ANTI-VIRAL ACTIVITY INDUCED BY CULTURING LYMPHOCYTES WITH TUMOR-DERIVED OR VIRUS-TRANSFORMED CELLS

Identification of the Anti-Viral Activity as Interferon and Characterization of the Human Effector Lymphocyte Subpopulation*

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Interferons are cellular glycoproteins that inhibit viral replication and that may be involved in the defensive response of an animal to virus infection. Virus infection of cells induces the synthesis and release of interferons, which render uninfected cells resistant to virus infection. Interferon can be induced by agents other than viruses, such as micro-organisms (1), substances of microbial origin (2), and synthetic polymers (3). Although interferon is not specific for a particular virus, it displays a characteristic cell specificity, being more active on homologous than on heterologous cells. Interferons not only display anti-viral activity, but also have been shown to affect various cellular mechanisms that are not directly related to viral replication. Interferons inhibit cell growth (4), facilitate or suppress interferon induction (priming and blocking effects) (5, 6), and increase the susceptibility of cells to the toxicity of double-stranded RNAs (7). Moreover, they affect several immunological functions: interferons inhibit antibody formation in vivo and in vitro (8, 9), and enhance the phagocytic activity of macrophages (10), the cytotoxicity of lymphocytes (11–13), and IgE-mediated histamine release from basophils (14).

Exposure of lymphocytes to viruses induces the release of interferon molecules that can be distinguished immunologically from the interferon produced by other cell types (15). The synthesis of interferon from lymphocytes can also be induced by immune stimulation (bacterial [16] and viral antigens [17–19], allo-antigens [20]), anti-lymphocyte sera (21), and mitogenic lectins (22).

We have recently described that viral inhibitors are released in the supernates of mixed cultures of lymphocytes and certain tumor-derived or virus-transformed cell cultures (23). In the present study we identify these inhibitors

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as interferons and characterize some of their anti-viral and anti-cellular activities. Moreover we identify the producer cells in the human peripheral blood as Fc-receptor bearing lymphocytes that have no surface immunoglobulins and that do not form rosettes with sheep erythrocytes.

Materials and Methods

Reagents. The reagents used in these studies and their sources are as follows: cycloheximide, 2-aminoethylisothio-uronium bromide hydrobromide (AET),[1] and trypsin crystallized two times from bovine pancreas (3.4.4.4) from Sigma Chemical Co., (St. Louis, Mo.); actinomycin D from Calbiochem (San Diego, Calif.).

Cell Lines and Virus. The origin of and reference for the cell lines used in this study are summarized in Tables I and II. Most of the simian virus 40 (SV40)- and adenovirus 5-transformed cell lines were originated in our laboratories.

The 73-T strain of Newcastle disease virus (NDV) (allantoic fluid, 3.3×10^9 plaque-forming units (PFU)/ml on L929 cells), the Indiana strain of vesicular stomatitis virus (VSV, 3×10^8 PFU/ml on L-F_2 cells) and encephalomyocarditis virus (EMC, 4×10^8 PFU/ml on F-F_2 cells) were originally obtained from T. J. Wiktor (Wistar Institute). Vaccinia virus (Lister strain), provided by M. Herlyn (Wistar Institute), was originally obtained from Wyeth Laboratory, (10^6 TCID50/ml on WI38 cells). The Hong Kong/107/68 (H3N2) strain of influenza A virus (allantoic fluid, 850 hemagglutination units-ml) was obtained from W. Gerhard (Wistar Institute).

Human Lymphocyte Separation. Lymphocytes were obtained from heparinized human peripheral blood by centrifugation on a Ficoll-Hypaque gradient (31). The lymphocyte suspensions obtained was contaminated by 5–30% monocytes as identified by nonspecific acid esterase staining (acid α-naphthyl acetate esterase, ANAE) (32) and was further depleted of adherent cells by two 1-h incubations on glass Petri dishes at 37°C.

Further cell fractionation was obtained by separation of nylon fiber-adherent cells (33).

Cells bearing surface immunoglobulins or Fc-receptors were removed by passing the lymphocyte population through a Sepharose IgG-anti-IgG column. Cyanogen bromide-activated Sepharose 6B (Pharmacia, Uppsala, Sweden) was conjugated with normal human IgG (purified by DEAE chromatography, 10 mg/ml of gel), and columns containing 6 ml gel were prepared. 20 mg of IgG from a polyvalent rabbit anti-human IgG serum was run on the column, which was eluted several times before 10^8 lymphocytes in RPMI 1640 medium (1% FBS) were added to it and incubated 30 min at 4°C. The nonadherent cells were eluted from the column with ice-cold medium. Columns without anti-IgG serum were used as controls.

Cells forming rosettes with AET-treated sheep erythrocytes were separated from non-rosette-forming cells by the method of Pellegrino and Ferrone (34).

Mixed Cultures. Tumor-derived, virus-transformed, and fibroblast cell monolayers were grown in 16-mm wells (Disposo Trays FB16-24TC, Linbro Scientific, Inc. Div. of Flow Lab., Hamden, Conn.). Lymphocytes depleted of glass adherent cells were resuspended at 10^7/ml in RPMI-1640 medium supplemented with 10% fetal bovine serum (RPMI-FBS, Flow Laboratories, Inc., Rockville, Md.) and 1 ml was added to each well. The mixed cultures were incubated for 24 h at 37°C in a humidified 5% CO_2:95% air atmosphere, then the supernate was collected, centrifuged at 1,000 *g* for 30 min, and stored at −80°C until tested.

Interferon Tests. For testing human interferon, monolayers of fetal skin fibroblasts (FS2) were grown in the wells of microtiter plates (3040, BioQuest, BBL & Falcon Products, Becton, Dickinson & Co., Cockeysville, Md.). The medium was removed from the wells, and 0.1-ml samples of serial dilutions of the supernates to be tested were added. After an 18-h incubation, the supernates were removed and 0.2 ml of VSV (50,000 PFU) was added to each well. The anti-viral activity was measured by inhibition of the cytopathic effect of VSV. Anti-viral units are expressed

[1] *Abbreviations used in this paper*: AET, 2-aminoethylisothio-uronium bromide hydrobromide; ANAE, acid alphanaphthyl acetate esterase; EBV, Epstein-Barr virus; EMC, encephalomyocarditis virus; FBS, fetal bovine serum; HAU, hemagglutination units; MOI, multiplicity of infection; NDV, Newcastle disease virus; PBS, phosphate-buffered saline; PFU, plaque-forming unit; RDMC, rhabdomyosarcoma cell line; SV40, simian virus 40; $TCID_{50}$, tissue culture infectious dose 50%; VSV, vesicular stomatitis virus.

as the reciprocal of the highest dilution inhibiting 50% of the cytopathic effect. Each assay included a standard interferon preparation (NIH human Reference Interferon G-023-901-527 with a titer of 20,000 U). 1 anti-viral U in our testing procedure is equivalent to approximately 1 reference U of the NIH interferon.

Mouse interferon was similarly measured on monolayers of murine L-F_2 cells, by using EMC (50,000 PFU/well) as the challenge virus (35). Assays were standardized against an NIH mouse interferon reference standard. 1 anti-viral U in this testing procedure cells is equal to 1 U of reference standard.

Leucine, Uridine, and Thymidine Uptake. 10^4 trypsinized cells in 0.1 ml RPMI-FBS were added to flat bottom wells of microtiter plates (3040, Falcon), and 0.1 ml of dilutions of the supernates to be tested were added to triplicate wells. The cells were incubated for 18 h at 37°C, then 2 μCi of [^{3}H]labeled precursors (New England Nuclear Corp., Boston, Mass.) were added to each well. The cells were incubated for another 6 h, then the medium was removed, 0.2 ml of trypsin (0.25%) was added, and the cells were collected on to glass fiber filters (GF/B, Whatman, Inc., Clifton, N. J.) by means of a semi-automatic collector (O. Hiller, Madison, Wis.). Radioactivity was assessed by liquid scintillation spectrometry. [^{3}H]thymidine uptake inhibitory units are defined at the reciprocal of the dilution inhibiting 50% [^{3}H]thymidine uptake in rhabdomyosarcoma (RDMC) cells.

Results

Anti-Viral Activity in the Supernates of Mixed Cultures. The results obtained by testing for presence of anti-viral activity in the supernates of mixed cultures of human lymphocytes with cells obtained from fibroblast, virus-transformed, tumor-derived, and lymphoid cell lines are summarized in Table I. No anti-viral activity was usually observed in the supernates from human lymphocytes cultured alone. None of the 13 human fibroblast cultures tested induced anti-viral activity when mixed with human lymphocytes, whereas 13 out of 21 tumor-derived cell lines were inductive. The association between the tumor origin of a line and its ability to induce interferon was highly significant (P = 0.00022, Fisher's exact test). None of the cell lines, including the lymphoid lines, spontaneously released detectable anti-viral activity when cultured alone under conditions comparable to those of the mixed cultures. Moreover, the supernates from inducer cell lines, which included one EBV- and two SV40-producing lines, were unable to stimulate human lymphocytes to produce the virus inhibitor(s).

Table II summarizes the results of culturing murine cells from several different lines with human lymphocytes or with mouse spleen cells. The supernate from BALB/c spleen cells cultured alone usually contained 1–2 U of anti-viral activity. The lines that induced anti-viral activity when cultured with human lymphocytes also induced such activity in mouse spleen cells, although the activity obtained with mouse lymphocytes was always much lower.

Allogeneic and Syngeneic Mixed Cultures. As a control in each experiment, two allogeneic human lymphocyte preparations were cultured together. When tested for anti-viral activity after a 24-h incubation, the supernates from these cultures were always negative. Lymphocytes from donor H. K., from whom the lymphoid line HK was established by transformation of peripheral blood lymphocytes with Epstein-Barr virus (EBV), were able to produce anti-viral activity when mixed with autologous HK cells. When spleen cells from C57BL/6 or BALB/c mice were cultured with cells from lines able to induce interferon production in human lymphocytes, anti-viral activity was detected in the supernate of xenogeneic, allogeneic, and syngeneic combinations (Table III). The anti-viral inhibitors in the supernates from mixed cultures of lymphocytes and

TABLE I

Origin of Human Cells and Ability to Induce Anti-Viral Activity When Cultured with Human Lymphocytes

Cell	Reference	Origin	Passages	Anti-viral Units Induced* (Mean ± standard error)
		Fibroblasts		
FS1 and 2		Fetal skin	14–20	<1
Two strains		Fetal skin	35–45	<1
Seven strains		Skin	5–20	<1
Two strains		Brain	2–15	<1
		SV40-transformed		
Five lines		Skin	24–255	<1
Nine lines		Brain	4–22	<1
S1054TR		Brain	32–37	193 ± 137
S10033WTR		Brain	8–12	125 ± 0
		Adenovirus 5-transformed		
293/31	24			<1
NP1	24	293/31 tumor in nude mouse		<1
		Kirsten murine sarcoma virus-transformed (nonproducer)		
2-970-S	25	Osteosarcoma (TE85, Cl F5)		337 ± 152
A172-10‡		Melanoma		1,562 ± 587
		Tumor-derived lines		
SW690§		Melanoma	80–90	850 ± 552
SW691		Melanoma	80–85	6,000 ± 3,000
SW843		Melanoma	30–40	244 ± 119
SW489		Melanoma	30–40	25 ± 0
A375		Melanoma	76	25 ± 0
A375		Melanoma	95	194 ± 137
Four lines	26	Colorectal carcinoma	10–95	<1
SW620	26	Colorectal carcinoma	160–165	362 ± 137
SW480	26	Colorectal carcinoma	105–110	125 ± 0
D98 (HeLa)	27	Cervical carcinoma	50–60	312 ± 165
A673		Sarcoma		1,081 ± 649
HT1080	28	Fibrosarcoma	110–115	<1
SW80		Rhabdomyosarcoma	48–50	<1
RDMC	29	Rhabdomyosarcoma	150–200	3,494 ± 1,008
TE85, Cl F5	25	Osteosarcoma	15	<1
		Lymphoid lines		
Daudi	30	Burkitt lymphoma		<1
SB		Lymphatic leukemia		1,562 ± 823
PGIP7		Lymphatic leukemia		1,800 ± 693
CMG		PBL-EBV‖		75 ± 28
EB-P8		PBL-EBV		2,120 ± 559
HK		PBL-EBV		600 ± 0
(B95)		(Marmoset, EBV producer)		1,800 ± 693

* Mean of results obtained with at least four lymphocyte preparations from different donors.

‡ Cell lines with A as first letter originated in Dr. S. A. Aaronson's laboratory (National Cancer Institute, Viral Carcinogenesis Branch).

§ Cell lines with SW as first letters originated in Dr. A. Leibovitz' laboratory (Scott and White Clinic, Temple, Texas).

‖ Human peripheral blood lymphocytes, Epstein-Barr virus-transformed.

TABLE II

Origin of Murine Cells and Ability to Induce Anti-Viral Activity When Cultured with Lymphocytes

Cell line	Mouse strain	Origin	Passages	Anti-viral units produced	
				Human lympho-cytes	Mouse lympho-cytes
BICR	Random bred albino	Brain	17–19	<1	ND*
3T3	BALB/c	Embryo	97–101	65	ND
NTG2	BALB/c	Embryo (3T3)	80–85	16	ND
IT22	Random bred albino	Embryo (3T3)	47–70	<1	ND
Cl 1D	C3H/HeJ	Earle's L cells	210–213	125	16
L-F_2	C3H/HeJ	Earle's L cells		ND	16
MG57G	C57BL/6	Methylcholanthrene-induced tumor	310–325	255	ND
P815Y	DBA/2	Mastocytoma		128	32
L-5178Y	DBA/2	Lymphoma		64	32
F9	129/J	Embryo-derived tera-tocarcinoma		ND	<2
		SV40-Transformed cell lines			
C57SV	C57B1/6J	Embryo	160–170	60	8
SV3T3	BALB/c An	Embryo	90–110	722	16
16 lines	Various	Kidney	13–90	<1	≤2
K129SV	129/J	Kidney	93–96	1,200	32
KG$^-$SV	129 G^{ix-}	Kidney	77–87	2,000	64
KT6SV	CBA/H-T6J	Kidney	75–80	125	64
K4RSV	B10.A(4R)	Kidney	70–77	600	32
4 lines	Various	Spleen	13–80	<1	≤2
5 lines	Various	Liver	21–30	<1	≤2
BT6SV	CBA/H-T6J	Blood	50–60	<1	1
BICR-TR	Random bred albino	BICR brain	6–8	<1	ND
		Adenovirus 5-transformed cell lines			
7 Lines	Various	Kidney	17–53	<1	<2

* ND = not done.

TABLE III

Induction of Anti-Viral Activity in Mixed Cultures Containing Mouse Lymphocytes and Xenogeneic, Allogeneic, or Syngeneic Cultured Cells

Cell line	Origin	Lymphocytes			
		H-2	BALB/c	C57BL/6	(BALB/c × B6)F_1
None	–	–	2*	<1	<1
RDMC	Human	–	32	8	8
SV3T3	BALB/c	d	16	4	8
P815	DBA/2	d	64	32	16
C57SV	C57BL/6J	b	16	4	4
K129SV	129/J	b	32	4	8
KT6SV	CBA/H-T6J	k	16	4	4

* Anti-viral units produced in the supernate of the mixed cultures.

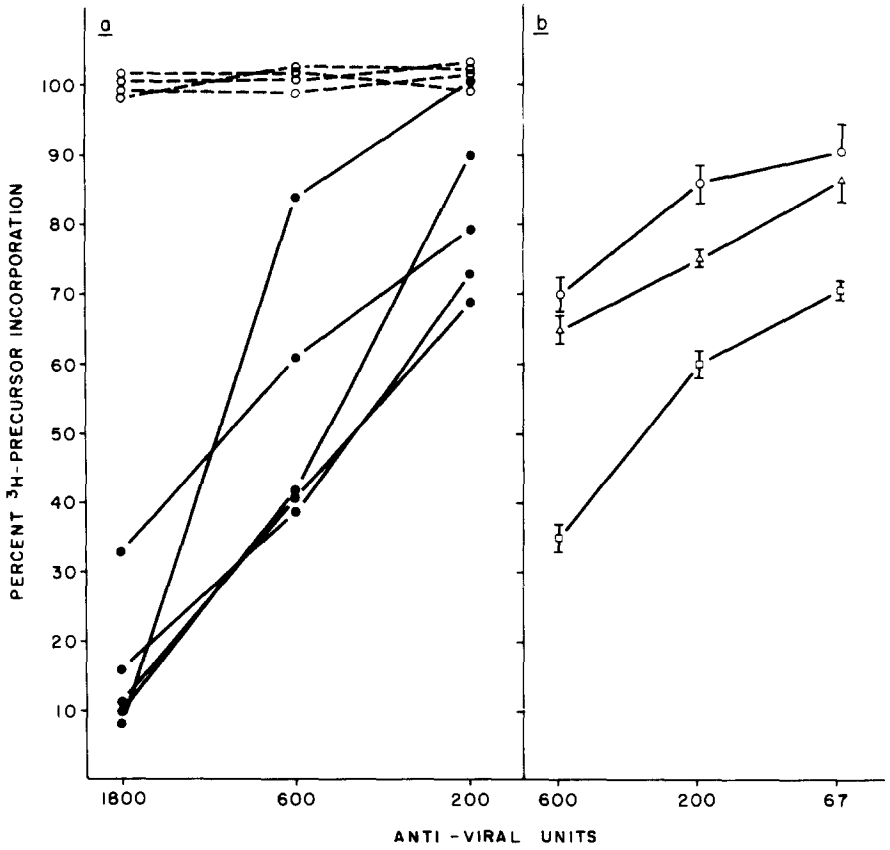

FIG. 1. Inhibition of thymidine, uridine, and leucine uptake in human and murine cells treated with supernates from mixed cultures of lymphocytes and RDMC cells. The experimental points are expressed as percent of the incorporation in control cultures. a:[^{3}H]thymidine incorporation in five different human cell lines (●——●) and four mouse cell lines (○——○). b: ○——○, [^{3}H]leucine uptake; ▽——▽, [^{3}H]uridine uptake; □——□, [^{3}H]thymidine uptake in RDMC cells. Vertical bars indicate standard deviation.

cell lines displayed an almost complete specificity for cells of the same species of the lymphocytes. Supernates from xenogeneic cultures of human lymphocytes mixed with murine cells or of mouse lymphocytes with human cells inhibited viral replication only in human and in mouse cells, respectively.

Characterization of the Viral Inhibitor. Supernate from human mixed cultures was not toxic for human fibroblast cells. Pretreatment for 18 h at 37°C of [^{51}Cr]labeled FS1 cells with a dilution of supernate from a mixed lymphocyte-RDMC culture containing 10^{3} anti-viral U did not increase the spontaneous release of ^{51}Cr from the cells (36). The preparations containing anti-viral activity effectively inhibited viral replication at a dilution at which cellular DNA, RNA, and protein synthesis were not affected (Fig. 1).

The anti-viral activity in the supernate of mixed human lymphocyte human cell cultures was active on FS1 cells by using different challenge viruses (NDV, EMC, vaccinia virus, and influenza A (H3N2) virus).

The viral inhibitor in the supernate did not inhibit virus replication when

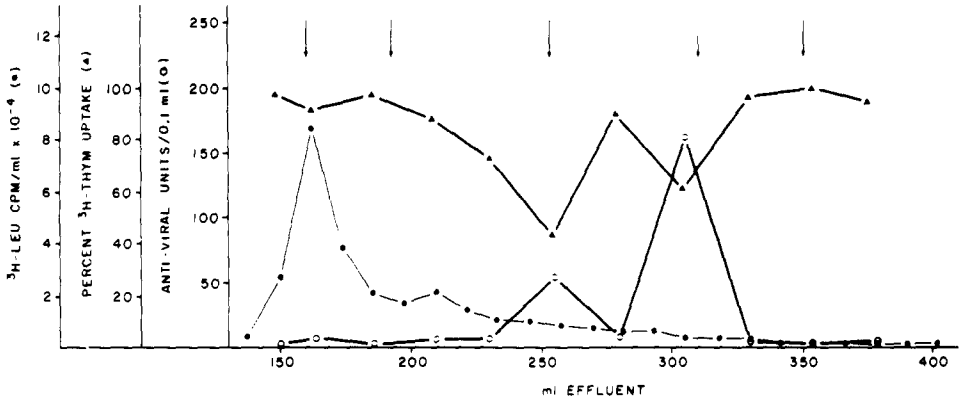

FIG. 2. Gel filtration on Sephadex G100 column of concentrated supernate from a mixed culture of human lymphocyte-RDMC cells. Cells were cultivated in the presence of 10 μCi [^{3}H]leucine/ml of medium. 30 ml of supernate was concentrated to 3 ml by vacuum dialysis and applied to the column (25 × 960 mm, 471 cm^3, void volume 160 ml). Arrows indicate molecular weight markers; from left to right: blue dextran 2,000 (mol wt 2,000,000), aldolase (158,000), ovalbumin (45,000), chymotrypsinogen A (25,000), ribonuclease A (13,700). The fractions were dialyzed against RMPI 1640, sterilized by filtration, and tested for anti-viral activity and ability to inhibit [^{3}H]thymidine uptake in RDMC cells.

added to the indicator cells at the same time as the virus; FS1 cells pretreated with the supernate and then carefully washed were unable to replicate VSV. Actinomycin D (1 μg/ml) or cycloheximide (250 μg/ml) present during treatment of FS1 with dilutions of supernates containing up to 200 anti-viral U completely prevented the inhibition of VSV replication.

The viral inhibitor was completely destroyed by treatment of diluted supernate with 0.1 mg/ml of trypsin (1 h incubation at 37°C). After gel filtration of concentrated supernate on a Sephadex G100 fine column, the anti-viral activity was recovered in two peaks of apparent mol wt of approximately 45,000 and 25,000 daltons (Fig. 2). In some experiments, a variable amount of activity was in front of the albumin peak. Chromatography of NDV-induced lymphocyte interferon (obtained after treatment at pH 2.0) gave only one peak of anti-viral activity with mol wt 25,000. When NDV interferon was not pretreated at pH 2.0, the same two peaks (45,000 and 25,000) were obtained. The anti-viral activity in the 25,000 peak was resistant to treatment at pH 2.0, whereas the activity in the 45,000 peak was destroyed under the same conditions.

The above characterization of the viral inhibitor in the supernates of mixed cultures allows their acceptance as interferons.

Characterization of the Human Lymphocyte Population Producing Interferon. Human lymphocytes, preincubated at 22°C for a period of up to 24 h, maintained the ability to produce interferon when induced by RDMC cells in mixed culture or by infection with NDV. In contrast, after 17 h of preincubation at 37°C, the lymphocytes were unable to be induced by RDMC cells, but still responded to NDV infection with an anti-viral activity reduced to about 20% of that observed with fresh cells.

The results of experiments with cell fractions of the lymphocyte population are presented in Table IV. Depletion of glass-adherent cells from Ficoll-Hy-

TABLE IV

Ability of Human Lymphocyte Subpopulations to Produce Interferon in Mixed Lymphocyte-RDMC Cultures

Fraction	ANAE*	S-Ig‡	E-RFC§	Fc-rec‖	Anti-viral units produced
	%	%	%	%	
Exp. A Ficoll-Hypaque separated	22.3	ND	ND	ND	125
Glass nonadherent	6.7	ND	ND	ND	3,000
Exp. B Ficoll-Hypaque separated	17.9	22.7	62.9	36.6	160
Nylon column nonadherent	1.4	2.4	86.4	20.2	640
Nylon column adherent	30.4	55.8	24.2	66.0	10
Exp. C Nylon column nonadherent	0.9	1.7	88.4	22.9	600
AET-SRBC Nonrosetting	11.4	32.0	0	48.5	3,000
AET-SRBC Rosetting	0	0	97.6	10.2	25
Exp. D Ficoll-Hypaque separated	23.6	14.2	77.2	38.2	80
IgG-Anti-IgG column nonadherent	9.3	0.9	89.4	9.8	<5
IgG Column adherent	21.0	14.9	73.1	29.4	40

* Staining for nonspecific acid esterase, strongly positive cells (monocytes) scored (32).

‡ Positive fluorescence for surface immunoglobulin [fluorescein-rabbit $F(ab')_2$ anti-human $F(ab')_2$] (37).

§ Cells forming rosettes with AET-treated sheep erythrocytes (18 h incubation at 4°C) (38).

‖ Positive fluorescence for Fc-receptor with heat aggregated human IgG (39) and fluroescein-labeled anti-human IgG.

ND, not done.

SRBC, sheep erythrocytes.

paque-separated lymphocytes increased the level of anti-viral activity obtained by induction with RDMC cells (Exp A). The major population responsible for interferon production in mixed cultures of lymphocytes and RDMC cells was not adherent to nylon fiber columns, did not form rosettes with AET-treated sheep erythrocytes, and was retained on an Ig-anti-IgG column. The cell fractions in Exp. C of Table IV were all incubated with erythrocytes and subjected to hypo-osmotic treatment to avoid artifactual results due to different handling of the subpopulations.

Anti-Cellular Activity. Supernates from mixed cultures of human lymphocytes and cells from RDMC or any other interferon-inducing cell line inhibited [^{3}H]thymidine incorporation in human cells from several lines, but not in cells from murine lines (Fig. 1 a). [^{3}H]leucine and [^{3}H]uridine incorporation were also inhibited in human cells, but not to as great an extent as was [^{3}H]thymidine incorporation. The supernates from unmixed cultures of lymphocytes or of cells from the various lines tested and the supernates from mixed cultures of lymphocytes and cell lines unable to induce interferon did not inhibit incorporation. The activity inhibiting thymidine incorporation and the anti-viral activity were significantly correlated in 15 supernates from mixed cultures of lymphocytes from different donors and RDMC cells ($r = 0.865$, $P < 0.001$, Fig. 5). About 500 anti-viral U were required to inhibit 50% uptake in RDMC cells (Figs. 1 b and 3). In gel filtration experiments the activity inhibiting thymidine incorporation was eluted in the same two peaks of the anti-viral activity.

Inhibition of Cell Growth. Supernates from mixed cultures of lymphocytes and cells from RDMC or other interferon-inducing lines were able to inhibit cell growth in different human cell lines (Fig. 4). This effect was detected only when supernate was used in concentrations containing more than 500 anti-viral U. The supernate from lymphocytes cultured alone or from RDMC cells cultured alone had no significant effect (Fig. 4).

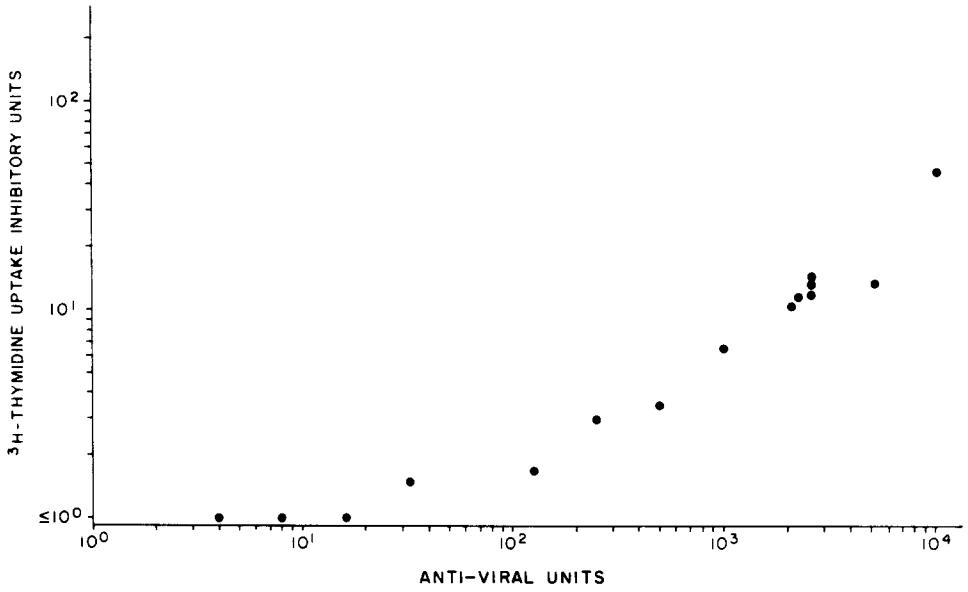

FIG. 3. Anti-viral and anti-cellular (inhibition of thymidine uptake) activity in 15 supernates from mixed cultures of RDMC cells and human lymphocytes from different donors. Each dot represents the results obtained with one interferon preparation.

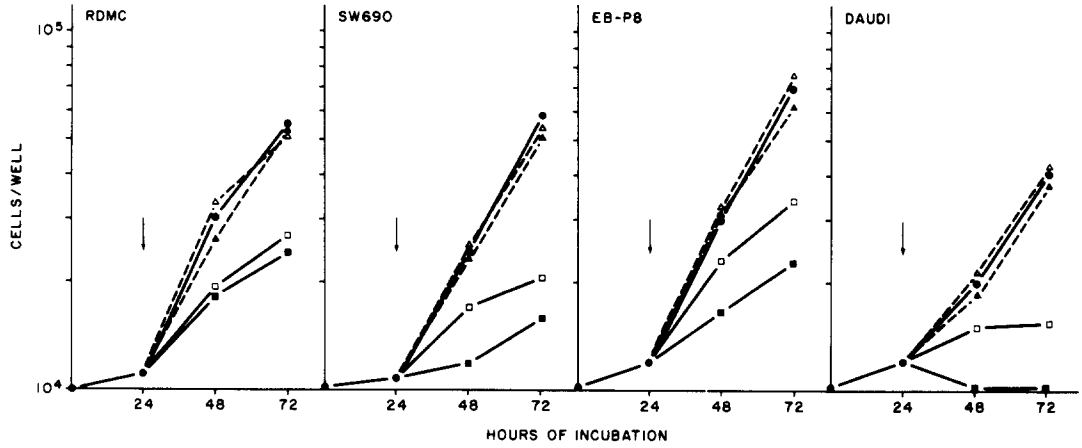

FIG. 4. Inhibition of cell growth of four different cell lines (RDMC, SW690, EB-P8, and Daudi) by supernate from mixed lymphocyte-RDMC cultures. The arrows indicate the times when supernates (0.1 ml) were added to cells (incubated in 0.1 ml medium). ●——●, medium only; ■——■, mixed lymphocyte-RDMC supernate, 1,800 anti-viral U; □——□, 600 anti-viral U; △——△, lymphocyte supernate; ▲——▲, RDMC supernate.

Discussion

A virus inhibitor(s) is released in the supernate of mixed cultures of lymphocytes and certain tumor-derived and virus-transformed cell lines after a few hours of incubation (23). The species preference of the inhibitor produced in the xenogeneic mixed cultures identifies the lymphocytes as the producers of the inhibitor. The viral inhibitor(s) from human mixed cultures meets the

criteria established by Lockart (40) for acceptance of a viral inhibitor as an interferon: (*a*) it is a protein that is readily destroyed by incubation with trypsin; (*b*) it does not inhibit viral replication through a toxic effect on the cells; (*c*) it is active against all viruses tested; (*d*) it does not directly inactivate the virus; instead it induces an anti-viral status in the cells that persists when the inhibitor is removed from the medium; synthesis by the cells of both RNA and protein are required for induction of the anti-viral status. In addition, the viral inhibitor shows other properties that are common to most interferons: it is more active in homologous than heterologous cells; it is not released spontaneously but only in the presence of the inducer cell line; one of the two molecular populations identified by gel filtration is stable at pH 2.0.

The gel filtration experiments show that at least two molecular species of interferon are present in the supernate of the mixed cultures. The viral inhibitor which elutes in a peak corresponding to a mol wt approximately 25,000 represents the majority of the activity. This interferon is stable at pH 2.0, and is probably corresponding to the type I interferon of Youngner and Salvin (41). Type I interferon in the mouse is produced by cells other than T cells and can be induced by viruses and by B-cell stimulants (41, 42). In humans, NDV-induced leukocyte interferon (43, and our results) also has a mol wt of approximately 25,000. The viral inhibitor in the supernate of mixed cultures, which is eluted with a peak of approximately 45,000, is unstable at pH 2.0 and shares most of the characteristics of the interferon designed as type II (41) or immune (44) interferon. In the mouse, this type of interferon is considered to be a product of T cells activated mainly by antigenic or mitogenic stimulation (42, 44). However, we have found some anti-viral activity in a peak corresponding to a mol wt of 45,000 after gel filtration of NDV-induced interferon that had not been pretreated at pH 2.0. We have also found the presence of a variable amount of anti-viral activity eluting in front of the albumin peak possibly due to molecules of interferons that bind to albumin or other proteins (45).

Cell separation experiments have been performed to identify the population of human peripheral blood lymphocytes necessary for interferon production in the mixed cultures. Monocytes are not only unnecessary for interferon production in mixed cultures but also have an inhibitory effect. Depletion of B cells (surface immunoglobulin-bearing cells) and monocytes by incubation on nylon fiber columns increased interferon production by the eluted cell population. The B-cell-enriched populations recovered from the columns produced very little interferon. On the other hand, lymphocytes depleted of both surface immunoglobulin-bearing cells and Fc-receptor-bearing cells (on IgG-anti-IgG columns) were capable of producing only minimal amounts of interferon in the mixed culture experiments. These data suggest that the major effector cell population is composed of lymphocytes without surface immunoglobulins but with Fc-receptors. To evaluate the role of T cells in the production of interferon we employed AET-treated sheep erythrocytes, reported to be the most sensitive for rosetting techniques (38). When corrected for the different numbers of cells recovered in the two populations (usually 4–10 times more cells in the rosetting fraction), the data indicate that most of the interferon-producing lymphocytes do not bind to AET-treated sheep erythrocytes. These results exclude a major role of T cells in the production of interferon in this system. All the lymphocyte

subpopulations able to produce interferon also contained most of the activity of the natural killer cells. Natural killer cells not only spontaneously interact with and lyse inducer cells in the mixed cultures (46), thus possibly playing some role in the induction of interferon itself, but their cytotoxic activity is also strongly enhanced by the presence of interferon in the culture (36).

In addition to the anti-viral activity, an anti-cellular activity is present in the supernate of mixed human lymphocyte human cell cultures. The major effect on cellular metabolism is inhibition of DNA synthesis, as measured by inhibition of [^{3}H]thymidine uptake. That this assay actually measures inhibition of DNA synthesis and not an artifactual inhibition of the uptake of the labeled precursor, as observed with supernate from macrophage and lymphocyte cultures (47, 48), is suggested by these facts: the inhibitory activity is not dialyzable and it inhibits cell proliferation. Although a close correlation between anti-cellular and anti-viral activity has been observed in the present experiments, as in several other studies with virus-induced interferon (49–52), no conclusion can be derived on the unsettled issue of whether or not the anti-cellular and the anti-viral activities in interferon preparations are mediated by the same molecules (45, 49–53).

The characteristics that make certain cell lines able to induce interferon and the nature of the stimulus that triggers the lymphocytes are still unknown (23). Although the association of ability to induce interferon with transformation by EBV or mouse sarcoma virus or with the tumor origin of the line is statistically significant, any speculation on the biological relevance of such an association seems premature. That stimulation by allogeneic (or heterologous) antigens are responsible for stimulation may be excluded by the induction of interferon in autologous or syngeneic mixed cultures, both in human and in the mouse.

The possibility that viruses present in the cell lines are directly responsible for the induction seems to be unlikely. The possibility remains that some viruses usually associated with inducer cell lines (murine sarcoma virus, EBV), unknown viruses or other agents derived from the original tumor or acquired in culture induce particular antigenic or structural surface characteristics that are responsible for the interaction with the lymphocytes and their stimulation.

It is not possible to extrapolate the data obtained in vitro with cell lines to infer a stimulation of interferon in vivo by spontaneous tumors. Indeed, a hyporeactive factor, which inhibits interferon production, is present in the serum of tumor-bearing mice (54, 55). This factor could mask a stimulation by tumor cells. However, at least in the case of transplantable tumors in mice, our experiments and a preliminary report by others (56) show interferon induction by syngeneic tumor cells in vivo: because of the effect of interferon both on the host defense mechanisms and on the tumor cell metabolism (2) this phenomenon might affect the progress of the transplanted tumor.

Summary

A viral inhibitor(s) is released in the supernate of mixed cultures containing human or mouse lymphocytes and cells from certain lines. The inhibitor is active against a variety of unrelated viruses and is a protein that is not toxic for cells. It does not inactivate viruses directly, but inhibits viral replication through an intracellular mechanism that involves synthesis by the cells of both

RNA and protein. These characteristics identify the inhibitor as an interferon. The anti-viral activity is contained in at least two molecular species, of approximately 25,000 and 45,000 daltons, respectively. In addition to the anti-viral activity, the supernates of the mixed cultures display an anti-cellular activity, the inhibition of DNA synthesis and of cell multiplication. The anti-viral and the anti-cellular activities are positively correlated in supernates from various cultures and in partially purified preparations. The human cell population responsible for interferon production is composed mainly of Fc-receptor positive, surface immunoglobulin negative, non-T-cell lymphocytes. The ability of certain cell lines to induce interferon seems to be preferentially associated with tumor origin or with in vitro transformation by certain viruses (Epstein-Barr virus, murine sarcoma virus).

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