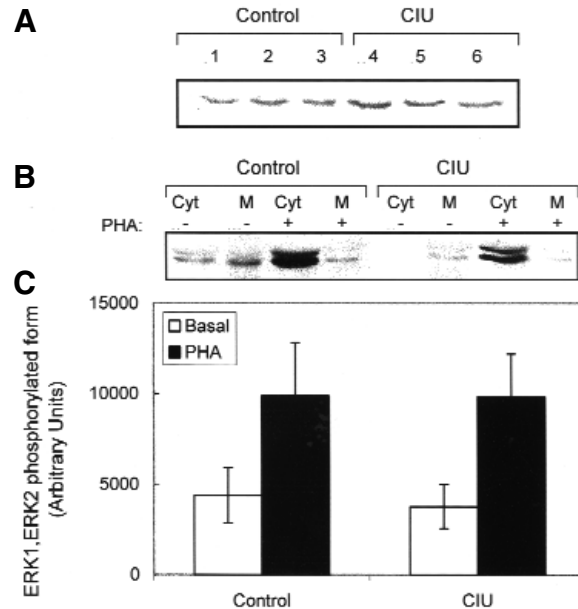


FIG 5. Similar MAP-kinase (ERK1 and ERK2) activity in patients with CIU and control subjects. **A**, Representative Western blot demonstrating the basal ERK1 and ERK2 activity (phosphorylated form) in nonstimulated PBMCs of normal control subjects (lanes 1, 2, and 3) and patients with CIU (lanes 4, 5, and 6). **B**, Representative Western blot of phosphorylated ERK1 and ERK2 in fractions of cytoplasm (Cyt) and membranes (M) before and after stimulation. **C**, Levels of phosphorylated ERK1 and ERK2 were determined before (open bars) and after (filled bars) PHA stimulation by means of photodensitometry in whole-cell lysates (cytoplasm plus membrane fractions) of patients with CIU and control subjects and expressed in arbitrary units. Data are means $\pm$ SEM of each group.

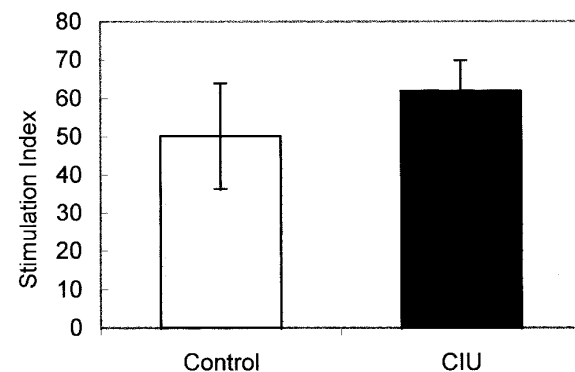


FIG 6. In vitro proliferative response to PHA of PBMCs from patients with CIU and normal control subjects. PBMCs were isolated from patients (filled bars) and control subjects (open bars), as previously described. Maximal proliferative response to PHA (2.5-20 μ g/mL) was assessed after 3 days by means of tritiated thymidine uptake (20-hour pulse). Values $\pm$ SEM are expressed in SI units calculated as the maximal proliferation in counts per minute divided by the background unstimulated value of each patient.

cytes of patients with CIU. The expression of p21Ras and its stimulatory regulatory element hSOS1, but not p120GAP, was abnormal in our patients. Furthermore, the basal nonstimulated activity of the p21Ras pathway, as

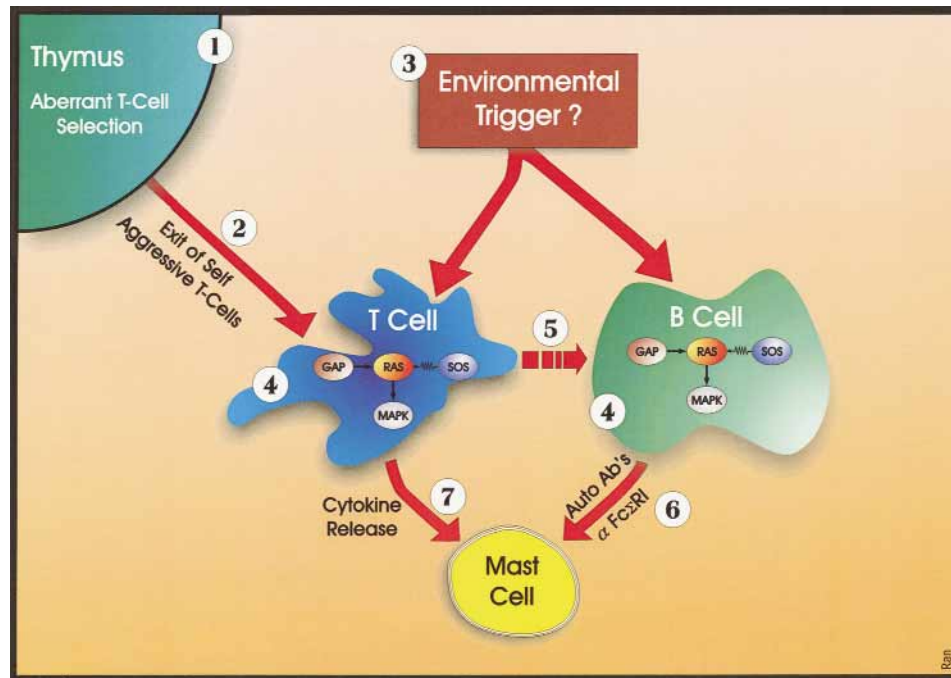


FIG 7. Aberrant regulation of the p21Ras pathway in lymphocytes of patients with CIU: possible mechanisms leading to autoimmune disease. Genetically determined aberrant p21Ras signaling interferes with thymic T-cell selection (1), leading to a release of self-aggressive T cells (2). Alternatively, exposure to an unknown environmental trigger (3) results in an aberrant signaling in B and T cells (4) and disrupts immune balance. T cell-assisted (5) production of anti-FcεRI autoantibodies (*auto Abs*) by B cells (6) and a direct interaction of autoimmune T cells with mast cells (7) lead to autoimmune chronic urticaria.

reflected by the hSOS1 M/C ratio, was upregulated in these patients, and cellular activation resulted in different kinetics of hSOS1 redistribution in patients with CIU compared with normal control subjects. Taken together, these data demonstrate that the p21Ras pathway activity is aberrant in patients with CIU. It is of interest that the decreased hSOS1 expression was present only in patients with positive skin test responses. This suggests that these 2 findings (ie, autoimmune skin reaction and decreased hSOS1 expression) are associated and lends further support to the autoimmune basis of this disease. How aberrant p21Ras signaling may contribute to the development of autoimmune chronic urticaria remains speculative. P21Ras has a major role in membrane receptor-mediated T-cell signaling,^{15,29} which is a key determinant in the early immune cells' positive and negative selection in the thymus and in the induction of self-tolerance. Thus an aberrant T-cell signaling through the p21Ras pathway during the early phase of T-cell maturation may result in the release of self-aggressive autoimmune T cells and ultimately promote autoimmune disease.^{30,31} It should be kept in mind that our findings were present in a mixed lymphocyte population. This may suggest that aberrant expression and function of the p21Ras pathway may be present in both T and B lymphocytes of patients with CIU. Indeed, it was previously reported that the p21Ras pathway is involved not only in regulation of T-cell activ-

ity but also in the B-lymphocyte response to antigenic stimuli.³² Thus aberrant p21Ras signaling in B cells of these patients may disrupt B-cell regulatory mechanisms, resulting ultimately in autoantibody production and autoimmune disease. These postulated mechanisms (Fig 7) derive further support from our findings of aberrant p21Ras expression and regulation in 2 other autoimmune diseases, namely SLE and type I diabetes.²²⁻²⁴

The expression of the p21Ras stimulatory element hSOS1 was decreased, and the expression of its inhibitory element p120GAP remained normal. This was associated with both increased p21Ras expression and hSOS1 M/C ratio. These seemingly contradictory findings could suggest that the increase in p21Ras expression and hSOS1 M/C ratio compensate for the low expression of hSOS1. It is also possible that p21Ras is being activated by a parallel signaling pathway or by an hSOS1-independent mechanism.³³ This is supported by the normal basal and stimulated MAP kinase activities, demonstrating that the early aberrant p21Ras signaling does not significantly affect the late p21Ras signaling, the overall p21Ras signaling, or both. Furthermore, the adequate lymphocyte proliferative response of these patients also suggests that the T-cell receptor-mediated p21Ras signaling is intact. Although this may be the case, we recently showed, in an animal model²² and in human patients,²³ that downstream signaling along the p21Ras pathway, namely accumulation of

GTP-p21Ras, is decreased in the presence of normal PBMC proliferative response. This suggests that the in vitro proliferative response is not a sensitive indicator of the p21Ras activity in these cells. Thus the absence of p21Ras GDP/GTP-binding studies that directly examine the activation of p21Ras does not allow us to determine whether the activation of p21Ras itself is altered in patients with CIU. Moreover, we used a nonspecific stimulation (ie, PHA) to examine the stimulated activity of the p21Ras pathway. It has been demonstrated in hematopoietic cells that the extent and mechanisms of the membrane receptor-mediated stimulation of the p21Ras pathway is ligand dependent.¹⁵ This indicates that further functional analysis of this pathway with other ligands or use of autologous serum as an antigenic stimulus is necessary to determine the extent and characteristics of the p21Ras pathway aberration in these patients. Moreover, several other downstream signaling pathways, such as JNK and p38, interact with the p21Ras pathway to regulate T-cell activation and function.³⁴ These pathways should be examined to determine whether additional signaling aberrations are present in patients with CIU.

It remains also to be determined whether p21Ras aberrant signaling can be exploited in the classification and management of CIU. We previously reported that decreased activity of the p21Ras pathway correlated with disease progression in autoimmune diabetes and that correction of this defect prevented disease.^{23,35} We also found recently that activity of the p21Ras/MAP-kinase pathway correlates with disease activity in patients with SLE (unpublished data, M. Rapoport). It is tempting to assume that similar findings may be found in patients with CIU. Indeed, the presence of a decreased hSOS1 expression restricted to patients with positive skin reactions may further define this subgroup of patients. Larger numbers of patients, including control groups with antigen-specific urticaria, and longitudinal studies are necessary to establish whether the M/C hSOS1 ratio or other parameters of the p21Ras pathway activity may be used to classify different patient populations, as well as to guide therapeutic approaches.

In conclusion, the clinical significance of aberrant expression and function of the p21Ras pathway in lymphocytes of patients with active CIU remains uncertain. Nevertheless, our data offer additional evidence in support of the hypothesis of autoimmune process as the pathogenic mechanism in CIU. They also underscore the need for further evaluation of the function of the p21Ras pathway in these patients.

REFERENCES

- Greaves M. Chronic urticaria. *J Allergy Clin Immunol* 2000;105:664-72.
- Elias J, Boss E, Kaplan AP. Studies of the cellular infiltrate of chronic idiopathic urticaria: prominence of T-lymphocytes, monocytes and mast cells. *J Allergy Clin Immunol* 1986;78:914-8.
- Grattan CEH, Wallington TB, Warin RP, Kennedy CTC, Bradfield JW. A serological mediator in chronic idiopathic urticaria—a clinical, immunological and histological evaluation. *Br J Dermatol* 1986;114:583-90.
- Grattan CEH, Boon AP, Eady RAJ, Winkelmann RK. The pathology of the autologous serum skin test response in chronic urticaria resembles IgE mediated late-phase reactions. *Int Arch Allergy Appl Immunol* 1990;93:198-204.
- Nathony SF, Philips ME, Elias JM, Godfrey HP, Kaplan AP. Histologic studies of chronic idiopathic urticaria. *J Allergy Clin Immunol* 1983;71:177-83.
- Leznoff A, Josse RG, Denberg J, Dolovich J. Association of chronic urticaria and angioedema with thyroid autoimmunity. *Arch Dermatol* 1983;119:636-40.
- Grattan CEH, Francis DM, Hide M, Greaves MW. Detection of circulating histamine releasing autoantibodies with functional properties of anti-IgE in chronic urticaria. *Clin Exp Allergy* 1991;21:695-704.
- Riske F, Hakimi J, Mallamaci M, Griffin M, Pilson B, Tobkes N, et al. High affinity human IgE receptor (Fc epsilon RI). Analysis of functional domains of the alpha-subunit with monoclonal antibodies. *J Biol Chem* 1991;266:11245-51.
- Hakimi J, Seals C, Kondas JA, Pettine L, Danho W, Kochan J. The α -subunit of the human IgE receptor (Fc ϵ RI) is sufficient for high affinity IgE binding. *J Biol Chem* 1990;265:22079-81.
- Hide M, Francis DM, Grattan CEH, Hakimi J, Kochan JP, Greaves MW. Autoantibodies against the high affinity IgE receptor as a cause of histamine release in chronic urticaria. *N Engl J Med* 1993;328:1599-604.
- Fiebiger E, Maurer D, Holub H, Reininger B, Hartmann G, Woisetschlager M, et al. Serum IgG autoantibodies directed against the alpha chain of Fc epsilon RI: a selective marker and pathogenic factor for a distinct subset of chronic urticaria patients? *J Clin Invest* 1995;96:2606-12.
- Ferrer M, Kinet JP, Kaplan AP. Comparative studies of functional and binding assays for IgG, anti-Fc epsilon RIalpha (alpha-subunit) in chronic urticaria. *J Allergy Clin Immunol* 1998;101:672-6.
- Niimi N, Francis DM, Kermani F, O'Donnell BF, Hide M, Kobza-Black A, et al. Dermal mast cell activation by autoantibodies against the high affinity IgE receptor in chronic urticaria. *J Invest Dermatol* 1996;106:1001-6.
- Bourne HR, Sanders DA, McCormick F. The GTPase superfamily: a conserved switch to diverse cell functions. *Nature* 1990;348:125-31.
- Izquierdo PM, Reif K, Cantrell D. The regulation and function of p21ras during T-cell activation and growth. *Immunol Today* 1995;16:159-64.
- Boguski MS, McCormick F. Proteins regulating Ras and its relatives. *Nature* 1993;366:643-54.
- Pawson T. Protein modules and signaling networks. *Nature* 1995;373:573-80.
- Li N, Batzer A, Dali R, Yajnik V, Skolnik E, Chardin P, et al. Guanine-nucleotide-releasing factor hSOS1 binds to Grb2 and links receptor tyrosine kinases to Ras signalling. *Nature* 1993;363:85-8.
- Buday L, Downward J. Epidermal growth factor regulates p21ras through the formation of a complex of receptor, Grb2 adapter protein, and SOS nucleotide exchange factor. *Cell* 1993;73:611-20.
- Salojin K, Zhang J, Cameron M, Gill B, Arreaza G, Ochi A, et al. Impaired plasma membrane targeting of Grb2-murine son of sevenless (mSOS) complex and differential activation of the Fyn-T cell receptor (TCR)-V-cbl pathway mediate T cell hyporesponsiveness in autoimmune nonobese diabetic mice. *J Exp Med* 1997;186:887-97.
- Rubinfeld H, Hanoch T, Seger R. Identification of a cytoplasmic retention sequence in ERK2. *J Biol Chem* 1999;274:30349-52.
- Rapoport MJ, Lazarus AH, Jaramillo A, Delovitch TL. Deficient T-cell receptor regulated p21Ras activation mediates thymic T-cell anergy and may predispose to diabetes in autoimmune NOD mice. *J Exp Med* 1993;177:1221-6.
- Rapoport MJ, Vardi P, Mor A, Ramot Y, Levi O, Bistrizter T. Defective activation of p21ras in activated peripheral blood mononuclear cells from patients with insulin dependent diabetes mellitus. *Autoimmunity* 1999;29:147-54.
- Rapoport MJ, Mor A, Amit M, Rosenberg R, Ramot Y, Mizrahi A, et al. Decreased expression of the p21ras stimulatory factor hSOS in PBMC from inactive SLE patients. *Lupus* 1999;8:24-8.
- Fagiolo U, Cancian M, Bertollo L, Peserico A, Amadori A. Inhibitory effect of heparin on skin reactivity to autologous serum in chronic idiopathic urticaria. *J Allergy Clin Immunol* 1999;103:1143-7.
- Yung Y, Dolginov Y, Yao Z, Rubinfeld H, Michael D, Hanoch T, et al. Detection of ERK activation by a novel monoclonal antibody. *FEBS Lett* 1997;408:292-6.
- Egan SE, Giddings BW, Brooks MW, Buday L, Sizeland AM, Weinberg RA. Association of Sos Ras exchange protein with Grb2 is implicated in signal transduction and transformation. *Nature* 1993;363:45-51.
- Huang DCS, Marshall CJ, Hancock JF. Plasma membrane-targeted GTPase-activating protein is a potent suppressor of p21ras function. *Mol Cell Biol* 1993;13:2420-31.
- Downward J, Graves J, Cantrell D. The regulation and function of p21ras in T cells. *Immunol Today* 1992;13:89-92.
- Finkel TH, Marrack P, Kappler JW, Kubo RT, Cambier JC. Alpha beta T

- cell receptor and CD3 transduce different signals in immature T cells. Implications for selection and tolerance. *J Immunol* 1989;142:3006-12.
31. Marrack P, Kappler J, Kotzin BL. Autoimmune disease: why and where it occurs. *Nat Med* 2001;7:899-905.
32. Lazarus AH, Kawauchi K, Rapoport MJ, Delovitch TL. Antigen-induced B lymphocyte activation involves the p21ras and rasGAP-signaling pathway. *J Exp Med* 1993;178:1765-9.
33. Izquierdo M, Downward J, Graves JD, Cantrell DA. Role of protein kinase C in T-cell antigen receptor regulation of p21Ras: Evidence that two p21Ras regulatory pathways coexist in T cells. *Mol Cell Biol* 1992;12:3305-12.
34. Dong C, Davis RJ, Flavell RA. Signaling by the JNK group of MAP kinases. C-jun N-terminal Kinase. *J Clin Immunol* 2001;21:253-7.
35. Rapoport MJ, Weiss L, Mor A, Bistritzer T, Ramot Y, Slavin S. Prevention of autoimmune diabetes by linomide in nonobese diabetic (NOD) mice is associated with up-regulation of the TCR-mediated activation of p21(ras). *J Immunol* 1996;157:4721-5.