

## INTERFERON AS A MEDIATOR OF HUMAN LYMPHOCYTE SUPPRESSION\*

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One of the major concerns of contemporary immunology is the elucidation of mechanisms of regulation of the immune response. The study of suppressor and helper functions in experimental animal models has uncovered important roles for immune response genes (1, 2). There is limited evidence that there are genetic restrictions operating to control suppression of certain *in vitro* immune responses in man (3, 4). The principal experimental model for studying human suppressor cell generation and function remains the mitogen-induced suppressor response *in vitro* (5, 6). Suppressor functions induced by exposure of lymphocytes to concanavalin A operate in a nonspecific and apparently genetically nonrestricted fashion and appear capable of suppressing both human cell-mediated and humoral functions. Human mitogen-induced lymphocyte suppression has been shown to be effected by a specific subset of T lymphocytes (7); however, the mechanism of suppression remains largely unknown.

Originally defined by their antiviral activity, interferons have recently been found to exert a variety of other biological activities, which include inhibition of cell division (8), enhancement of phagocytosis (9), alteration of expression of cell surface antigens (10), and regulation of immune responses. Included in the immunoregulatory activities of interferons are their ability to augment cytotoxic activity of natural killer (NK)<sup>1</sup> cells (11) and their ability to suppress cell-mediated responses both *in vivo* (12) and *in vitro* (13, 14). It is now clear that there are different molecular entities that possess antiviral activities. Perhaps the best studied interferon (IF) is that produced by challenge of fibroblasts or tissue culture cell lines with viruses. Stimulation of human leukocytes with virus causes the production of an immunologically noncross-reactive species of IF known as type I or leukocyte IF. Activation of primed T cells by specific antigens such as purified protein derivative (PPD) or mitogens is known to engender the production of a third type of IF known as type II or immune IF. These three species of IF have been described as possessing distinct molecular characteristics and are thought to be antigenically distinct (15-17). Both leukocyte (type I) and fibroblast IF have been described as being stable at acid pH and heat labile. Type II IF is acid labile and stable at 56°C.

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<sup>1</sup> Abbreviations used in this paper: Con A, concanavalin A; [<sup>3</sup>H]TdR, tritiated thymidine; IF, interferon(s); NDV, Newcastle disease virus; NK, natural killer; PBL, peripheral blood leukocytes; PPD, purified protein derivative; VPA, virus plaque assay; V-PFC, virus plaque-forming cells; VSV, vesicular stomatitis virus.

Because of the well-described immunosuppressive activities of IF, and the fact that mitogens are known to stimulate the production of IF by lymphocytes, we have examined the role of IF in the in vitro suppressive activity of concanavalin A (Con A)-activated human peripheral blood mononuclear cells. The results presented here will indicate that the suppressor activity of Con A-activated cells is mediated largely by IF and that similar suppressor activity can be generated by exposure of human peripheral blood leukocytes (PBL) to certain tumor cell lines, viruses, and specific antigen, which induce the production of IF.

### Materials and Methods

*Lymphocyte Preparation and Culture.* PBL were prepared from venous blood by heparinization followed by Ficoll-Hypaque (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) sedimentation. For those experiments with the virus plaque assay (VPA) defibrination was used (18). Culture medium for all experiments was RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 10% pooled human AB serum, glutamine, and antibiotics. PBL were cultured at  $1 \times 10^6$  cells/ml of medium in 5-ml plastic tubes for the VPA (2 ml of cell suspension/tube) or in flat-bottomed microtiter plates (0.2 ml/well) for measurement of tritiated thymidine ( $^3\text{H}$ ]TdR) incorporation. All cultures were done in triplicate. Con A (Miles Laboratories Inc., Miles Research Products, Elkhart, Ill.) was added to cultures at a final concentration of 30  $\mu\text{g}/\text{ml}$ . Cultures were incubated at 37°C in a humidified  $\text{CO}_2$  incubator for 72 h before addition of  $^3\text{H}$ ]TdR or infection with virus.

*VPA and Assay for Incorporation of  $^3\text{H}$ ]TdR.* The VPA for enumerating activated human lymphocytes was carried out as described previously (18). Briefly, lymphocytes were infected with vesicular stomatitis virus (VSV) at the end of the culture period, treated with guinea pig anti-VSV serum to neutralize free virus, washed, and plated over an indicator monolayer of L929 cells in an infectious centers assay. Virus plaque-forming cells (V-PFC) were then enumerated and expressed as V-PFC per  $10^6$  cells plated. Aliquots (0.2 ml) of the same cultures tested for V-PFC were removed before infection with virus and placed in microtiter wells. For assay of lymphocyte transformation, 1  $\mu\text{Ci}$  of  $^3\text{H}$ ]TdR (New England Nuclear, Boston, Mass.) was added to each sample in microtiter wells. Samples were harvested 6 h later in a multiple cell culture harvester (Skatron, Flow Laboratories, Inc., Rockville, Md.) and counted in a Beckman liquid scintillation counter (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.).

*Generation of Modulator Cells and Supernates with Con A and PPD.* Modulator cells were prepared by culture of  $2 \times 10^6$  PBL in 2 ml in plastic tubes with or without Con A (30  $\mu\text{g}/\text{ml}$ ) or PPD (20  $\mu\text{g}/\text{ml}$ ) for 72 h. PPD was obtained from the Ministry of Food, Fishery, and Agriculture, Weybridge, Surrey, England. In the case of Con A, modulator cells were washed with 0.1 M  $\alpha$ -methylmannoside to remove residual Con A. Modulator cells ( $1 \times 10^6$ ) were then treated with mitomycin C (50  $\mu\text{g}/\text{ml}$  for 30 min at 37°C) and added to equal numbers of freshly prepared autologous or allogeneic responder cells, cultured for an additional 72 h in the presence or absence of Con A, and then assayed for V-PFC or incorporation of  $^3\text{H}$ ]TdR. In the case of PPD-activated modulator cells, PPD-negative responder PBL were used. In experiments with modulator supernates, supernates from Con A- or PPD-activated PBL and from PBL cultured in medium alone were added to responder lymphocytes at a final dilution of 1:1 in culture medium in the presence and absence of Con A and assayed for  $^3\text{H}$ ]TdR incorporation after a 72-h incubation.

*Tumor Cell-Lymphocyte Cocultures.* Tumor cell-lymphocyte cultures were carried out as reported by Trinchieri and Santoli (11) with  $5 \times 10^5$  tumor cells and  $5 \times 10^6$  PBL in 16-mm wells. As controls, lymphocytes were cultured in the absence of tumor cells, and tumor cells in the absence of lymphocytes. Lymphocytes and supernates were harvested after 24 h. Lymphocytes to be used as modulator cells were treated with mitomycin C and added to equal numbers of freshly prepared autologous or allogeneic responder cells. Supernates from these cultures were centrifuged at 400 g, millipored to remove cell debris, and added to responder cells at a final concentration of 10% in culture medium. Modulator-responder cultures were cultured with or without Con A for 72 h before infection with virus or addition of  $^3\text{H}$ ]TdR. Tumor lines

tested included a human melanoma line, MeWo (19); a human breast carcinoma line, MCF-7 (20) (obtained from Jorgen Fogh, Sloan-Kettering Institute for Cancer Research, Rye, N. Y.); two human bladder carcinoma lines, T24 (21); and TCCSUP (22) (obtained from Carol O'Toole of the London Hospital Medical College, London, England), HeLa, and HeLa-Ms, a HeLa line persistently infected with measles virus (23) (obtained from John Holland of the University of California, San Diego, Calif.). All tumor lines were shown to be free of contamination by mycoplasma when stained with Hoechst dye 33258 (Hoechst Ag., Frankfurt, Federal Republic of Germany) (24).

*Newcastle Disease Virus (NDV) Treatment of PBL.* NDV was grown in chick embryo fibroblasts, harvested at the time of maximal cytopathic effect, yielding  $5 \times 10^7$  PFU/ml, and inactivated with  $\beta$ -propiolactone (0.15%, 37°C, 2 h) (25). Ficoll-Hypaque-prepared PBL were cultured at  $2 \times 10^6$  cells/ml with inactivated NDV (10 $\lambda$ /ml) at 37°C for 48 h. IF-containing supernates were centrifuged at 30,000 *g* at 4°C for 3 h to remove virus. The supernate used in all experiments contained 3,170 U of antiviral activity/ml.

*Computation of Percent Suppression.* Percent suppression was calculated as follows:

$$\% \text{ suppression} = \left[ 1 - \frac{\text{Con A response of treated modulator} - \text{responder cultures}}{\text{Con A response of control modulator} - \text{responder cultures}} \right] \times 100,$$

where the Con A response was the Con A-induced V-PFC or counts per minute minus the background (unstimulated) V-PFC or counts per minute. Treated modulators included cells exposed to Con A, PPD, NDV, or tumor cell lines or their supernates. Control modulators were PBL cultured in medium alone or their supernates. For tumor coculture experiments, supernates from tumor cells and lymphocytes alone were used as controls in determining percent suppression by coculture supernates.

*Assay for Antiviral Activity.* IF was assayed by the reduction of the cytopathic effect of VSV on human 21 trisomic fibroblasts (Detroit 532, American Type Cell Culture Collection, Rockville, Md.). 0.10-ml samples of supernate and serial half log dilutions were added to fibroblast monolayers ( $10^4$  cells/well) in microtiter wells. After an 18 h incubation,  $5 \times 10^4$  PFU of VSV were added to each well. The IF titer was determined 48 h later as the reciprocal of the highest dilution that inhibited 50% of the cytopathic effect. Each assay included a human reference IF preparation (G-023-901-527, National Institutes of Health). 1 U of IF in our assay system corresponded to 0.6 reference units.

*Anti-Human Leukocyte IF Sera.* Rabbit anti-human leukocyte IF serum was prepared by Dr. K. Paucker (Medical College of Pennsylvania, Philadelphia, Pa.) (and kindly provided by Dr. G. Galasso, Infectious Disease Branch, National Institute of Allergy and Infectious Diseases, Bethesda, Md.). This serum was raised against Sendai virus-induced leukocyte IF and has an antibody titer of 10,000 U/ml against human leukocyte IF and 240 U/ml against human fibroblast IF. The antiserum was added to modulator-responder cultures at a final dilution of 1:100 at the start of the culture period. A sheep anti-human leukocyte interferon serum was kindly provided by Dr. K. Cantell (Central Public Health Laboratory, Helsinki, Finland) and was similarly used in some experiments.

*Treatment at pH 2.* Supernates were dialyzed 24 h at 4°C in RPMI (pH 7.4) or in RPMI acidified to pH 2 by addition of 4 N HCl and then dialyzed an additional 24 h in RPMI. All supernates were checked for pH at the end of the dialysis period and millipored. Supernates were assayed for antiviral activity and suppression before and after dialysis at pH 7.4 and pH 2.

## Results

*Suppression by Con A-activated Modulator Cells.* In our initial studies, we compared the effects of Con A-induced modulator cells in two assays for lymphocyte activation, the lymphocyte transformation test and the VPA. The latter enumerates activated T cells by their ability to permit replication of VSV (18). Lymphocytes treated for 3 d with or without Con A were treated with mitomycin C and used as modulator cells, which could influence the response of normal responder lymphocytes to Con A. The degree of suppression obtained with Con A-activated modulator cells on the Con A

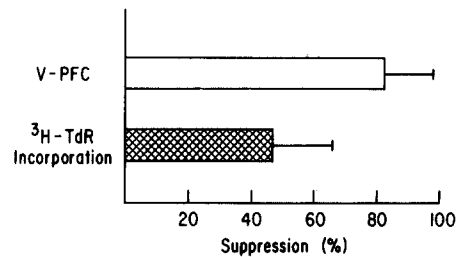


FIG. 1. Con A-induced suppression of the mitogen response measured in the VPA and in the thymidine incorporation assay. The data represent the mean suppression obtained from 15 separate experiments with autologous or allogeneic responder-modulator combinations. Suppression in the virus assay is significantly greater than in the [<sup>3</sup>H]TdR incorporation assay ( $P < 0.001$ ).

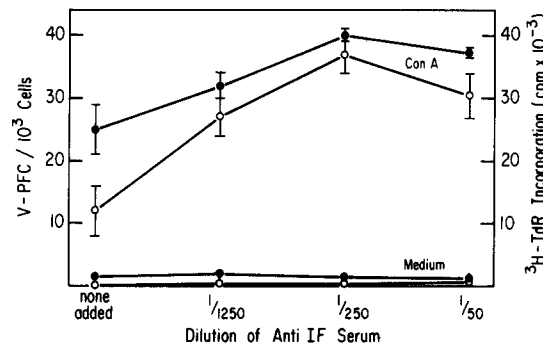


FIG. 2. Effect of anti-human leukocyte IF serum on the Con A response as measured in the VPA (open circles) and in the [<sup>3</sup>H]TdR incorporation assay (closed circles). The lower two curves represent the values for unstimulated PBL, the upper for Con A-stimulated cultures. Values shown are means of triplicate cultures  $\pm$  SD.

response of freshly prepared PBL was invariably greater in the VPA than in the [<sup>3</sup>H]TdR incorporation assay done on the same cultures (Fig. 1). The mean percent suppression in a group of 15 normals observed in the VPA was 82.7% compared with 47.2% in the [<sup>3</sup>H]TdR assay. As reported by others (5), Con A-treated modulator cells were equally effective in suppressing the mitogen response of autologous or allogeneic responders.

*Effect of Anti-Human Leukocyte IF Serum on Con A-stimulated PBL.* To explain the increased sensitivity of the VPA to the action of Con A-activated modulator cells, we considered the possibility that mitogen-induced IF might contribute to the observed suppression, perhaps affecting the generation of cells permissive to viral replication to a greater extent than the incorporation of [<sup>3</sup>H]TdR. To ascertain whether IF was involved in the suppression, we first investigated the effect of adding rabbit anti-human leukocyte IF serum to mitogen-stimulated cultures, and found that the number of Con A-stimulated V-PFC was increased, often as much as 200%, whereas the [<sup>3</sup>H]TdR incorporation in the same cultures increased by only 10–30% in most experiments. Unstimulated cultures (no Con A added) were unaffected by addition of the antiserum. A representative experiment is shown in Fig. 2. There was some variation from donor to donor in sensitivity to the anti-IF serum, perhaps reflecting genetic differences in levels of IF production between different individuals, analogous to strain differences among mice (26) or production of different proportions of types I and II IF (see below).

TABLE I  
Effect of Anti-Human Leukocyte IF Serum on Suppression of Con A Responses by Con A-stimulated Modulator Cells

Treatment of modulator cells*	IF‡		VPA		[ <sup>3</sup> H]TdR incorporation assay	
	U/ml	V-PFC/10 <sup>6</sup> cells§	% sup-pression	cpm§	% sup-pression	
Control	10	48,936 (2,463)	—	118,699 (16,425)	—	
Con A	100	19,132 (4,821)	61%	62,765 (4,243)	47%	
Control + Anti-IF serum	0	54,142 (6,310)	—	116,032 (11,031)	—	
Con A + Anti-IF serum	10	46,875 (9,722)	13%	98,650 (5,834)	15%	

\* Modulator cells prepared by preincubation in medium (control) or Con A for 72 h and added to freshly prepared responder cells in the presence and absence of Con A. Rabbit anti-human leukocyte IF added at a final concentration of 1:100 in culture medium at the start of the culture period.

‡ IF titers of modulator-responder cultures assayed on D532 cells.

§ V-PFC and cpm values cited represent Con A-stimulated values minus background (unstimulated) values. Standard deviations of the mean are shown in parentheses.

Supernates of Con A-activated PBL from normal individuals generally contained 100 U of IF/ml. Antiviral activity was virtually all (90–100%) eliminated after dialysis at pH 2, compatible with type II or immune IF. Addition of rabbit anti-human leukocyte IF to supernates of Con A-activated PBL before IF assay reduced antiviral titers by 70% in most experiments. This antiserum, which was raised against Sendai virus-induced leukocyte (type I) IF, thus was able to neutralize a portion of the antiviral activity of pH 2 labile mitogen-induced IF, which suggested either antigenic cross-reactivity between viral- and mitogen-induced IF or, alternatively, that the antiserum has multiple specificities. Similar reduction of the antiviral activity of supernates of Con A-activated PBL was observed with a sheep anti-human leukocyte IF serum in two experiments.

*Addition of Anti-Human Leukocyte IF Serum to Con A-treated Modulator-Responder Cultures.* The role of IF in the Con A suppressor system was established when it was found in five separate experiments that the presence of anti-IF serum largely abrogated the Con A-induced suppression of the mitogen response. A representative experiment is shown in Table I. IF titers were often, but not always, significantly higher in the supernates of suppressed cultures. Culture supernates that contained anti-IF serum showed partial loss of antiviral activity in parallel with loss of suppression. Similar results were obtained with both sheep and rabbit anti-IF sera. Suppression of mitogen responses by supernates of Con A-activated cells was also abrogated by addition of anti-IF to supernate-treated responder cells.

*Suppression by PPD-activated Modulator Cells.* When PBL from PPD-positive donors were cultured in the presence of PPD for 48 h, significant suppressor activity was obtained when these modulator cells were added to PPD-negative responder PBL stimulated with Con A. Supernates of such PPD-treated modulator cells contained 100–1,000 U of antiviral activity and suppressed the Con A responses of responder PBL. Conversely, modulator cells or supernates prepared from PPD-negative PBL treated with PPD failed to suppress or to exhibit antiviral activity. A representative experiment is shown in Table II. PBL from some PPD-negative donors did produce 1–3 U of IF that was acid stable, neutralized by anti-IF serum, and capable of

TABLE II  
*Suppression of Con A Response by Modulator Supernates from PPD-Positive and PPD-Negative Donors*

	[ <sup>3</sup> H]TdR incorporation		IF*
	cpm	% suppression	U/ml
PPD-positive donor‡			
Modulator supernate from:§			
Lymphocytes cultured alone	124,942 (6,812)	—	<3
Lymphocytes cultured with PPD	81,236 (8,858)	36%	300
Lymphocytes cultured with Con A	101,460 (3,726)	19%	30
PPD-negative donor‡			
Modulator supernate from:§			
Lymphocytes cultured alone	116,596 (8,712)	—	<3
Lymphocytes cultured with PPD	114,407 (2,488)	2%	3
Lymphocytes cultured with Con A	83,794 (6,107)	25%	30

\* IF titers of modulator (PPD- or Con A-treated) or control supernates.

‡ PPD-positive donor gave a [<sup>3</sup>H]TdR response of 22,432 cpm over background in the presence of PPD. PPD-negative donor gave [<sup>3</sup>H]TdR response of 300 cpm over background.

§ 48-h supernates of PBL cultured in medium or PPD and added to PPD-negative responder cells in the presence of Con A at a final concentration of 1:1 in culture medium.

|| Results shown are mean cpm of triplicate cultures stimulated by Con A. Standard deviations of the mean are shown in parentheses.

priming for the induction of IF by virus, thus appearing to be type I IF. At these low levels (0.5–1.5 U/ml in final culture medium), significant suppression by modulator cells or supernates was not seen. As will be shown in a later section (Fig. 5) supernates of PPD-activated PPD-sensitive PBL had antiviral activity that appeared to be a mixture of type I and type II IF.

*Generation of Suppressor Cells, Soluble Suppressor Factors, and IF by Lymphocyte-Tumor Cell Coculture.* To test the generality of the suppression of lymphocyte activation by IF, normal human PBL were cultured overnight on monolayers of several tumor cell lines in a protocol similar to that described by Trinchieri and Santoli (11) for inducing IF production by NK cells. Results of a representative experiment with MeWo MCF-7 cell lines are shown in Table III. Lymphocytes removed from the MeWo cells after overnight incubation were themselves suppressed and strongly suppressed the Con A responses of freshly prepared responder cells, whereas lymphocytes cultured in the absence of melanoma cells failed to suppress. Supernates from the mixed cultures contained significant amounts of IF, whereas supernates from lymphocytes or melanoma cells cultured alone had no measurable antiviral activity. In some experiments, supernates from MeWo cells cultured alone suppressed and induced IF. IF induction and suppression by these supernates could be abolished by milliporing or by repeated freeze-thawing, a procedure that did not affect the antiviral or suppressor activities of IF-containing supernates. When added to responder cells after milliporing or freeze-thawing, only the MeWo-lymphocyte supernates suppressed. In experiments with modulator lymphocytes cultured on tumor lines, we were concerned that tumor cells might be carried over into modulator-responder cultures. Mitomycin treatment of PBL did not significantly affect their ability to make IF on coculture with MeWo cells; however, mitomycin-treated MeWo cells were unable to induce IF or suppressor

TABLE III  
*Generation of Suppressor Cells, Soluble Suppressor Factor, and IF in Tumor Cell-Lymphocyte Cultures*

	IF‡		VPA		[ <sup>3</sup> H]TdR incorporation assay	
	U/ml	V-PFC/10 <sup>6</sup> cells§	% sup- pression	cpm§	% sup- pression	
<b>Modulator cells*</b>						
Lymphocytes cultured alone	—	20,138 (2,333)	—	60,166 (7,813)	—	
Lymphocytes cultured with MeWo cells	—	1,414 (12)	93%	24,269 (3,018)	60%	
<b>Modulator supernates from:*</b>						
Lymphocytes cultured alone	0	36,052 (13,412)	—	71,541 (11,031)	—	
Lymphocyte-MeWo culture	317	177 (230)	99%	37,911 (2,990)	41%	
MeWo cells alone	0	24,342 (1,885)	—	56,916 (3,646)	—	
Lymphocytes cultured alone	0	ND		120,434 (7,532)	—	
Lymphocyte-MCF-7 culture	0	ND		127,969 (7,965)	-5%	
MCF-7 cells alone	0	ND		123,362 (4,036)	—	

\* Freshly prepared responder cells treated with modulator cells or modulator culture supernates and cultured in the presence and absence of Con A.

‡ IF titers of modulator supernates assayed on D532 cells.

§ V-PFC and cpm values cited represent Con A-induced values minus background (unstimulated) values. Standard deviations of the mean are shown in parentheses.

|| Percent suppression calculated with mean of the two control supernates (lymphocytes alone and tumor cells alone).

factor from PBL. It would thus appear that tumor cells carried over with modulator cells would not themselves cause suppression.

Both modulator cells and supernates exerted greater suppressive activity with the VPA than the [<sup>3</sup>H]TdR assay, as noted previously in experiments with Con A-treated modulators. A human mammary carcinoma line, MCF-7, failed to induce suppression of the mitogen response, and no antiviral activity could be detected in supernates of lymphocyte-MCF-7 cocultures. Similarly, the T24 and TCCSUP human bladder carcinoma lines and HeLa cells failed to induce IF or suppression. HeLa cells persistently infected with measles virus (HeLa-Ms) induced both IF and suppression. The results are summarized in Fig. 3.

Addition of anti-human leukocyte IF serum to lymphocyte-tumor cell coculture modulator-responder cultures largely eliminated suppression by modulator cells and significantly reduced suppression by supernates. Treatment at pH 2 did not affect suppression by lymphocyte-tumor cell supernates. Similarly, the antiviral activity of these preparations was unchanged by pH 2, but was fully neutralized by anti-human leukocyte IF serum. (Results summarized in Fig. 5.)

*Suppression of the Con A Response by NDV-induced IF.* A supernate from PBL treated with  $\beta$ -propiolactone-inactivated NDV contained 3,170 U of IF/ml. In the presence of varying dilutions of this material, the Con A response of normal PBL was suppressed. A representative experiment is shown in Fig. 4. Significant suppression of the Con A response was seen at final concentrations of < 1 antiviral U/ml of culture suspension, thus demonstrating that viral-induced leukocyte IF is a potent immunoregulatory substance even at concentrations undetectable by a sensitive antiviral

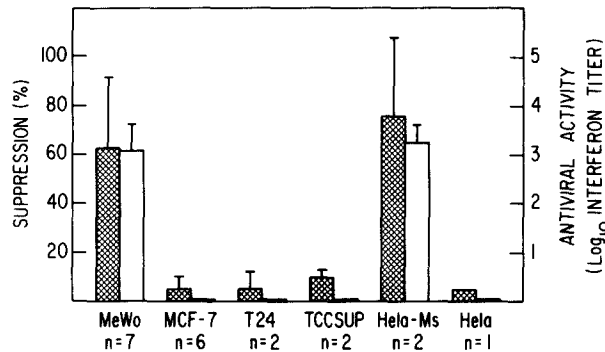


FIG. 3. Suppressive and antiviral activities of lymphocyte-tumor cell coculture supernates. The cross-hatched bars represent the mean suppression of the Con A response of responder PBL in the presence of coculture supernates ( $\pm$  SD). The open bars represent the antiviral activity ( $\log_{10}$  IF titer) of the various supernates. The darkened base lines over MCF-7, T24, TCCSUP, and HeLa represent antiviral titers of 0 obtained from coculture supernates. n is the number of experiments done with each cell line indicated.

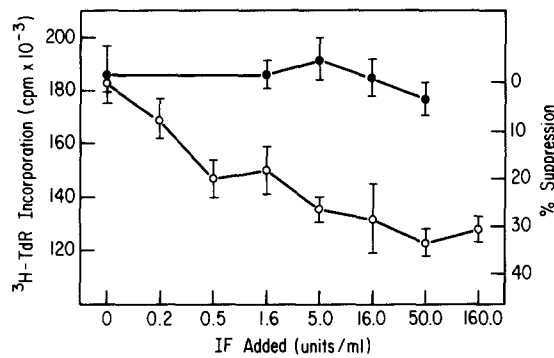


FIG. 4. Suppression of Con A response by NDV-induced leukocyte IF, and abrogation of suppression by anti-human leukocyte IF serum. Graded doses of IF were added to PBL stimulated with Con A in the presence of normal rabbit serum (open circles) or rabbit anti-human leukocyte IF (closed circles). Values shown represent the mean of triplicate cultures  $\pm$  SD.

assay. Sensitivity of responder PBL to added IF was variable; some donors failed to be suppressed at levels of  $< 3$  U/ml. Addition of anti-human leukocyte IF serum to these cultures completely eliminated suppression of the Con A response. Similarly the antiviral activity of this preparation was completely abolished by this antiserum. Antiviral activity was unaffected by treatment at pH 2.

*Antiviral and Suppressive Activities of Supernates Produced by Various IF Inducers.* To determine whether suppression and antiviral activity are mediated by the same type(s) of IF, supernates from PBL cultured with Con A, NDV, PPD, and MeWo cells were dialyzed at pH 2, treated with anti-IF serum, or both, and were assayed for antiviral and suppressive activity. A representative experiment is shown in Fig. 5. From the results shown it can be seen that the IF induced by NDV and MeWo cell coculture are neutralized by anti-IF serum but unaffected by pH 2. Similarly, suppression by these supernates is eliminated or markedly reduced by anti-IF but unaffected by pH 2 treatment. As shown in Fig. 5, addition of anti-IF to tumor coculture supernates was unable to completely abrogate suppression as was the case with NDV-induced



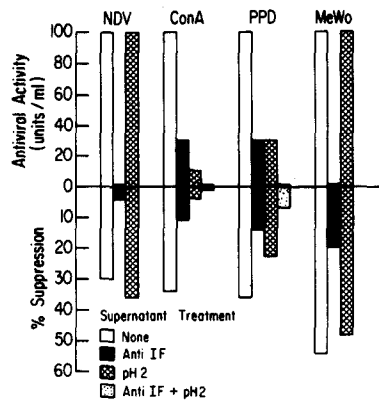


FIG. 5. Antiviral and suppressive activities of supernates of PBL treated with different IF inducers: effect of treatment with anti-human leukocyte IF serum and dialysis at pH 2. Supernates of PBL treated with NDV, Con A, PPD, and MeWo cells were diluted with medium to contain 100 U IF/ml. and dialysed at pH 2 or treated with anti-IF serum or both. Supernates of PBL cultured in medium alone were similarly treated. Modulator and control supernates were then added to responder PBL (PPD negative) in the presence of Con A.  $[^3\text{H}]\text{TdR}$  incorporation was measured after 72 h, and percent suppression was calculated. Treated and untreated modulator supernates were assayed for antiviral activity on D532 cells. Dialysis at pH 7.4 had no effect on suppressive or antiviral activity.

IF, which suggested that factors other than IF may also contribute to tumor cell-induced suppression. The Con A- and PPD-induced antiviral and suppressive activities appeared to be a mixture of classical type I and type II IF in that both pH 2 and anti-IF reduced both activities, whereas a combination of the two treatments eliminated them completely. It can be seen that suppressive and antiviral activities were affected in parallel in all cases, thus offering strong support that IF is a mediator of suppression in this system.

### Discussion

In the past several years two major mechanisms of immunological suppression have been described, one specific the other not. The first to be described (27, 28) was a nonspecific suppression of antibody formation *in vitro* induced by mitogen stimulation of lymphocytes from experimental animals. Both mitogen-treated lymphocytes and soluble factors produced by them were found capable of restricting cellular immune responses as well (29). The soluble factor responsible, termed "SIRS" (30), had properties similar to migration inhibitory factor and has more recently been shown to be capable of correcting the defect in NZB mice *in vivo* responsible for their developing autoimmune disease (31). The second mechanism of regulation demonstrated in experimental animals was the antigen-specific and genetically restricted control of antibody formation and cell-mediated immune reactions controlled by T cells (1). Recently, evidence has been provided that indicates that this type of suppression can be mediated by a molecule that possesses specificity for antigen and determinants of I-region gene products (32).

Because of the difficulty in carrying out studies on genetic restrictions in man, there are only a limited number of experiments that confirm the existence of human genetically restricted or antigen-specific T cell-mediated suppression (3, 4). The principal tool used to study defects in the cell-mediated immunosuppressive mecha-

nism has been the study of mitogen-induced suppressor cell activity. Considerable evidence exists that indicates that mitogen-activated human lymphocytes can suppress both *in vitro* cell-mediated and antibody responses, and that defects in this mechanism have been identified in such clinical entities as multiple sclerosis (33), lupus erythematosus (34–36), and autoimmune syndromes (37).

Although there is compelling evidence that T cells are involved in mitogen-induced suppression, it has not been possible to demonstrate rigorously that T cells are the only cells capable of mediating this response. In any case, the mechanism of the mitogen-induced suppressor activity of human lymphocytes and the nature of the soluble suppressor factor has not been elucidated.

The possible role of IF in Con A-induced suppressor activity of human lymphocytes was suggested by the finding that when two assays of lymphocyte activation were used as the detection system for monitoring Con A-induced suppression, the VPA invariably showed higher levels of suppression than did the incorporation of [<sup>3</sup>H]TdR. Because the virus used to detect lymphocyte activation in the VPA was VSV, known to be a sensitive indicator virus for IF assay, it seemed likely that the increase in sensitivity to Con A suppressor activity shown in the VPA was related to the sensitivity of the virus system for detecting IF activity. Although IF are defined by their antiviral activity, it is clear that they are capable of exerting a variety of functional and molecular changes in a variety of cell types (38). At least three molecular species that possess IF activity have been defined. A number of laboratories have reported that one or more types of IF were capable of inducing suppression of T cell or B cell responses *in vivo* and *in vitro* (12–14, 39).

The data presented here confirm an immunoregulatory function of IF and suggest that they may be the principal agents mediating mitogen-induced suppression of lymphocyte activation measured *in vitro*. The evidence in support of this conclusion is derived from the following observations: (a) There was a correlation between the suppressive activities of Con A-treated modulator lymphocytes and their ability to produce IF *in vitro*. (b) There was a parallel between the suppressive activities of supernates of Con A-stimulated lymphocytes and their antiviral activity. (c) Culture supernates that contained IF induced by virus or tumor cell coculture or by antigen (PPD) mimic the effect of mitogen-induced suppressor cells and supernatants. (d) The suppressive activities of Con A-stimulated lymphocytes or their culture supernates were markedly inhibited by two different antisera prepared against human leukocyte IF. At present, we are unable to exclude the possibility that factors other than IF contribute to suppression in this system.

By the indirect means available, it is difficult to define rigorously the species of IF that effects the nonantiviral activities being studied. In our experiments, NDV-induced IF from human leukocytes was found to be entirely pH 2 stable and completely neutralized by a rabbit and sheep anti-human type I leukocyte IF, and behaved as classically defined type I leukocyte IF. The PPD-induced immunosuppressive and antiviral activities were found to be partially pH 2 labile and partially affected by the antibodies to type I leukocyte IF, but completely neutralized by both treatments, thus having characteristics of a mixture of both types of IF. The immunosuppressive and antiviral activities produced by the interaction of normal human leukocytes and human tumor cells had the properties of type I leukocyte IF. The Con A-induced suppressor and antiviral activity shared properties with both type I and

type II IF. It was largely inactivated by treatment at pH 2, and yet 70% of the activity was abolished by treatment with anti-type I leukocyte IF antiserum. Although alternative explanations are possible, e.g., that anti-type I IF sera have some cross-reactivity with type II IF, or that there exists a subset of type I IF molecules that are pH 2 labile, the simplest interpretation would be that Con A- and PPD-stimulated human PBL produce both type I and type II leukocyte IF that mediate the observed suppressor activity. On the basis of the antibody neutralization studies, we would assume that ~ 70% of the suppressor activity of Con A-activated cells is mediated by type I leukocyte IF, and that 30%, not neutralizable with these sera, would represent type II IF. Because it has been clearly demonstrated that T cells can produce type II IF (40) and that a non-B non-T subset with characteristics of NK cells can produce type I IF (41), we would conclude that both lymphocyte subsets may be stimulated by Con A to carry out suppressor function *in vitro*.

We believe that the results described here might have useful application to the study of a number of human diseases, including lupus erythematosus and multiple sclerosis, in which a defect of mitogen-induced suppression has been described. Whether the primary defect is truly an immunoregulatory one, or is related to the failure to produce or to respond to IF might provide useful insights into the etiology and pathogenesis of these conditions. In this regard, it is of interest that there was a defect in virus-induced suppression in 32 of 36 patients with multiple sclerosis that was paralleled precisely by their inability to produce IF (42). Recently it has been reported that immune IF is elevated in the serum of some patients with autoimmune disease (43).

Secondly, Trinchieri and Santoli (11) reported that some human tumor cell lines are capable of stimulating the production of IF from normal human lymphocytes, whereas others are not. In the present experiments, we have confirmed this observation and extended it to demonstrate a parallel between their ability to induce IF and their ability to engender suppressor cells. Because of the importance of IF as an immunoregulatory molecule capable of stimulating NK cell activity and its demonstrated suppressor activity on lymphocyte, presumably T cell, activation, it will be of interest to study the antiviral and suppressive response of lymphocytes from patients with various forms of cancer to search for immunoregulatory defects in that disease.

### Summary

Evidence is presented that interferon (IF) is a major mediator of the human concanavalin A (Con A) suppressor cell. The suppressive effects of Con A-activated lymphocytes on the mitogen responses of normal responder cells were largely abrogated by addition of anti-human leukocyte IF serum. Similar suppressor activity was generated by coculture of peripheral blood leukocytes (PBL) with a melanoma cell line (MeWo) and a HeLa cell line persistently infected with measles virus that induced the production of IF by lymphocytes. A human mammary carcinoma line (MCF-7) and two bladder carcinoma lines (T24 and TCCSUP) failed to induce IF or suppression. Addition of anti-human leukocyte IF serum to suppressor cells and supernates from tumor cell-lymphocyte cocultures largely abolished suppression and neutralized the antiviral activity of such supernates. Exposure of PBL from purified protein derivative (PPD)-positive donors to PPD caused the production of suppressor activity and IF. PBL from PPD-negative donors failed to produce significant amounts

of IF or to suppress on exposure to PPD. Supernates from PBL treated with virus (Newcastle disease virus [NDV]) contained IF and suppressed the mitogen responses of responder PBL. Both the suppressive and the antiviral activities of this material were eliminated after treatment with anti-IF serum. To ascertain whether antiviral and suppressive activities were mediated by the same types of IF, supernates from PBL cultured with Con A, PPD, NDV, and tumor cells were treated with anti-IF serum or acid pH. In all cases antiviral activity was neutralized in parallel with abrogation of suppressor activity. These results provide strong evidence for the role of IF as a mediator of human suppressor cell activity.

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