Proc. Natl. Acad. Sci. USA
Vol. 78, No. 8, pp. 5099–5103, August 1981
Immunology

Conversion of soluble immune response suppressor to macrophage-derived suppressor factor by peroxide

(lymphokine/suppression/hybridoma/peroxidase)

THOMAS M. AUNE* AND CARL W. PIERCE*†

*Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis; and Departments of *Pathology and †Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri 63110

Communicated by Baruj Benacerraf, April 15, 1981

ABSTRACT **After incubation with soluble immune response suppressor (SIRS), a product of concanavalin A-activated Ly^{2+} T cells, macrophages release a factor that suppresses *in vitro* antibody responses, DNA synthetic responses to T-cell and B-cell mitogens, and division of several tumor cell lines. This factor, macrophage-derived suppressor factor (Mϕ-SF), is a protein with an apparent M_r of 55,000 that is inactivated by sulfhydryl compounds, certain amines, and iodide but not by other halides. In experiments reported here, conventional SIRS and SIRS produced by a cloned T-cell hybridoma were used to analyze formation of Mϕ-SF by SIRS-treated macrophages. Formation of Mϕ-SF was insensitive to inhibitors of protein and prostaglandin synthesis but was sensitive to catalase and cyanide, indicating that Mϕ-SF was not a newly synthesized product and that peroxide was important to its formation. As Mϕ-SF and SIRS have similar molecular weights and other properties, it is possible that Mϕ-SF is SIRS modified by peroxide. To test this possibility, SIRS was treated with H_2O_2 and Mϕ-SF activity was determined. H_2O_2 at 0.1–1 pM was sufficient to convert SIRS to Mϕ-SF; the reaction required ≈15–20 min and was sensitive to cyanide. Several conventional peroxidase substrates inactivated Mϕ-SF produced by the SIRS–H_2O_2 reaction or by SIRS-treated macrophages. In addition, catalase and several of the compounds that directly inactivate Mϕ-SF also partially interfere with SIRS-mediated suppression of antibody responses. Collectively, these data suggest that SIRS-treated macrophages produce H_2O_2, which converts SIRS to Mϕ-SF, which has properties of an oxidized peroxidase-like protein and acts by oxidizing cellular components essential for cell division.**

Soluble immune response suppressor (SIRS) is a glycoprotein product of concanavalin A (Con A)-activated murine Ly^{2+} T cells (1–3) that suppresses immune responses through its action on macrophages (Mϕ) (4–7). SIRS-treated Mϕ release a second factor, macrophage-derived suppressor factor (Mϕ-SF) that is directly responsible for the immunosuppressive activity of SIRS (6, 7). Mϕ-SF suppresses primary and secondary IgM and IgG antibody responses *in vitro*, DNA synthetic responses by spleen cells to T-cell and B-cell mitogens, and division and DNA synthesis by several tumor cell lines. Suppression or inhibition mediated by Mϕ-SF appears to be due to oxidation of cellular components essential for cell division as its effects can be reversed by high concentrations of 2-mercaptoethanol or dithiothreitol (6, 7).

The experiments presented here examined the relationship between SIRS and Mϕ-SF and determined the requirements for formation of Mϕ-SF. The results show that SIRS can be converted to Mϕ-SF by Mϕ or very low concentrations of hydrogen peroxide (H_2O_2) in the absence of Mϕ. The data also suggest that SIRS (and Mϕ-SF) has certain peroxidase-like properties.

MATERIALS AND METHODS

Mice. C57BL/10 mice were bred in the animal facility at the Jewish Hospital and maintained on laboratory chow and water ad lib. Mice were used when 2 to 4 months old.

Spleen Cell Cultures and Hemolytic Plaque Assay. Single-cell suspensions of spleen were incubated under modified Mishell–Dutton conditions at 37°C in 10% CO_2/7% O_2/83% N_2 atmosphere in 16-mm wells of 24-well tissue culture plates (FB-16-24-TC; Linbro Division, Flow Laboratories, Hamden, CT) at 5×10^6 cells/0.5 ml in fully supplemented Eagle's minimal essential medium/10% fetal calf serum (Lot 32301, Reheis Chemical, Kankakee, IL) containing 10^6 sheep erythrocytes (SRBC) for 4 or 5 days. SRBC (GIBCO) were prepared for use as antigen in culture or as indicator cells in the hemolytic plaque-forming cell (PFC) assay as described (1). IgM PFC responses to SRBC were assayed with the slide modification of the Jerne hemolytic plaque assay (1).

Preparation of Conventional SIRS. Spleen cells were cultured in fully supplemented Eagle's minimal essential medium/2% fetal calf serum containing phenylmethylsulfonyl fluoride at 10 μg/ml (Sigma) and concanavalin A (Con A) at 2 μg/ml (Lot 127, Research Div., Miles) under Mishell–Dutton conditions for 48 hr (1, 7). Con A was removed by absorbing supernatant fluids with Sephadex G-50 (Pharmacia); SIRS was concentrated by ultrafiltration (Amicon stirred cell, Model 52, Amicon, Lexington, MA) and fractionated by gel filtration on Sephacryl S-200 (Sigma) using phosphate-buffered saline. Fractions containing SIRS activity were pooled, concentrated to 1/10 the original volume by ultrafiltration, and stored at −70°C.

Production of T-Cell Hybridomas Producing SIRS. C57BL/10 spleen cells were incubated for 24 hr under modified Mishell–Dutton conditions with Con A at 2 μg/ml and then fused with the hypoxanthine/guanine phosphoribosyltransferase-deficient AKR thymoma, BW5147, using polyethylene glycol (8, 9). Cells were cultured in hypoxanthine/aminopterin/thymidine medium (9, 10), and primary cultures were tested for SIRS activity by using suppression of *in vitro* PFC responses to SRBC as the assay. Cells from primary wells that yielded supernatant fluids that suppressed PFC responses >75% were cloned by limiting dilution in soft agar over 3T3 cells (9–12). Individual colonies were plated and supernatant fluids were retested for SIRS activity. The results presented are from one selected clone, 393.D2.6; however, at least six others secrete factors with properties of SIRS. No significant differences have

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: SIRS, soluble immune response suppressor; Con A, concanavalin A; Mϕ, macrophage; Mϕ-SF, macrophage-derived suppressor factor; SRBC, sheep erythrocytes; PFC, plaque forming cell.

been detected between hybridoma-derived SIRS and conventional SIRS (12). Supernatant fluids from cultures of BW5147 cells alone or several other clones derived from the initial fusion are devoid of SIRS activity (12). Hybridoma-derived SIRS was fractionated on Sephacryl S-200, concentrated by ultrafiltration, and stored at −70°C. Conventional and hybridoma-derived SIRS were adjusted and used at approximately equivalent suppressive activity.

Preparation of Mϕ-SF. The RAW 264.7 Mϕ-like cell line was maintained in 75-cm^2 plastic tissue culture flasks (Falcon) in fully supplemented RPMI 1640/10% horse serum (GIBCO)/30 mM Hepes without 2-mercaptoethanol. RAW 264.7 cells were cultured in suspension in spinner flasks (Belco Glass) in fully supplemented Eagle's minimal essential medium/2% fetal calf serum containing phenylmethylsulfonyl fluoride at 10 μg/ml under modified Mishell–Dutton conditions at 4×10^5 cells per ml in total volumes of 50–100 ml. Conventional or hybridoma-derived SIRS was added at an activity equivalent to a final dilution of 1:100 of conventional SIRS, and cells were cultured for 24 hr, washed, and cultured in fresh medium for an additional 48 hr. Supernatant fluids were harvested, dialyzed, concentrated by ultrafiltration, and fractionated on a 2.5 × 40 cm Sephacryl S-200 column equilibrated with phosphate buffer. Fractions with Mϕ-SF activity were pooled, concentrated to 1/10 the original volume by ultrafiltration, and stored at −70°C. Mock Mϕ-SF were prepared by culturing RAW 264.7 cells with conventional SIRS-control supernatant fluids (6, 7) or with hybridoma-derived fluids devoid of SIRS activity.

RESULTS

Inhibition of Mϕ-SF Formation. Supernatant fluids from RAW 264.7 Mϕ treated with conventional and hybridoma-derived SIRS contained Mϕ-SF, which suppressed day 4 PFC responses by 70–80% when added on day 3 (Table 1). SIRS has no suppressive activity when added on day 3 of culture (1, 4, 7) and mock Mϕ-SF has no activity. Inhibitors of protein synthesis (puromycin or cycloheximide) had no effect on Mϕ-SF production at concentrations of inhibitor that reduced incorporation of [^{3}H]leucine into acid-precipitable protein by 80%. Indomethacin, at concentrations reported to effectively inhibit prostaglandin synthesis by spleen cells (13–15), did not inhibit formation of Mϕ-SF; the yield of Mϕ-SF was somewhat greater in the presence of indomethacin. However, both catalase and cyanide completely inhibited production of Mϕ-SF. The inhibition by catalase implies that peroxide is required for Mϕ-SF formation, that by cyanide suggests that a hemoprotein may be necessary at some stage in the pathway (16, 17).

Table 1. Inhibition of Mϕ-SF production

Conditions for Mϕ-SF production	IgM, PFC per culture: Conventional SIRS	Hybridoma SIRS
No Mϕ-SF (control response)	3850	3850
Mϕ	3975 (0)	3975 (0)
SIRS	4005 (0)	4240 (0)
Mϕ + control SIRS (mock Mϕ-SF)	3890 (0)	4100 (0)
Mϕ + SIRS	1155 (70)	850 (78)
Mϕ + SIRS + cycloheximide (10 μg/ml)	924 (76)	N.T.
Mϕ + SIRS + puromycin (25 μg/ml)	1195 (69)	N.T.
Mϕ + SIRS + 1 μM indomethacin	580 (85)	N.T.
Mϕ + SIRS + 10 μM CN^-	2656 (31)	2156 (44)
Mϕ + SIRS + 1 mM CN^-	3388 (12)	3620 (6)
Mϕ + SIRS + catalase (5000 units/ml)	3920 (0)	3660 (5)

RAW264.7 Mϕ were treated with conventional (Con A-activated spleen cells) or hybridoma-derived SIRS alone or with SIRS and the indicated compounds. Conventional and hybridoma-derived SIRS were adjusted to activity equivalent to final dilution of 1:100 of conventional SIRS. Supernatant fluids were harvested and processed as described in *Materials and Methods* to remove inhibitors. Mϕ-SF activity was tested by adding 100 μl of supernatant fluid to C57BL/10 spleen cells stimulated with SRBC on day 3; PFC responses were assayed on day 4. Data in parentheses are % suppression of control responses. NT, not tested.

Reaction of SIRS with H_2O_2 Yields Mϕ-SF. As SIRS and Mϕ-SF have comparable molecular sizes—≈55,000 daltons—and share other properties, such as pH, temperature, and protease sensitivities (7), one interpretation of our results was that Mϕ treated with SIRS release H_2O_2, which converts SIRS to Mϕ-SF. To test this possibility, conventional or hybridoma SIRS was incubated with various concentrations of H_2O_2 and Mϕ-SF activity was determined by addition to culture on day 3 and assaying PFC responses to SRBC on day 4 (Fig. 1). Reaction of SIRS with as little as 0.1 pM H_2O_2 was sufficient to reproducibly generate Mϕ-SF that suppressed PFC responses by 75%; higher concentrations of H_2O_2 failed to generate significantly greater Mϕ-SF activity. By contrast, 10–100 μM H_2O_2 was necessary to significantly inhibit PFC responses in the absence of SIRS. Control SIRS (conventional or hybridoma derived) after reaction with H_2O_2 had no Mϕ-SF activity.

Several factors can influence the yield of Mϕ-SF obtained by treating SIRS with H_2O_2. First, hemoglobin or met-hemoglobin in supernatant fluids, especially from Con A-activated spleen cells, can compete with SIRS for H_2O_2 and shift the concentration of H_2O_2 necessary to convert SIRS to Mϕ-SF from 0.1 pM to 0.5 mM. Second, low protein concentrations (<100

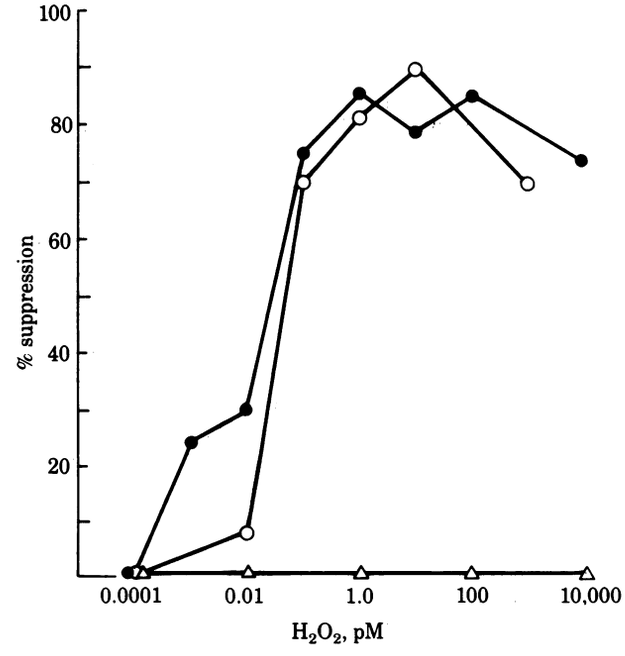

FIG. 1. Concentration of H_2O_2 necessary to convert SIRS to Mϕ-SF. SIRS was treated with the indicated concentration of H_2O_2 for 20 min at 4°C, and the mixture was dialyzed to remove excess H_2O_2. Mϕ-SF activity was tested by adding 10 μl (conventional SIRS from Con A-activated spleen cells, ○), 20 μl (1:100 dilution of hybridoma SIRS, ●), or 10 μl (control SIRS; △) of the reaction mixtures to SRBC-stimulated spleen cell cultures on day 3; PFC responses to SRBC were assayed on day 4. SIRS or control Mϕ-SF added on day 3 had no effect on day 4 PFC responses. Control response: 9470 IgM PFC per culture.

μg/ml) reduce the stability of the Mϕ-SF obtained by reaction of SIRS with H_2O_2. Third, extended reaction times with higher concentrations of H_2O_2 decrease the yield of Mϕ-SF (see Fig. 2).

Rate of Conversion of SIRS to Mϕ-SF by H_2O_2. The time required to convert SIRS to Mϕ-SF with H_2O_2 was determined by treating SIRS with 1 pM H_2O_2; 500 units of catalase were added as indicated to stop the reaction (Fig. 2). Conversion of SIRS to Mϕ-SF required 15–20 min at 4°C; longer reaction times resulted in some loss of activity. This is in marked contrast to the 2 or 3 days required for SIRS-treated Mϕ to produce Mϕ-SF (5–7).

Conversion of SIRS to Mϕ-SF by H_2O_2 Is Cyanide Sensitive. Generation of Mϕ-SF by SIRS-treated Mϕ was sensitive to cyanide (Table 1). However, it was not certain whether cyanide affected peroxide formation, the reaction of SIRS with peroxide, or some other cellular process necessary for formation of Mϕ-SF. The simplest method to distinguish these possibilities was to test the effect of cyanide on the SIRS–H_2O_2 reaction. Cyanide inhibited formation of Mϕ-SF from SIRS plus H_2O_2 (Table 2). Cyanide added to SIRS 20 min after addition of H_2O_2 did not prevent formation of Mϕ-SF nor did it inactivate Mϕ-SF derived from SIRS-treated Mϕ or from the SIRS–H_2O_2 reaction.

Inactivation of Mϕ-SF by Peroxidase Substrates. Some toxic properties of cyanide arise from its affinity for heme groups; cyanide causes the reversible inactivation of a variety of heme-containing enzymes, including peroxidases (17). As SIRS appeared to react with H_2O_2 to yield Mϕ-SF in a cyanide-sensitive process, SIRS may have other peroxidase-like properties. To examine these properties of SIRS and to compare further Mϕ-SF produced by SIRS-treated Mϕ and the reaction of SIRS with H_2O_2, a number of peroxidase substrates were tested for their ability to inactivate Mϕ-SF (Fig. 3). All peroxidase substrates tested inactivated Mϕ-SF produced by Mϕ or H_2O_2 at similar concentrations. For instance, ascorbic acid was only effective at high concentration (1–100 μM), while phenylenediamine was effective at 1–100 nM. Other compounds ranged in effectiveness from hydroquinone (1–1000 nm) to *p*-aminobenzoic acid

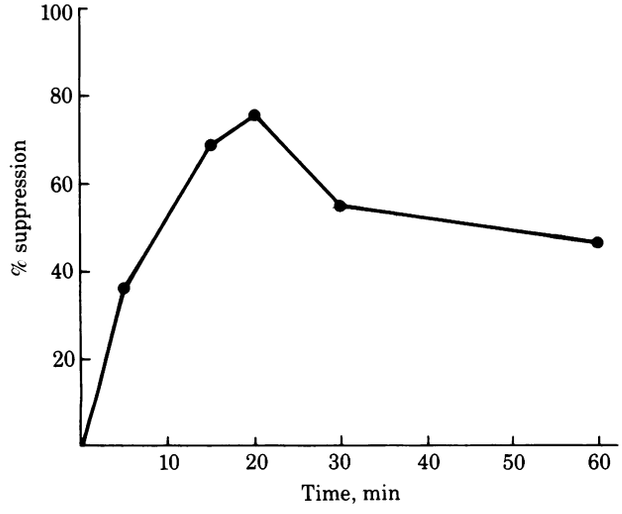

FIG. 2. Rate of conversion of SIRS to Mϕ-SF by H_2O_2. SIRS was treated with 1 pM H_2O_2 at 4°C for the indicated times. Catalase (500 units) was used to stop the reaction. The zero time point was obtained by adding H_2O_2 to SIRS and catalase. Mϕ-SF activity (●) was tested by adding 10 μl of the reaction mixture to spleen cell cultures on day 3; PFC responses to SRBC were assayed on day 4. Control response: 12,480 IgM PFC per culture.

Table 2. Cyanide sensitivity of conversion of SIRS to Mϕ-SF by H_2O_2

Reaction conditions	Day 4 IgM, PFC per culture	
	Conventional SIRS	Hybridoma SIRS
No Mϕ-SF (control response)	5140 (0)	6270 (0)
SIRS	5270 (0)	6410 (0)
SIRS + 1 pM H_2O_2	1235 (76)	1620 (74)
SIRS + 10 μM CN^- + 1 pM H_2O_2	4520 (12)	6340 (0)
SIRS + 1 pM H_2O_2, then 10 μM CN^-	1590 (69)	2200 (65)

Conventional and hybridoma-derived SIRS at equivalent activities were treated at 4°C for 15 min with the indicated compounds and then dialyzed and tested for Mϕ-SF activity. Mϕ-SF activity was tested by adding 10 μl of reaction product to C57BL/10 spleen cell cultures stimulated with SRBC on day 3; PFC responses were assayed on day 4. Data in parentheses are % suppression of control responses.

and *o*-dianisidine (1–100 μM). Tyrosine inactivated Mϕ-SF at 10–100 μM (data not shown).

Interference with SIRS-Mediated Suppression of PFC Responses. These data suggested that SIRS-mediated suppression may be achieved by the following mechanism: SIRS binds to Mϕ, which produces H_2O_2; SIRS reacts with H_2O_2 and is released from Mϕ as Mϕ-SF within 3 days; Mϕ-SF reacts with and inhibits antibody-producing cells. Therefore, any of the compounds that block Mϕ-SF production or inactivate Mϕ-SF should inhibit SIRS-mediated suppression of PFC responses. Several of the compounds given in Fig. 3 and catalase and 2-mercaptoethanol partially interfere with SIRS-mediated suppression (Table 3, optimal conditions are shown); 2-mercaptoethanol, KI, and catalase were the most effective, while ascorbic acid and *p*-aminobenzoic acid were least effective. Increasing the concentration of any of these compounds further resulted in toxicity and suppression of PFC responses in the absence of SIRS. In experiments not shown, the effects of each

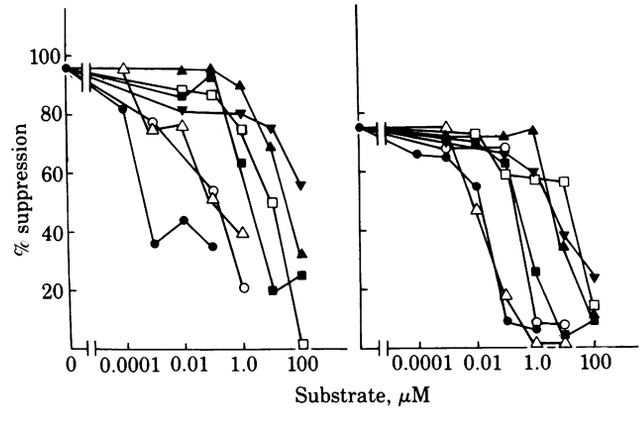

FIG. 3. Inactivation of Mϕ-SF and products of the SIRS–H_2O_2 reaction by peroxidase substrates. SIRS was treated with 1 pM H_2O_2 for 20 min at 4°C, and the mixture was dialyzed to remove excess H_2O_2. Mϕ-SF was prepared by culture of SIRS with RAW 264.7 Mϕ as described in *Materials and Methods*. The products of the SIRS–H_2O_2 reaction (*A*) and Mϕ-SF (*B*) were treated with the indicated concentrations of phenylenediamine (●), potassium iodide (○), *o*-dianisidine (□), hydroquinone (△), pyrogallol (■), *p*-aminobenzoic acid (▲), or ascorbic acid (▼) for 2 hr at 4°C. Activity was tested by adding 10 (*A*) or 25 (*B*) μl reaction mixture to spleen cell cultures on day 3; PFC responses to SRBC were assayed on day 4. Equivalent amounts of the various compounds did not suppress PFC responses at the highest concentrations used. Control responses: *A*, 3480 PFC per culture; *B*, 2100 PFC per culture.

Table 3. Compounds that prevent SIRS-mediated suppression

Culture conditions	Day 5 IgM, PFC per culture	
	Conventional SIRS	Hybridoma SIRS
Control response	8775 (0)	17910 (0)
SIRS	1053 (88)	1612 (91)
SIRS + 0.1 mM 2-mercaptoethanol	6140 (30)	11015 (38)
SIRS + 10 μM ascorbic acid	4210 (52)	7520 (58)
SIRS + 1 mM *p*-aminobenzoic acid	4125 (53)	6450 (64)
SIRS + 2 mM KI	5705 (35)	3940 (78)
SIRS + 0.5 mM tyrosine	4565 (48)	8420 (53)
SIRS + 10,000 units of catalase	5440 (38)	9850 (45)

C57BL/10 spleen cells were cultured with SRBC and 5 μl of conventional SIRS or 25 μl of a 1:100 dilution of hybridoma SIRS added at culture initiation. Compounds listed were added on day 2. PFC responses were assayed on day 5. Compounds tested had no significant effect on PFC responses alone. Data in parentheses are % suppression of control responses.

of these compounds could be reversed by addition of more SIRS.

DISCUSSION

SIRS, a product of Con A-activated murine Ly^{2+} T cells, nonspecifically suppresses *in vitro* PFC responses (1–3); the target cell of SIRS is the Mϕ, and Mϕ-treated with SIRS release a soluble factor (Mϕ-SF) that is responsible for the suppression (5–7). Mϕ-SF suppresses primary and secondary IgM and IgG PFC responses, proliferative responses by spleen cells to T- or B-cell mitogens, and cell division and DNA synthesis by several tumor cell lines (6). Mϕ-SF is inactivated by low concentrations of sulfhydryl reagents, reducing reagents such as sodium borohydride, amines, and iodide. Under certain conditions, Mϕ-SF-mediated suppression or inhibition is reversible by high concentrations of dithiothreitol or 2-mercaptoethanol. These data suggest that Mϕ-SF contains oxidizing equivalents and that the effects of Mϕ-SF may be due to the oxidation of cellular components essential for the activity suppressed, primarily cell division (unpublished data; ref. 6). We have investigated the nature of the oxidizing equivalents on Mϕ-SF and provide evidence that Mϕ-SF is modified SIRS. These studies were facilitated by the availability of a T-cell hybridoma that secretes monoclonal SIRS without further stimulation with Con A. Hybridoma-derived SIRS is ≈100-fold more active than conventional SIRS and is devoid of enhancing factors and present in preparations of conventional SIRS, which interfere with or reverse the effects of SIRS (7, 12). In addition, preparations containing conventional SIRS activity also have macrophage migration inhibition factor activity: these two activities have not been dissociated (3). Hybridoma-derived SIRS also has migration inhibition factor activity (unpublished data). The availability of a SIRS-producing T-cell hybridoma should allow complete biochemical characterization.

Reaction of very low concentrations of H_2O_2 (0.1 pM) with conventional or hybridoma-derived SIRS was sufficient to form Mϕ-SF; this reaction was sensitive to catalase and to cyanide. Production of Mϕ-SF by SIRS-treated Mϕ was insensitive to inhibitors of protein synthesis but was sensitive to catalase and cyanide. Therefore, Mϕ appeared to serve only as a source of peroxide. Cyanide reversibly binds to heme groups and inactivates hemoglobin, peroxidases, and catalases (17). The inhibition of Mϕ-SF production by Mϕ and H_2O_2 with cyanide suggested that SIRS may be a hemoprotein having peroxidase-like characteristics. To investigate the peroxidase-like nature of SIRS and to further compare Mϕ-SF produced by the SIRS–H_2O_2 reaction and SIRS-treated Mϕ, various peroxidase substrates were examined for their ability to inactivate Mϕ-SF. All compounds tested inactivated Mϕ-SF in similar concentration ranges, providing further evidence that Mϕ-SF is the oxidized product of the SIRS–H_2O_2 reaction and support for the hypothesis that SIRS and Mϕ-SF have peroxidase-like properties.

SIRS-mediated suppression of PFC responses to heterologous erythrocytes was partially circumvented by reagents that prevented formation of Mϕ-SF (catalase) or inactivated Mϕ-SF (iodide, tyrosine, *p*-aminobenzoic acid, ascorbic acid, and 2-mercaptoethanol). Sensitivity to catalase alone could be interpreted to mean that SIRS-dependent suppression was mediated solely by release of H_2O_2 by Mϕ. However, compounds that inactivated Mϕ-SF but did not react with H_2O_2 blocked suppression to an equivalent extent. This argues that Mϕ-SF was directly responsible for suppression and that H_2O_2 was necessary for formation of Mϕ-SF.

It should be possible to determine whether the SIRS-Mϕ-SF pathway is involved in other immunosuppressive systems by examining the ability of compounds that inactivate Mϕ-SF to block their suppressive activity. The immunosuppressive effects of murine viral (type I) interferon on PFC responses to heterologus erythrocytes by spleen cells were partially circumvented by iodide, tyrosine, 2-mercaptoethanol, *p*-aminobenzoic acid, or catalase at concentrations that inactivate Mϕ-SF. These data taken together imply that suppression of PFC responses by viral interferon is mediated at least in part by the SIRS-Mϕ-SF pathway (unpublished data).

Mϕ from mice injected with *Corneybacterium parvum* or thioglycollate suppressed proliferative responses by spleen cells (18–20). This suppression was partially blocked by catalase or indomethacin, and the combination of catalase and indomethacin completely blocked the Mϕ-mediated suppression. Increasing the number of Mϕ in the system partially overcame the effects of catalase and indomethacin (20). Mϕ from mice injected with bacille Calmette–Guerin or cultured with a lymphokine preparation released large quantities of H_2O_2 after triggering with phorbol myristate acetate (21, 22) and were also cytotoxic for tumor cells (23–25). Catalase or other agents that interfere with H_2O_2 formation reduced or prevented cytolysis of tumor cells by activated Mϕ (25).

These and other studies have demonstrated that H_2O_2 has an important role in the cytolytic and immunosuppressive functions of Mϕ. The extent to which Mϕ-SF mediates the effects of H_2O_2 is not known at present; it will be of considerable interest to determine whether activated Mϕ release Mϕ-SF in culture.

This work was supported by U.S. Public Health Service Grants AI-13915 and AI-15353 from the National Institute of Allergy and Infectious Diseases and Grant 1040 from the Council for Tobacco Research, U.S.A., Inc. T.M.A. was supported by U.S. Public Health Service Training Grant AI-07163 from the National Institute of Allergy and Infectious Diseases.

1. Rich, R. R. & Pierce, C. W. (1974) *J. Immunol.* **112**, 1360–1368.
2. Jandinski T., Cantor, H., Tadakuma, T., Peavy, D. L. & Pierce, C. W. (1976) *J. Exp. Med.* **143**, 1382–1390.
3. Tadakuma, T., Kuhner, A. L., Rich, R. R., David, J. R. & Pierce, C. W. (1976) *J. Immunol.* **117**, 323–330.
4. Tadakuma, T. & Pierce, C. W. (1976). *J. Immunol.* **117**, 967–972.
5. Tadakuma, T. & Pierce, C. W. (1978) *J. Immunol.* **120**, 481–486.
6. Aune, T. M. & Pierce, C. W. (1981) *J. Immunol.* **127**, 368–372.
7. Aune, T. M. & Pierce, C. W. (1981) *J. Immunol.*, in press.
8. Galfre, G., Howe, S. C., Milstein, C., Butcher, G. W. & Howard, J. C. (1977) *Nature (London)* **266**, 550–552.

9. Kapp J. A., Araneo, B. A. & Clevinger, B. L. (1980) *J. Exp. Med.* **152,** 225–240.
10. Littlefield, J. W. (1964) *Science* **145,** 709–710.
11. Coffino, P., Baumal, R., Larkov, R. & Scharff, M. D. (1972) *J. Cell. Physiol.* **79,** 429–440.
12. Aune, T. M. & Pierce, C. W. (1981) in *Monoclonal Antibodies and T Cell Hybridomas*, eds. Hammerling, G. J., Hammerling, U. & Kearney, J. F. (Elsevier, New York), in press.
13. Webb, D. R., Jamisson, A. J., & Nowowiejski, I. (1976) *Cell. Immunol.* **24,** 45–57.
14. Grimm, W., Seitz, M., Kirchner, H. & Gemsa, D. (1978) *Cell. Immunol.* **40,** 419–426.
15. Humes, J. L., Borney, R. J., Pelus, L., Dahlgre, M. E., Sadowski, S. J., Kuehl, A. Jr. & Davies, P. (1977) *Nature (London)* **269,** 149–151.
16. Schonbaum, G. R. & Chance, B. (1976) in *The Enzymes*, ed. Boyer, P. D., (Academic, New York), 3rd Ed., Vol. 13, pp. 363–408.
17. Morrison, M. & Schonbaum, G. R. (1976) *Ann. Rev. Biochem.* **45,** 861–888.
18. Kirchner, H., Holden, H. T. & Herberman, R. B. (1975) *J. Immunol.* **115,** 1212–1216.
19. Keller, R. (1975) *Cell. Immunol.* **17,** 542–551.
20. Metzger, Z., Hoffeld, J. T. & Oppenheim, J. J. (1980) *J. Immunol.* **124,** 983–988.
21. Nathan, C. F. & Root, R. K. (1977) *J. Exp. Med.* **146,** 1648–1662.
22. Nathan, C. F., Noguira, N., Juangbhanich, C., Ellis, J. & Cohn Z. (1979) *J. Exp. Med.* **149,** 1056–1068.
23. Meltzer, M. S., Tucker, R. W., Sandford, K. K. & Leonard, E. J. (1975) *J. Natl. Cancer Inst.* **54,** 1177–1184.
24. Nathan, C. F., Brukner, L. H., Silverstein, S. C. & Cohn, Z. A. (1979) *J. Exp. Med.* **149,** 84–99.
25. Nathan, C. F., Silverstein, S. C., Brukner, L. H. & Cohn, Z. A (1979) *J. Exp. Med.* **149,** 100–113.