0022-1767/87/1394-1185$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 139, 1185–1190, No. 4, August 15, 1987
Printed in U.S.A.

A ROLE FOR HISTAMINE TYPE II (H-2) RECEPTOR BINDING IN PRODUCTION OF THE LYMPHOKINE, SOLUBLE IMMUNE RESPONSE SUPPRESSOR (SIRS)[1]

H. WILLIAM SCHNAPER,[2*†] THOMAS M. AUNE,[3*†] AND RHONDA K. ROBY*

*From the *Department of Pathology, the Jewish Hospital of St. Louis, and the Departments of †Pediatrics and †Pathology, Washington University School of Medicine, St. Louis, MO 63110*

Soluble immune response suppressor (SIRS) is an immunosuppressive protein produced by human and murine suppressor cells activated by a variety of agents. Because histamine has been reported to activate suppressor cells, the possibility that it also induced SIRS production was investigated. Human lymphocytes treated with 10^{-4} M histamine for less than 1 hr released a suppressive substance into culture supernatants that was physically, functionally and antigenically similar to human SIRS. Cimetidine and ranitidine, structurally distinct histamine type II (H-2) receptor antagonists, prevented histamine-induced SIRS production. In further experiments, suppression of human polyclonal IgM PFC responses by Con A and interferons, substances that activate the SIRS pathway, was inhibited by H-2 receptor antagonists. Activation of lymphocytes to produce SIRS by Con A or interferons was blocked by cimetidine or ranitidine. These data demonstrate that production of SIRS is induced by histamine, and raise the possibility that H-2 receptor binding may play a role in the SIRS pathway.

Soluble immune response suppressor (SIRS)[4] is a protein, produced by interferon (IFN)- or concanavalin A (Con A)-activated suppressor T lymphocytes, which inhibits antibody production and tumor cell division in vitro (1) and plaque-forming cell (PFC) and delayed-type hypersensitivity responses in vivo (2). Murine SIRS has been purified to homogeneity (3), and experiments with human SIRS have determined that it has antigenic, functional, and physical similarities to its murine analog (4). The ability of monoclonal anti-murine SIRS to cross-react with human SIRS, and demonstration of a characteristic elution pattern on reversed-phase high-performance liquid chromatography (HPLC), have provided a means for detecting the presence of SIRS in other in vivo and in vitro systems. By these techniques it has been determined that SIRS is produced by humans with steroid-responsive nephrotic syndrome (5) and mice with experimental schistosomiasis (6).

The observation that SIRS is produced by activated human suppressor T lymphocytes raises the possibility that other agents which have been reported to activate suppressor cells could also induce SIRS production under appropriate conditions. One such agent that has received considerable attention is histamine. This substance activates suppressor cells to inhibit immunoglobulin production by B lymphocytes (7) and lymphocyte proliferation after mitogenic (8) or schistosomal antigenic (9) stimulation. Soluble suppressor factors produced by histamine-stimulated cells have been described by several groups of investigators (10–12). These factors may act through stimulation of prostaglandin synthesis (10, 11). Characterization of a histamine-induced suppressor factor has suggested that similar factors are also produced by antigen- or Con A-stimulated suppressor cells (13).

In the experiments reported in this paper, a potential role for histamine in SIRS production was investigated. Peripheral blood mononuclear cells (PBMC) incubated with histamine produced a soluble suppressor substance that was antigenically, functionally, and physically similar to SIRS. Histamine-induced SIRS production was prevented by the histamine H-2 receptor antagonists cimetidine and ranitidine. These agents also inhibited activation of the SIRS pathway by IFN or Con A. These data raise the possibility that histamine or binding of histamine H-2 receptors may play a role in the SIRS pathway.

MATERIALS AND METHODS

Unless otherwise noted, all chemicals were reagent grade and purchased from commercial sources. Con A and histamine were purchased from Sigma (St. Louis, MO). Human IFN-αA (IFLrA; Hoffman-La Roche, Nutley, NJ) prepared by recombinant DNA technology, was obtained from the Biopolymer Research and Immunotherapy Department of Hoffmann-La Roche. It was electrophoretically pure and had a specific activity of 2×10^8 U/mg protein. IFN-γ (Interferon Sciences, New Brunswick, NJ) was derived from PHA-activated human leukocytes and had a specific activity of 10^6 U/mg protein. Ranitidine powder was kindly provided by Glaxo, Inc., Morrisville, NC.

Production of SIRS by PBMC. Heparinized whole blood was obtained from healthy volunteers by venipuncture under sterile conditions, diluted 1/1 with Hanks' balanced salt solution, and centrifuged over a Ficoll-sodium diatriazoate gradient according to the methods of Böyum (14). PBMC were harvested from the plasma-Ficoll interface, washed with Hanks' balanced salt solution, and suspended at 2×10^6 cells/ml in RPMI 1640 medium supplemented with 10% fetal calf serum (Rehatuin; Armour Pharmaceuticals, Phoenix, AZ), L-glutamine, penicillin/streptomycin, and nonessential amino acids. It was previously determined (4) that the optimal protocol for activation of suppressor cells to produce SIRS is 24-hr incubation of PBMC with Con A, 30 μg/ml; IFN-αA, 10,000 U/ml; or

Received for publication March 11, 1987.
Accepted for publication May 11, 1987.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported by Council for Tobacco Research USA, Inc., Grant No. 1746, and U.S. Public Health Service Grant CA40567 from the National Cancer Institute.
[2] Recipient of Clinical Investigator Award DK01317 from the National Institute of Diabetes, Digestive and Kidney Diseases. Address reprints to Dr. Schnaper at Jewish Hospital of St. Louis, CSB-7, 216 S. Kingshighway, St. Louis, MO 63110.
[3] Current address: Genentech, Inc., 460 Point San Bruno Blvd., South San Francisco, CA 94080.
[4] Abbreviations used in this paper: SIRS, soluble immune response suppressor; HPLC, high-performance liquid chromatography; PBMC, peripheral blood mononuclear cell; $SIRS_{ox}$, activated SIRS.

IFN-γ, 300 U/ml. The cells are then washed, resuspended in medium for 24 hr, and centrifuged to obtain culture supernatant. The timing and dosage for histamine-induced SIRS production are discussed in *Results*.

Splenocyte culture assay for SIRS activity. Human spleen cells were obtained aseptically from cadaver kidney transplant donors at the time of organ harvest, processed as described (5), and preserved frozen in liquid nitrogen until used. Cultures of 2×10^6 cells in 1 ml supplemented RPMI 1640 medium were stimulated with pokeweed mitogen, 5 μg/ml, and incubated in 5% CO_2, humidified 37°C atmosphere for 6 days. These cultures were assayed for polyclonal IgM production by a slide modification of the Jerne hemolytic PFC assay (15) as described previously (16). Fluids to be assayed for SIRS activity were added to cultures at initiation. Those that caused greater than 50% suppression of PFC response compared with the response of control cultures were considered to show significant suppressive activity. In some experiments, a titration curve was generated and the quantity of supernatant causing 50% suppression was determined graphically. This volume was considered to contain one unit of activity. Generally, PFC response is reported in graphs and tables characterizing functional effects of lymphocyte supernatant, and units of activity are shown in experiments demonstrating the presence or absence of SIRS in a fraction or supernatant. Suppression was confirmed to be SIRS mediated by the ability of monoclonal anti-SIRS antibody coupled to Sepharose 4B (Sigma) to absorb suppressive activity from solution (5).

Separation of T lymphocyte subpopulations. Monoclonal antibodies to human helper/inducer T cell (CD4) and suppressor/cytotoxic T cell (CD8) antigens were used to isolate these two populations of cells as described (16). Briefly, 10^7 PBMC/ml were incubated with 5 μl/ml monoclonal anti-OKT4 and anti-OKT8 antibody reconstituted according to the package insert (Ortho-mune; Ortho Diagnostic Systems, Raritan, NJ), at 4°C for 45 min, washed, and resuspended in rabbit complement at 37°C for 45 min. Alternatively, cells treated with antibody were layered onto 100 x 15-mm sterile culture plates (Fisher Scientific Co., Pittsburgh, PA) coated with goat anti-mouse IgG (0.75 μg/ml) for plate separation. Nonadherent cells were obtained after the plates were gently swirled and adherent cells were removed by vigorous pipetting. After complement treatment or plate separation, cells were washed and resuspended at 2×10^6 cells/ml in culture medium.

Fractionation of culture supernatant fluids. Culture supernatant (1.5 ml) was fractionated by gel filtration on a 1.75 x 28-cm (67.3-cm^3) column of Sephacryl S-200 (Sigma) in phosphate-buffered saline (PBS) or 0.4 M pyridine-0.4 M acetic acid. The column was calibrated by using aldolase (M_r = 158,000), bovine serum albumin (M_r = 68,000), chymotrypsinogen A (M_r = 25,000) and cytochrome *c* (M_r = 13,000). Fractions of 2 ml were collected; those eluted in pyridine-acetic acid were lyophilized and resuspended in PBS. All samples were filter sterilized before testing in culture.

Reversed-phase HPLC was performed by dialyzing supernatant against 1.0 M pyridine-0.5 M acetic acid, pH 5.5 buffer. After clearing of particulate matter by centrifugation for 2 min in a Beckman (Palo Alto, CA) microfuge, the supernatant was applied to a Lichrosorb RP-8 (10 μm) (E. Merck, Darmstadt, F.R.G.) column (250 x 4 mm) equilibrated in the same pyridine-acetic acid buffer. Protein was eluted with an increasing gradient of *n*-propanol. HPLC fractions were lyophilized and resuspended in culture medium before testing for bioactivity. Protein content of the fractions was measured by optical density at 280 nm or by the method of Lowry et al. (17).

RESULTS

Suppression of PFC responses by histamine-treated PMBC. Supernatant fluids from histamine-treated PBMC were assayed for ability to suppress PFC responses of splenocyte cultures. In preliminary experiments, it was determined that optimal production of suppressive activity occurred when PBMC were incubated for 45 min to 1 hr with histamine, washed, and cultured for 24 hr more. Supernatants from cultures of cells incubated with histamine for 3 hr or 24 hr before washing did not suppress PFC responses and were comparable to supernatants of cells cultured in medium without histamine (data not shown). Figure 1 shows one of three experiments in which supernatants of PBMC treated with different concentrations of histamine were added to splenocyte cultures. Although supernatant of cells treated with 10^{-3} M histamine did not contain a suppressor substance, cells treated with 10^{-4} to 10^{-5} M histamine released significant suppressive activity into the supernatant. Treatment of PBMC with 10^{-4} M histamine was optimal for inducing detectable suppressive activity. Incubation of supernatant fluid from the 10^{-4} M histamine-treated cells with monoclonal anti-murine SIRS antibody coupled to Sepharose 4B removed suppressive activity from the supernatant. These data show that histamine activates PBMC to release a suppressive moiety that is antigenically similar to SIRS.

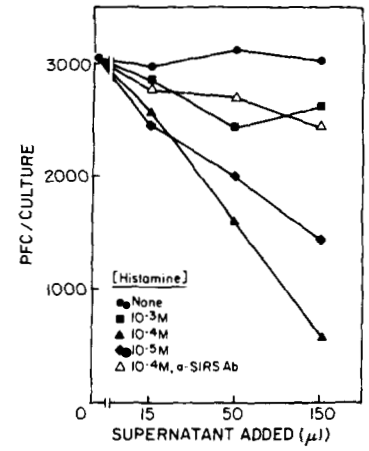

Figure 1. Effect of supernatant of histamine-treated PBMC on PFC responses. PBMC were treated with 10^{-3} M, 10^{-4} M, 10^{-5} M histamine, or with medium alone, for 1 hr, washed, and cultured in medium for 24 hr. Culture supernatant was harvested, and the indicated volumes were added to pokeweed mitogen-stimulated splenocyte cultures. A portion of the supernatant of PBMC treated with the 10^{-4} histamine was incubated with monoclonal anti-murine SIRS antibody bound to Sepharose before addition to culture (*open triangles*). After 6 days, the number of IgM-producing cells per culture was determined by PFC assay.

TABLE I

SIRS production by histamine-stimulated T lymphocyte subpopulations[a]

Subpopulation[b]	Treatment	SIRS Activity (U/ml)
PBMC	None	<5
PBMC	Histamine	12
OKT4-adherent	Histamine	<5
OKT8-adherent	Histamine	66
OKT8-adherent	None	<5
anti-OKT4 + C-treated	Histamine	23
anti-OKT8 + C-treated	Histamine	<5
C-treated	Histamine	18

[a] PBMC or selected subpopulations were incubated at 2×10^6 cells/ml in plain medium or in medium containing 10^{-4} M histamine for 1 hr, washed, and cultured for 24 hr. Supernatants were harvested and assayed for SIRS activity.

[b] Cells were incubated for 30 min with either anti-OKT4 (helper/inducer) or anti-OKT8 (suppressor/cytotoxic) antibody at 4°C. Subpopulations expressing these markers were either positively selected by "panning" with plates coated with goat anti-mouse Ig or lysed by incubation in rabbit complement (C). Cells not adherent by panning with a given antibody behaved in a manner similar to that of cells treated with antibody and C.

Lymphocyte subset activated to produce SIRS by histamine. To determine the lymphocyte subpopulation(s) required to produce SIRS after stimulation with histamine, PBMC were incubated with anti-CD4 (helper/inducer) or anti-CD8 (suppressor/cytotoxic) antibody, and subpopulations were either obtained by adherence to anti-mouse IgG-coated plates or removed by lysis with rabbit complement. In the experiment shown in Table I, CD4-positive cells treated with 10^{-4} M histamine did not produce SIRS, whereas CD8-positive, histamine-treated cells did produce SIRS. No suppression was obtained from

cells treated with OKT8 (anti-CD8) antibody and complement, but cells treated with OKT4 (anti-CD4) antibody and complement produced SIRS after activation with histamine. Nonadherent cells from the plate separation experiments were functionally similar to cells treated with antibody and complement. Similar results from two other experiments confirm that histamine activates a CD8-positive cell to produce SIRS.

Physicochemical characterization of histamine-induced SIRS. SIRS secreted by IFN- or Con A-activated cells or produced by patients with nephrotic syndrome characteristically has a high m.w. on gel filtration in PBS and a lower m.w. in 0.4 M pyridine-0.4 M acetic acid. To determine whether a similar finding is obtained with histamine-induced SIRS, supernatants of histamine-treated PBMC were fractionated on a Sephacryl S-200 column in PBS or pyridine-acetic acid, and the fractions were tested for SIRS activity. Figure 2 shows the results of one of two similar experiments. The m.w. in PBS was 110,000 to 160,000, identical to that previously found for other forms of human SIRS. Further, fractionation of the same supernatant in pyridine-acetic acid yielded suppressive activity in the 10,000 to 15,000 dalton fractions, identical to the m.w. of other forms of human SIRS under similar conditions.

Histamine-induced SIRS was also evaluated by HPLC

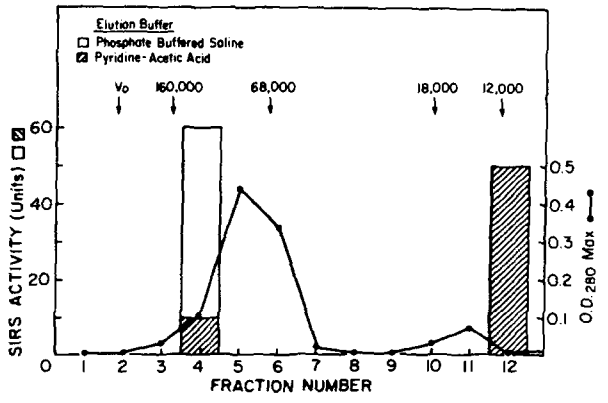

Figure 2. Molecular weight determination of histamine-induced SIRS. Supernatants of histamine-treated lymphocytes were fractionated by Sephacryl S-200 gel chromatography in either phosphate-buffered saline (*open bar*) or 0.4 M pyridine-0.4 M acetic acid (*hatched bar*). One unit of activity was defined as the amount of material required to cause 50% suppression compared with control responses (determined graphically). The column was calibrated with the use of aldolase, BSA, chymotrypsinogen A, and cytochrome c.

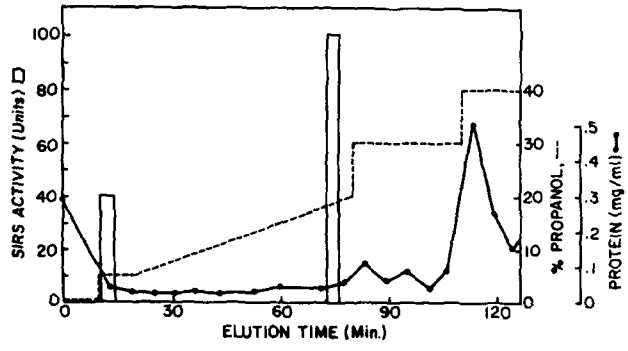

Figure 3. Reversed phase HPLC of histamine-induced SIRS. Supernatant fluid from histamine-treated lymphocytes was dialyzed against 1.0 M pyridine-0.5 M acetic acid buffer, and applied to a Lichrosorb RP-8 (10 μM) column equilibrated in the same buffer. Protein was eluted by using an increasing gradient of *n*-propanol. Protein concentration of the eluted samples was determined by the method of Lowry et al. (17).

TABLE II

Physical characteristics of histamine-induced SIRS[a]

Factor	Treatment[b]	PFC/Culture
None	—	5,920
100 μl	None	1,320
100 μl	pH 4	1,600
100 μl	pH 2	4,280
100 μl	Protease	5,840

[a] Supernatant fluid from histamine-treated PBMC cultures was added at the initiation of pokeweed mitogen-stimulated splenocyte cultures, which were assayed for polyclonal IgM PFC responses 6 days later.

[b] Supernatant was adjusted to pH 4 or pH 2 with HCl, maintained at 4°C for 3 hr, and then neutralized with NaOH. Alternatively, factor was treated with protease (Proteinase K; Sigma), 10 μg/ml, for 1 hr at 37°C.

TABLE III

Functional characterization of SIRS from histamine-treated PBMC[a]

Factor	Day Added	Inhibitor	PFC/Culture
None	—	—	1140
SIRS, 100 μl	0	—	540
SIRS, 100 μl	5	—	900
$SIRS_{ox}$[b], 100 μl	5	—	500
$Control_{ox}$[b], 100 μl	5	—	1120
SIRS, 100 μl	0	Levamisole, 5 μg/ml (day 0)	1100
SIRS, 100 μl	0	2-mercaptoethanol, 10^{-4} M (day 3)	1220
SIRS, 100 μl	0	Catalase, 5000 U (day 3)	1060

[a] Supernatant fluid from PBMC incubated for 1 hr with 1×10^{-4} histamine, washed, and cultured for 24 hr, was added to pokeweed mitogen-stimulated splenocyte cultures at the indicated time during the culture period. After 6 days the cultures were assayed for polyclonal IgM PFC response.

[b] SIRS was reacted with 2×10^{-6} M H_2O_2 for 10 min at 25°C before addition to culture. In the control preparation, medium alone was treated for 10 min with 2×10^{-6} M H_2O_2 before addition to culture.

TABLE IV

Effect of cimetidine on histamine-induced SIRS production[a]

Cell Treatment	Cimetidine	SIRS Activity (U/ml)
None	—	<5
Histamine	—	50
Histamine	1×10^{-3} M	5
Histamine	3×10^{-4} M	7
Histamine	1×10^{-4} M	<5
Histamine	3×10^{-5} M	10
Histamine	1×10^{-5} M	18
None	1×10^{-4} M	<5

[a] PBMC were incubated in plain medium or with 1×10^{-4} histamine, with or without cimetidine, for 1 hr, washed, and cultured for 24 additional hours. Supernatant fluids from these cultures were assayed for SIRS activity.

TABLE V

Effect of cimetidine on suppression of PFC responses by Con A or IFN[a]

Suppressive Agent	Cimetidine	PFC/Culture
None	None	2600
None	1×10^{4} M	2240
Con A, 3 μg/ml	None	220
Con A, 3 μg/ml	1×10^{-4} M	1560
IFN-αA, 500 U/ml	None	1200
IFN-αA, 500 U/ml	1×10^{-4} M	2360
IFN-γ, 30 U/ml	None	1160
IFN-γ, 30 U/ml	1×10^{-4} M	2760

[a] Con A or IFN was added to 1-ml cultures of pokeweed mitogen-stimulated splenocytes with or without cimetidine at the time of culture initiation. Polyclonal IgM PFC response of the splenocytes was assayed 6 days later.

by eluting protein from an RP-8 column with an increasing gradient of *n*-propanol. Figure 3 shows the results of one of three similar experiments. All of the suppressive activity bound to the column, and SIRS eluted at the step-up in the gradient from 0 to 5% propanol and again at 18 to 19% propanol. This pattern is identical to that previously found with HPLC of human SIRS derived from other sources (5), and suggests that the SIRS derived from each

TABLE VI

Effect of cimetidine on activation of SIRS production by Con A or IFN[a]

PBMC Treatment	SIRS Activity (U/ml) in PBMC Supernatant Fluids	
	Without cimetidine	Cimetidine, 10^{-4} M
None	<5	<5
Con A, 30 μg/ml	35	<5
IFN-αA, 10,000 U/ml	29	<5
IFN-γ, 300 U/ml	30	<5

[a] PBMC from a normal donor were incubated with the indicated agent for 24 hr, with or without cimetidine. The cells were washed and resuspended in medium for an additional 24 hr. Culture supernatants were harvested and tested for SIRS activity by ability to suppress PFC responses of pokeweed mitogen-stimulated splenocyte cultures.

TABLE VII

Effect of ranitidine on activation of SIRS production by Con A or IFN[a]

PBMC Treatment	SIRS Activity (U/ml) in PBMC Supernatant Fluids	
	Without ranitidine	Ranitidine, 3×10^{-4} M
None	<5	<5
Con A, 30 mg/ml	100	<5
IFN-αA, 10,000 U/ml	45	<5
IFN-γ, 300 U/ml	33	<5

[a] PBMC were incubated with the indicated agent for 24 hr, with or without ranitidine, washed, and cultured for 24 hr more before supernatants were assayed for SIRS activity.

of these different sources is similar, if not identical.

Conventional SIRS is inactivated by protease and acidification to pH 2. In the representative experiment shown in Table II, histamine-induced SIRS acidified to pH 4 was as suppressive as untreated factor. However, acidification to pH 2 or treatment with protease (Proteinase K; Sigma) abolished suppressive activity. Taken together with the results of the gel filtration and HPLC experiments, these data show that SIRS from histamine-treated PBMC shares physicochemical characteristics with SIRS previously described from other sources.

Functional characteristics of histamine-induced SIRS. SIRS requires either activation by peroxide to $SIRS_{ox}$, or a prolonged period of time in culture, to suppress PFC responses. To determine whether histamine-induced SIRS also requires activation by peroxide, factor was added to splenocyte cultures at the time of initiation or on day 5 of a 6-day culture. Table III shows the results of one of three similar experiments in which addition of 100 μl of factor on day 0 suppressed responses. Addition of factor on day 5, 24 hr before PFC assay, did not significantly suppress responses unless the factor was incubated with 10^{-6} M H_2O_2 before addition. Further, SIRS activity is inhibited by levamisole and catalase, which block activation of SIRS to $SIRS_{ox}$, and by 2-mercaptoethanol, which inhibits $SIRS_{ox}$. In the experiment shown in Table III, these agents also inhibited suppression by histamine-induced SIRS. These results show that SIRS from histamine-treated PBMC is functionally as well as physically similar to human SIRS derived from nephrotic patients or from suppressor cells activated by Con A or IFN.

Effect of cimetidine on activation of SIRS production by histamine. Studies of histamine-induced suppression in other systems suggest that binding to the H-2 receptor is critical for expression of suppressive activity, since cimetidine blocks suppression (9, 18). To determine whether H-2 receptor blockade also prevents SIRS production, PBMC were incubated with histamine and various concentrations of cimetidine. Table IV shows data from a representative experiment in which amounts of cimetidine equimolar to histamine inhibited SIRS production. Although a titration range of 10^{-3} to 10^{-5} M partially inhibited SIRS production, these other concentrations were less effective than 10^{-4} M. These data indicate that cimetidine inhibits histamine-induced activation of PBMC to secrete SIRS.

Effect of cimetidine on suppression of PFC responses by Con A or IFN. Con A, IFN-αA and IFN-γ all activate CD8-positive suppressor cells to secrete SIRS. Further, because levamisole, 2-mercaptoethanol, or anti-SIRS antibody prevent suppression of PFC responses by Con A or IFN, suppression by these agents appears to be mediated largely by the SIRS pathway. Because cimetidine inhibits histamine-induced activation of CD8-positive cells to produce SIRS, experiments were performed to determine whether cimetidine would also prevent suppression of PFC responses by Con A, IFN-αA, or IFN-γ. Table V shows the results of one of three similar experiments. Con A, IFN-αA, or IFN-γ added at culture initiation suppressed PFC responses. Addition of 1×10^{-4} M cimetidine (Sigma Chemical Co., St. Louis, MO) completely inhibited suppression of responses by either IFN-αA or IFN-γ. The response of splenocytes treated with Con A and cimetidine was seven times that of cells treated with Con A alone, although it did not return to control values. Splenocytes treated with cimetidine alone consistently showed slightly but not significantly decreased responses. These results show that cimetidine inhibits suppression of PFC responses by agents which act via the SIRS pathway.

Inhibition of SIRS production by H-2 antagonists in PBMC treated with Con A or IFN. These results suggested that cimetidine may inhibit SIRS production by PBMC treated with a number of agents. To evaluate this activity more directly, PBMC were incubated with optimal concentrations of Con A, IFN-αA, or IFN-γ, with or without cimetidine for 24 hr, washed, and cultured for 24 hr more before culture supernatants were harvested and assayed for SIRS activity. Table VI shows the results of one of three similar experiments. PBMC incubated with Con A, IFN-αA, or IFN-γ released SIRS into culture supernatant. Cimetidine (1×10^{-4} M) prevented secretion of SIRS by lymphocytes treated with these agents. Supernatant of lymphocytes treated with cimetidine alone did not significantly affect responses (data not shown). These results show that cimetidine inhibits activation of suppressor cells to produce SIRS by Con A or IFN, as well as by histamine.

Effect of ranitidine on SIRS-mediated suppression. These data raise the possibility that binding to the H-2 receptor may play a role in the SIRS pathway. However, cimetidine has been reported to have immunoenhancing effects, and binding of cimetidine to the H-2 receptor could inhibit binding to other, nearby receptors through steric interactions. To begin addressing these issues, experiments were carried out with the use of the structurally different H-2 antagonist ranitidine, which does not enhance the mitogenic response of lymphocytes (19). In preliminary experiments, ranitidine prevented secretion of SIRS by histamine-treated cells at concentrations from 1×10^{-5} to 3×10^{-4} M; optimal inhibition was found at 3×10^{-4} M ranitidine. This concentration of ranitidine also prevented suppression of PFC responses by Con A,

IFN-αA, or IFN-γ added directly to splenocyte cultures (data not shown). In the experiment shown in Table VII, ranitidine (3×10^{-4} M) also inhibited secretion of SIRS by PBMC treated with Con A, IFN-αA, or IFN-γ. Supernatant from lymphocytes treated with ranitidine alone did not significantly affect PFC responses. These data show that two structurally different histamine H-2 receptor antagonists inhibit suppression via the SIRS pathway.

DISCUSSION

SIRS is a lymphokine that suppresses delayed-type hypersensitivity and PFC responses in vivo, and PFC responses and tumor cell division in vitro (1, 2). Previously, human SIRS has been found to be produced in vitro by untreated lymphocytes from patients with steroid-responsive nephrotic syndrome (5), and by normal lymphocytes stimulated in vitro with Con A, IFN-αA, IFN-γ, or serum from nephrotic patients (4, 20). Data reported in this paper show that histamine also activates suppressor cells to produce SIRS. A soluble product of lymphocytes treated with histamine (10^{-4} M) suppressed polyclonal, IgM PFC responses of human splenocytes; this substance is antigenically, physically, and functionally similar to SIRS derived from previously described sources. These data suggest that SIRS production is a potentially common phenomenon, since several disparate agents have now been described that are biologically relevant to humans and that activate SIRS production.

Histamine-induced suppressive activity has been described in lymphocytes expressing either suppressor (21) or helper/inducer (22) T cell phenotypes. Unlike SIRS, the soluble products of these cells are typically of low m.w. under physiologic conditions. However, both the low m.w. factor(s) (23) and SIRS (24) may require an adherent cell population for expression of suppression. One histamine-induced factor augments prostaglandin production by monocytes (25); inhibitors of prostaglandin synthesis block suppression by these factors (13). To date, we have not been able to demonstrate consistent effects of prostaglandin inhibitors in our system (H. W. Schnaper, unpublished observation).

Activation of SIRS production by a variety of agents was inhibited by cimetidine. To determine whether this phenomenon was specific for cimetidine or might relate more generally to H-2 receptor antagonists, similar experiments were performed with ranitidine. This latter substance lacks the imidazole ring structure of cimetidine and instead contains a furan ring structure (19). In our hands, the optimal antisuppressive concentration for ranitidine was slightly higher than that for cimetidine; this is in contrast to the findings of Badger et al. (19) regarding histamine-mediated suppression of mitogen-induced lymphocyte proliferation, and also differs somewhat from the known relative potencies of ranitidine and cimetidine in decreasing gastric acid secretion. Nonetheless, the data clearly show that structural diversity in H-2 receptor antagonists does not prevent a common inhibitory effect on the SIRS pathway.

The decreased time requirement for activation of SIRS production by histamine compared with that required for activation by Con A or IFN is of interest. This may reflect the toxicity inherent in prolonged exposure of SIRS-producing lymphocytes to pharmacologic concentrations of histamine. The brief interval required between initial exposure to histamine and recovery of SIRS activity also suggests that histamine could act at a later point on the pathway of suppressor cell activation than does Con A, IFN-αA, or IFN-γ. Because the cellular events immediately preceding production of SIRS by $CD8^+$ cells are not known, it is not possible to determine whether histamine or H-2 receptor binding is essential for induction of SIRS production. However, the experiments in which cimetidine and ranitidine prevented activation of the SIRS pathway raise the possibility that histamine release is required for SIRS production. Alternatively, the data are equally consistent with the interpretation that H-2 receptor antagonists prevent either binding of Con A or IFN to the appropriate receptors, or triggering of suppressor cell activity once the ligand has bound to the receptor. A definitive answer to these questions requires more extensive studies of receptor-ligand interactions during suppressor cell activation. The precise role of H-2 receptor binding in SIRS production and the relationship of histamine-induced suppressor factor(s) to the SIRS pathway remain to be determined.

Acknowledgments. We appreciate the assistance of the Transplant office of the Department of Surgery, Washington University School of Medicine, in obtaining human spleen. April Main, Susana Zahn, and Nancy Fieldhammer provided superb technical assistance. Review of the manuscript by Dr. Carl W. Pierce was helpful. Carole Shieber and Paula Ryan provided essential secretarial and administrative assistance.

REFERENCES

1. **Aune, T. M, and C. W. Pierce.** 1984. Mechanism of SIRS action at the cellular and biochemical level. In *Lymphokines, Vol. 9.* E. Pick, ed. Academic Press, New York. P. 257.
2. **Schnaper, H. W., and T. M. Aune.** 1986. Suppression of immune responses to sheep erythrocytes by the lymphokine soluble immune response suppressor (SIRS) in vivo. *J. Immunol. 137:863.*
3. **Webb, D. R., K. Mason, G. Semenuk, T. M. Aune, and C. W. Pierce.** 1985. Purification and analysis of the isoforms of soluble immune response suppressor (SIRS). *J. Immunol. 135:3238.*
4. **Schnaper, H. W., C. W. Pierce, and T. M. Aune.** 1984. Identification and initial characterization of concanavalin A- and mitogen-induced human suppressor factors: evidence for a human equivalent of murine soluble immune response suppressor (SIRS). *J. Immunol. 132:2429.*
5. **Schnaper, H. W., and T. M. Aune.** 1985. Identification of the lymphokine soluble immune response suppressor (SIRS) in urine of nephrotic children. *J. Clin. Invest. 76:341.*
6. **Aune, T. M., G. L. Freeman, and D. G. Colley.** 1985. Production of the lymphokine soluble immune response suppressor (SIRS) during chronic experimental schistosomiasis mansoni. *J. Immunol. 135:2768.*
7. **Birch, R. E., and S. H. Polmar.** 1982. Pharmacological modification of immunoregulatory T lymphocytes. I. Effect of adenosine, H_1 and H_2 histamine antagonists upon T lymphocyte regulation of B lymphocyte differentiation *in vitro. Clin. Exp. Immunol. 48:218.*
8. **Rocklin, R. E.** 1977. Histamine-induced suppressor factor (HSF): effect on migration inhibitory factor (MIF) production and proliferation. *J. Immunol. 118:1734.*
9. **Barsoum, I. S., H. S. S. Dahawi, F. M. Gamil, M. Habib, M. A. El Alamy, and D. G. Colley.** 1984. Immune responses and immunoregulation in relation to human schistosomiasis in Egypt. II. Cimetidine reversal of histamine-mediated suppression of antigen-induced blastogenesis. *J. Immunol. 133:1576.*
10. **Suzuki, S., and R. Huchet.** 1982. Properties of histamine-induced suppressor factor in the regulation of lymphocyte response to PHA in mice. *Cell. Immunol. 68:349.*
11. **Rocklin, R. E., A. Blidy, and M. Kamal.** 1983. Physiochemical characterization of human histamine-induced suppressor factor. *Cell. Immunol. 76:243.*
12. **Nair, M. P. N., J. M. Cilik, and S. A. Schwartz.** 1986. Histamine-induced suppressor factor inhibition of NK cells: reversal with interferon and interleukin 2. *J. Immunol. 136:2456.*
13. **Beer, D. J., L. J. Rosenwasser, C. A. Dinarello, and R. E. Rocklin.**

1982. Cellular interactions in the generation and expression of histamine-induced suppressor activity. *Cell. Immunol. 69:101.*

14. **Böyum, A.** 1968. Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest. 97 (Suppl.):77.*
15. **Pierce, C. W., B. M. Johnson, H. E. Gershon, and R. Asofsky.** 1971. Immune responses *in vitro*. IV. Development of primary μM, γG and γA plaque-forming cell responses in mouse spleen cell cultures stimulated with heterologous erythrocytes. *J. Exp. Med. 134:395.*
16. **Schnaper, H. W., T. M. Aune, and C. W. Pierce.** 1983. Suppressor T cell activation by human leukocyte interferon. *J. Immunol. 131:2301.*
17. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem. 193:265.*
18. **Griswold, D. E., S. Alessi, A. M. Badger, G. Poste, and N. Hanna.** 1986. Differential sensitivity of T suppressor cell expression to inhibition by histamine type 2 receptor antagonists. *J. Immunol. 137:1811.*
19. **Badger, A. M., J. Young, and G. Poste.** 1984. Reversal of histamine-mediated immunosuppression by structurally diverse histamine type II (H-2) receptor antagonists. *Int. J. Pharmacol. 6:467.*
20. **Schnaper, H. W., and T. M. Aune.** 1987. Steroid-sensitive mechanism of soluble immune response suppressor production in steroid-responsive nephrotic syndrome. *J. Clin. Invest. 79:257.*
21. **Rocklin, R. E., J. Breard, S. Gupta, R. A. Good, and K. L. Melmon.** 1980. Characterization of the human blood lymphocytes that produce a histamine-induced suppressor factor (HSF). *Cell. Immunol. 51:226.*
22. **Birch, R. E., A. K. Rosenthal, and S. H. Polmar.** 1982. Pharmacological modification of immunoregulatory T lymphocytes. II. Modulation of T lymphocyte cell surface characteristics. *Clin. Exp. Immunol. 48:231.*
23. **Beer, D. J., C. A. Dinarello, L. J. Rosenwasser, and R. E. Rocklin.** 1982. Human monocyte-derived soluble product(s) has an accessory function in the generation of histamine and concanavalin A-induced suppressor T cells. *J. Clin. Invest. 70:393.*
24. **Aune, T. M., and C. W. Pierce.** 1981. Identification and initial characterization of a nonspecific suppressor factor (Mϕ-SF) produced by soluble immune response suppressor (SIRS)-treated macrophages. *J. Immunol. 127:1828.*
25. **Rocklin, R. E., I. Kiselis, D. J. Beer, P. Rossi, F. Maggi, and J. A. Bellanti.** 1983. Augmentation of prostaglandin and thromboxane production *in vitro* by monocytes exposed to histamine-induced suppressor factor (MSF). *Cell. Immunol. 77:92.*