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Lymphocyte Transformation and Interferon Production in Human Mononuclear Cell Microcultures for Assay of Cellular Immunity to Herpes Simplex Virus

SVEN HAAHR,[1] LUCY RASMUSSEN,* AND THOMAS C. MERIGAN

Department of Medicine, Stanford University School of Medicine, Stanford, California 94305

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Interferon production and transformation in response to herpes simplex virus antigen were studied in microcultures of human mononuclear cells. Mononuclear cells consisting of monocytes and both T and B lymphocytes were purified by Ficoll-Hypaque gradients. Lymphocytes, predominantly T with 5% B, were obtained by passage of buffy-coat cells through nylon fiber columns. For some experiments, autochthonous macrophages and column-purified lymphocytes were stimulated with herpesvirus antigen. The effect of specific antibody and cell concentration on reactivity is described. Crude and purified antigens were compared as cell culture stimulants. Significant differences in transformation and interferon were observed between donors with a history of herpes labialis and donors with no detectable antibody, both in cultures prepared by Ficoll-Hypaque gradients and by column purification of lymphocytes. Cultures from seronegative donors prepared by Ficoll-Hypaque gradients produced interferon but did not transform when stimulated by herpes simplex antigen. "Immune" interferon production, that is, type II as opposed to type I, occurred only with autochthonous macrophage and columm-purified lymphocyte cultures. Interferon produced by Ficoll-Hypaque-purified mononuclear cultures was type I, and its production was unrelated to immune status. Similarly, column-purified lymphocytes responded to herpes simplex virus antigen with type I interferon if obtained from a seropositive donor.

In our laboratory, cellular immunity to herpes simplex virus (HSV) infections in humans is measured by interferon production and blastogenesis in cultures of autochthonous macrophages and T lymphocytes. The characteristics of this system have been described in previous publications (4, 9). To review briefly, the interferon produced by macrophage-lymphocyte (M-L) combined cultures is detected at highest titer after recent herpes labialis recurrence and subsequently declines. In contrast, blastogenesis correlates with presence of HSV antibody and does not alter quantitatively after a recurrent HSV infection (9). The memory for interferon production in M-L cultures depends upon the presence of T lymphocytes from an individual who has had recent HSV infection (18). The interferon produced in such cultures demonstrates the characteristics of an "immune" interferon (18–20), that is, lability to treatment at pH 2, instability to heating at 56 C, and failure to be neutralized by antisera to human leukocyte interferon.

The various methods used to prepare cultures of mononuclear cells for in vitro studies of cellular immunity to specific infections yield different populations of cells. Our system for study of HSV cellular immunity show the results of interaction of antigen with fully differentiated macrophages and predominantly ($P > 95\%$) T lymphocytes (18). Ficoll-Hypaque (F-H) gradient purification of mononuclear cells, used in many laboratories to prepare cultures for cellular immunity assays, yields T and B lymphocytes with monocytes. The following study was done to determine whether the products of an in vitro cell-mediated immune reaction varied according to the method used to prepare mononuclear cell cultures. Interferon production and blastogenesis were compared in cultures of F-H-purified mononuclear cells, purified T lymphocytes, and autochthonous M-L cultures. Microculture systems for studying these two parameters of cellular immunity and the influence of various factors such as serum supplement and physical state of the stimulating antigen are described.

MATERIALS AND METHODS

Viruses and antigens. The preparation of HSV type 1 viral and control antigens from HeLa cells

[1] Present address: University of Aarhus, Institute of Medical Microbiology, Aarhus, Denmark.

has been described (9). These antigens are designated as "crude" and contained 2×10^6 plaque-forming units (PFU) per ml before heat inactivation. Cytoplasmic extracts of infected HeLa cells were purified as described by Spear and Roizman (14). Infected HeLa cells were scraped from 32-oz (about 960-ml) bottles, pelleted by low-speed centrifugation, and then resuspended in approximately 2 volumes (vol/vol) of hypotonic phosphate buffer at pH 7.4. Cells were disrupted with four strokes of a Dounce homogenizer, and then nuclei were stabilized by addition of 60% (wt/vol) sucrose, added to a final concentration of 0.25 M. Two milliliters of cytoplasmic fraction, obtained as supernatant from low-speed centrifugation of the homogenized cell extract, was layered onto 36 ml of continuous gradient of dextran 10, ranging from densities of 1.05 to 1.14 g/cm^3, and centrifuged for 1 h at 20,000 rpm in a Beckman SW27 rotor. When [^{3}H]thymidine-pulsed HSV-infected cell extracts were used, this gradient resulted in distinct separation of the peak of [^{3}H]thymidine label (coincident with virus infectivity by PFU assay) from other acid-insoluble radioactivity. Infectivity titers in collected fractions were done by PFU assay. Fractions with peaks of virus infectivity were pooled, diluted two to three times in tris(hydroxymethyl)aminomethane buffer at pH 7.4, and then pelleted through a 10% sucrose gradient at 25,000 rpm for 1 to 2 h in a Beckman SW27 rotor. The resultant virus pellet was resuspended in 2 to 10 ml of tris(hydroxymethyl)aminomethane buffer and dialyzed for 24 h against McCoy medium. Recovery of ^{3}H-labeled virus from the sucrose gradient-derived virus pellet was quantitative. Virus infectivity was inactivated by heating to 56 C for 2 h. Because of loss of infectivity of HSV during the sucrose gradient step, antigen infectivity titer calculations are based on those determined on pooled peak fractions from dextran 10 centrifugations. Purified HSV antigen contained 6×10^6 PFU per ml before heat inactivation. Purified control antigen was prepared from uninfected HeLa cells, using these same procedures. The fractions from the dextran 10 gradient with the same density as those with peak virus yields were used as control antigens, omitting the sucrose gradient step.

Vesicular stomatitis virus (VSV) was propagated in cultures of chicken embryo cells as previously described (8).

Cell cultures. All mononuclear cell cultures prepared from peripheral blood were maintained in McCoy medium supplemented with 30% serum. Unless otherwise indicated, serum was obtained from donors with AB blood (hereafter referred to as human AB sera). Only sera from donors with a complement-fixing (CF) antibody titer of less than 1:4 were used.

The preparation of M-L cultures has been described in detail (4, 9). To review briefly, peripheral blood monocytes were cultured for 7 days to obtain macrophages. Buffy-coat cells were passaged through a nylon fiber column at 37 C to obtain an effluent consisting of ±95% T and ±5% B lymphocytes, hereafter referred to as lymphocytes. Lymphocytes were either cultured alone or combined with autochthonous macrophages in Leighton tubes. For some experiments, 3×10^5 lymphocytes per well were cultured in Microtest II tissue culture plates (Cooke Engineering Co., Alexandria, Va.).

F-H solutions (2) were prepared by dissolving 23.89 g of Ficoll, molecular weight 400,000 (Pharmacia, Uppsala, Sweden), in 326 ml of distilled water and adding 50 ml of 75% Hypaque (Winthrop Laboratories, Rensselaer, N.Y.). F-H was sterilized by autoclaving. For gradients, approximately 15 ml of heparinized blood was mixed with 15 ml of McCoy medium and layered slowly onto 15 ml of the mixture of F-H in a 50-ml plastic centrifuge tube. The cells, layered on the gradient, were centrifuged for 40 min at $1,400 \times g$ and then removed from the interface of the media and F-H and centrifuged at $1,000 \times g$ for 10 min. Erythrocytes were lysed by mixing the pelleted cells from the interface with 20 ml of 0.83% NH_4Cl (wt/vol) and incubating for 10 min in a 37 C water bath. After three washes in McCoy medium, the cell suspension was adjusted to a concentration of 3×10^6 cells per ml in McCoy medium supplemented with 30% serum. Mononuclear cells from these gradients, hereafter referred to as F-H cells, consisted of monocytes and both T and B lymphocytes. One-tenth milliliter of the cell suspension was added to duplicate wells of Microtest II tissue culture plates. Such cultures will be referred to as microcultures. For stimulation of microcultures, one drop of HSV or control antigen (approximately 0.05 ml) was added to each well. Approximately 25 ml of heparinized peripheral blood was sufficient to yield cells for between 96 and 120 wells.

The preparation of human foreskin fibroblast (HFF) cells has been described previously (8).

Transformation assay. Transformation was measured by the incorporation of [^{3}H]thymidine into viral and control antigen-stimulated cultures. Transformation index represents the ratio of counts per minute incorporated into viral antigen-stimulated cultures as compared with control antigen-stimulated cultures. Cultures were assayed for [^{3}H]thymidine incorporation (New England Nuclear Corp., Boston, Mass.; specific activity 6.1 μCi/ml) after a 1-day pulse of 0.1 μCi per well or 0.2 μCi per Leighton tube culture 5 days after antigen addition, using a multiple automated sample harvester (MASH II) (Microbiological Associates, Bethesda, Md.) for microcultures. Cells were washed from microcultures with dual probes alternately delivering distilled water to the wells and aspirating the water washes onto a strip of glass fiber filter paper. The paper was immobilized on a manifold box containing 12 O-rings aligned in two rows to correspond to the series of wells in the Microtest II plates. After a 2-min continuous washing-aspiration cycle, the strip of filter paper was removed from the manifold box and dried for 1 h in a 56 C oven, and the filter paper disks were placed in vials for liquid scintillation counting by the method previously described for Leighton tube cultures (9). Transformation was assayed in Leighton tube cultures by washing the cells with distilled water onto a glass fiber filter, as previously described (9).

Interferon assay. Interferon assay in this study was done by using an adaptation of the Armstrong (1) semimicro-dye binding assay for HFF cells. Microtest II wells were seeded with 3×10^4 cells in a 0.1-ml volume, using minimal Eagle medium with 5% fetal calf serum. One day later, 0.05 ml of the test sample, harvested on day 5 except where indicated, was added directly to duplicate wells in the first row of the plate, resulting in an initial dilution of 1:3. Further dilutions were made with an automated microdiluter equipped with 0.05-ml diluting loops, sterilized for use by flaming. Seven horizontal rows of wells were used to make serial threefold dilutions ranging from 1:3 to 1:2,187. An interferon standard of known titer was included on each plate. The next day, plates were challenged by addition of 10^3 PFU of VSV in 0.05 ml of medium. When cytopathic effect involved 75% of the control HFF cultures, usually 1 day after VSV challenge, the cells were fixed by addition of a 1 part 37% formalin-9 parts 0.9% saline mixture. Staining after fixation was with a 0.5% (wt/vol) solution of crystal violet solubilized in 70% (vol/vol) ethanol and diluted 1:4 in distilled water. After copious washing in tap water, stained plates were dried and examined microscopically to determine the dilution of interferon sample that reduced VSV cytopathic effect by 50%. Type I interferon titers determined by this microassay method were comparable to those using plaque reduction assays (8). Supernatants from M-L cultures were assayed by plaque reduction (8).

Interferons were characterized according to stability on exposure to pH 2 for 24 h, exposure to 56 C for 1 h, and neutralization with anti-human leukocyte interferon sera as described by Valle et al. (19).

Study population. All donors were paid adult volunteers with either virologically documented HSV disease or serologically demonstrated immune status to HSV. Virological confirmation of HSV disease in donors was by isolation of virus within the first 48 h after recurrence of disease. Questionable isolates or isolates from "atypical" disease were identified by fluorescent-antibody staining with rabbit anti-HSV antisera. Documentation of serological status was by assay of CF antibody to HSV (13). Blood samples were drawn from donors within 2 to 6 weeks after HSV recurrence unless otherwise indicated.

RESULTS

Kinetics of interferon production and transformation in microcultures of F-H cells. Microcultures of F-H cells prepared from donors with a recent HSV recurrence were assayed at days 2, 5, and 7 after antigenic stimulation to determine the in vitro kinetics of interferon production and transformation. Interferon, first detected at 2 days, remained constant throughout a 7-day culture period (Fig. 1). In contrast, transformation reached a peak on the 5th day after stimulation. The kinetics of interferon production in cultures from donors either with or without detectable CF antibody were the same as in Fig. 1 (data not shown).

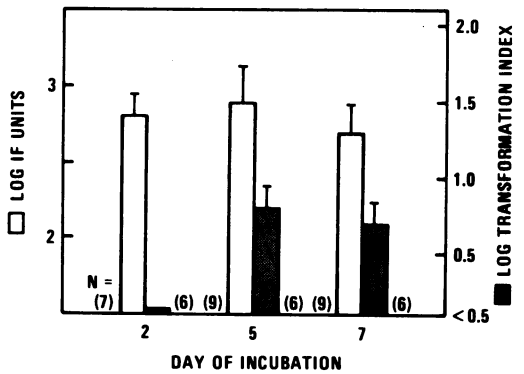

FIG. 1. *In vitro kinetics of blastogenesis and interferon production in Ficoll-Hypaque-purified mononuclear cell cultures. Mean ± standard errar.*

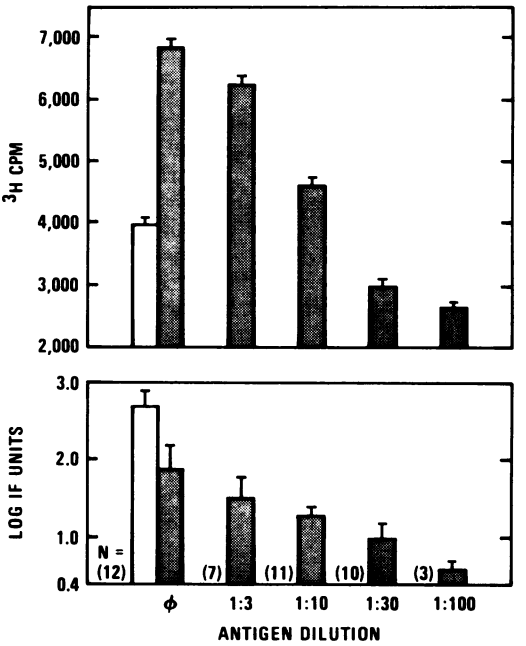

FIG. 2. *Comparison of crude (□) and dextran 10-purified (■) HSV antigen for blastogenesis and interferon production in Ficoll-Hypaque-purified mononuclear cell cultures. Undiluted purified antigen contained 6 × 10^6 PFU per ml, and crude antigen contained 2 × 10^6 PFU per ml before inactivation. Mean ± standard error.*

Comparison of crude and purified HSV antigen for cell culture stimulation. Figure 2 shows a comparison of crude and purified HSV antigens for their ability to induce reactivity in microcultures of F-H-purified cells prepared from donors with a recent HSV recurrence. In 12 experiments, the crude and purified HSV antigens were compared directly for their interferon-inducing and blastogenic capabilities.

The data show that undiluted crude antigen consisting of 2×10^6 PFU per ml before heating consistently stimulated nearly 90% more interferon than did undiluted purified antigen with approximately 6×10^6 PFU per ml before inactivation. However, purified antigen caused a near doubling of [^{3}H]thymidine incorporation as compared with crude antigen. The differences between crude and purified antigen were significant at the $P < 0.01$ level for transformation and $P < 0.05$ level for interferon production after analysis of the data by Student's t test. Crude HSV antigen, in several experiments, failed to reproducibly stimulate interferon or significant blastogenesis when used at concentrations less than one-third. Despite its poorer intrinsic ability to stimulate interferon, purified HSV antigen exhibited a consistently decreasing linear relationship with dilution for both interferon and transformation stimulation.

Determination of serum supplement required for maximum interferon production and lymphocyte transformation. Microcultures of F-H cells were prepared from donors between 2 to 6 weeks after recurrence of virologically confirmed HSV. The mononuclear cell cultures were tested for responses to undiluted HSV antigen after maintenance in either 30% human AB sera with a CF titer of less than 1:4 for HSV, human AB sera with a CF titer of 1:64 for HSV, or fetal calf serum. All three components were not compared in each experiment; however, in all experiments human AB sera with a CF titer less than 1:4 were included as a positive control for comparison with the sera to be tested. Both transformation and interferon production were highest when cultures were maintained with no detectable HSV antibody as compared with serum with antibody or fetal calf serum (Fig. 3). The differences between interferon production in cultures without antibody and the other sera tested were significant at the $P < 0.05$ level by Student's t test. Although transformation was lower in the serum with HSV antibody, by statistical analysis no significant differences were detected when compared with cultures without antibody. However, from a practical point of view, it is clear that overall responses in media with antibody-containing serum were not maximal. Transformation in cultures grown in fetal calf serum were significantly ($P < 0.05$) lower than in cultures with the other serum supplements.

Figure 4 shows the effect of increasing concentrations of antigen on interferon production in microcultures of F-H cells or lymphocytes both in the presence and absence of HSV antibody. Two representative experiments are

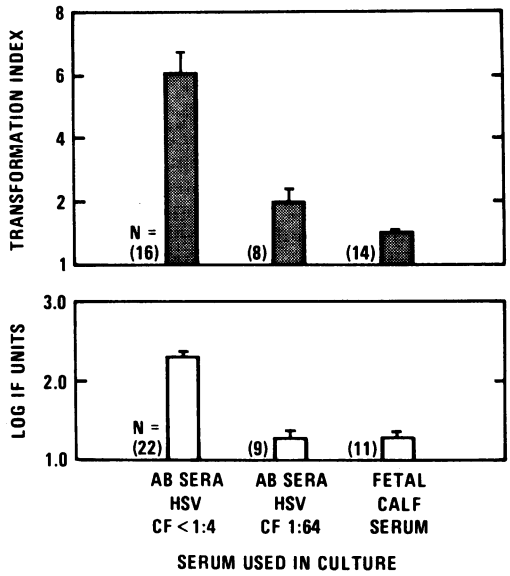

FIG. 3. *Effect of different sera on interferon production in Ficoll-Hypaque-purified cell cultures (upper graph) and nylon fiber-purified cells (lower graph). Medium was McCoy medium with either 30% AB serum, 30% AB serum with 1:64 CF titer of HSV antibody, or 30% fetal calf serum. Mean ± standard error.*

shown. In the absence of antibody (HSV CF titer < 1:4), maximum interferon production was obtained with 1×10^5 to 2×10^5 PFU equivalents of antigen. Maximum titers of interferon in the presence of HSV antibody (CF titer = 1:64) were not obtained until the stimulating antigen concentration was increased to 6×10^5 to 10×10^5 PFU equivalents.

Comparison of interferon production and transformation in F-H cells and T lymphocytes from donors with different HSV disease histories. Donors with either virologically documented HSV disease or serologically demonstrated presence or absence of HSV CF antibody were studied for in vitro reactivity to HSV antigen. Donors with a history of recurrent HSV disease were studied at several intervals after a single recurrence. Heparinized peripheral blood from the same phlebotomy was used to prepare both F-H cells and lymphocytes. From some donors, M-L cultures were also prepared (Table 1). Figure 5 (upper half) shows a comparison of interferon production and transformation in three categories of donors: those studied during a 12-week period after HSV recurrence, those studied more than 12 weeks after such disease, and those with no demonstrable CF antibody to HSV. Considerable variability in transformation indexes was observed both in the group studied within the first 12

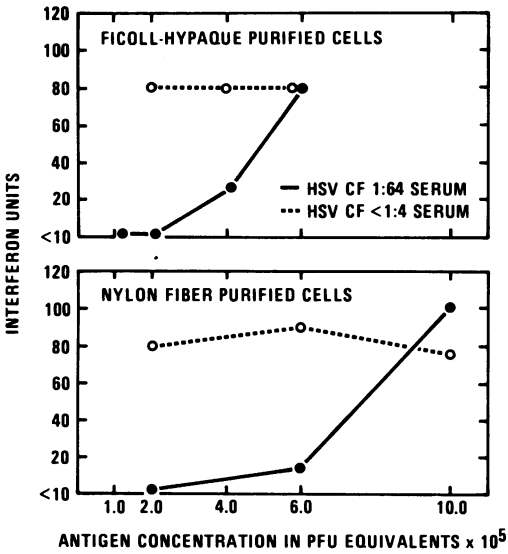

FIG. 4. *Effect of increased antigen concentration on interferon production in Ficoll-Hypaque-purified cell cultures (upper graph) and nylon fiber-purified cells (lower graph). Cultures were maintained in McCoy medium with either 30% AB serum with HSV CF titer <1:4 (----) or 30% AB serum with an HSV CF antibody titer of 1:64 (——).*

weeks after disease and the group studied at periods more distant (greater than 12 weeks) from HSV recurrences. However, by Mann-Whitney U test analysis, the groups were significantly different ($P = 0.01$). The transformation indexes for both groups with a history of disease were also significantly different ($P < 0.05$) from the seronegative group. This finding is in agreement with observations reported by several other groups (10, 12, 15-17). Additionally, interferon titers in the group studied 1 to 12 weeks after HSV were significantly higher ($P = 0.02$) than in the other groups. Two seronegative donors with transformation indexes of less than 3 but who produced interferon were restudied, using purified antigen as described in the previous section. Both the purified and crude antigens stimulated approximately 2,000 U of interferon.

Reactivity in microcultures of lymphocytes is shown in Fig. 5 (lower half). Both the interferon and transformation responses were somewhat lower than in F-H microcultures. Increasing the cell concentration of lymphocytes to 6×10^5 per well did not increase either the interferon or transformation response. In nine experiments the mean log interferon production from 3×10^5 lymphocytes was 1.7 ± 0.3 as compared with 2.1 ± 0.4 from 6×10^5 lymphocytes. Similarly, in ten experiments the mean transformation index with 3×10^5 lymphocytes was 3 ± 0.8 as compared with 2.6 ± 0.6 in 6×10^5 lymphocytes. Significant differences ($P < 0.05$ by Mann-Whitney U test analysis) in transformation indexes and interferon titers were calculated for both groups of donors with a disease history as compared with the seronegative group. In contrast to F-H microcultures, no interferon was detected in lymphocyte cultures from seronegative donors.

Quantitative comparison of interferon production in various cultures of mononuclear cells. Table 1 compares interferon production in four different types of mononuclear cell cultures. Donors were studied at two intervals, 2 to 5 weeks and 6 to 12 weeks, after HSV recurrence. The donor responses are ranked in Table 1 according to the magnitude of the interferon response in F-H cells. Among the 10 donors studied between 2 and 5 weeks after recurrence, no quantitative relationship among the interferon response in the different cell culture systems was obvious. F-H cultures prepared from donors 6 to 12 weeks after disease yielded high titers of interferon; however, none could be detected in either M-L or lymphocytes alone with one exception (600 U by donor FJ). Recurrent disease was observed only in the group of donors who had low (less than 10 U) interferon titers in the M-L cell culture system as previously observed (9).

Characteristics of the interferons produced in mononuclear cell cultures. Table 2 shows the characteristics of the interferons produced by the different mononuclear cell cultures. Three criteria were used: neutralization of antiviral activity by rabbit antisera to human leukocyte interferon; effect of treatment for 24 h at pH 2; and effect of heating at 56 C for 1 h. The interferons produced by both lymphocyte cultures and F-H cells were (i) neutralized by anti-interferon sera, (ii) stable to treatment at pH 2 for 24 h, and (iii) stable to heating at 56 C for 1 h. In contrast, interferon from M-L cultures was not neutralized by the anti-interferon sera and was inactivated by both treatment at pH 2 and heating for 1 h at 56 C.

DISCUSSION

This study has shown that the type of cell culture used for an in vitro assay of cellular immunity to HSV influences the nature of the reactivity observed. First, there were two distinguishable interferons produced according to the type of cell culture used to study reactivity. Both in cultures of T and B lymphocytes and monocytes (F-H microcultures) and in cultures of T (column-purified) lymphocytes, the interferon produced had characteristics of "classical" or type I interferon (20), that is, stable to heat-

TABLE 1. *Quantitative comparison and prognostic significance of interferon produced by different types of mononuclear cell cultures*

Time of assay after disease (wk)	Donor	Peak interferon titer in mononuclear cell culture of:				Disease within 12 weeks after assay
		F-H cells[b]	Lymphocytes[a]			
			Tube cultures[c]	Microcultures[d]	M + L[e]	
2–5	JW	5,300	<10	23	<10	–
	PP	1,740	23	80	38	–
	BM	980	<10	<3	<10	–
	CM	600	290	200	260	–
	JB	600	26	68	20	+
	MK	600	<10	1776	<10	+
	HH	120	79	160	162	–
	SN	68	ND	ND	15	–
	CK	68	<10	<3	<10	–
	ND	9	<10	ND[f]	<10	+
6–12	FJ	5,250	<10	600	<10	+
	BM	600	<10	0	<10	–
	HH	200	<10	0	<10	–
	SN	160	<10	0	<10	+

[a] Nylon fiber column-purified lymphocytes (approximately 95% T and 5% B).
[b] Ficoll-Hypaque cell cultures (monocytes, T lymphocytes, and B lymphocytes).
[c] 1×10^6 cells maintained in Leighton tubes.
[d] 3×10^5 cells maintained in Microtest II plate wells.
[e] Approximately 10^5 macrophages plus 10^6 T lymphocytes per Leighton tube culture.
[f] Not done.

ing at 56 C for 1 h, stable to pH 2 for 24 h, and neutralization of antiviral activity by antisera to leukocyte interferon. "Immune" or type II interferon was detected only in M-L cultures, as previously reported (18). The data suggest that the T lymphocytes, depending upon the HSV history of the donor, may have the ability to produce either type I or II interferon. This conclusion is based on the finding that type II interferon is produced only in the presence of fully differentiated macrophages and T lymphocytes, whereas T lymphocytes alone produce type I. The differentiated macrophage thus may be a regulatory mechanism for type II interferon production by T lymphocytes. This supposition is supported by the production of type I interferon in mixtures of monocytes and T and B lymphocytes (F-H cells). However, it is not possible to exclude a contribution of B cells to interferon production.

Second, the quantity of interferon produced was extremely variable in different cell culture systems. For example, when mononuclear cells were isolated in different ways from the same donor at the same time, there was no obvious quantitative pattern of interferon production; that is, high interferon production in one system was not necessarily reflected in the others studied. These quantitative differences may be related to the production of different types of interferon (Tables 1 and 2) according to the cell culture system used. In our study, the quantity of interferon produced was also influenced by antibody. The presence of HSV antibody reduced interferon more than transformation (Fig. 3). The effect of antibody, however, could be reversed by the addition of excess antigen. This suggests that antibody lowers interferon production by binding antigen. Whether the interferon response is diminished by the presence of specific antibody as in this report or is relatively unaffected, as reported by Fujibayashi et al. (5), is probably dependent upon the nature and relative proportions of antigen and antibody present in the neutralization mixture.

Third, the magnitude of the transformation response was affected both by the cell culture used for assay and the physical state of the inducing antigen. For example, transformation indexes in F-H cultures were generally greater than in T-lymphocyte microcultures. In our previous investigations, transformation indexes in M-L cultures were equal to or greater than those in F-H microcultures, but interferon titers were much lower, with a mean of 50 U. One possible explanation relates to the in vitro kinetics of interferon production. In F-H cultures and T-lymphocyte microcultures, interferon is detected at 2 days, but our earlier studies showed that M-L interferon was detected maximally at 7 days. The earlier production of interferon could have a "dampening" effect on

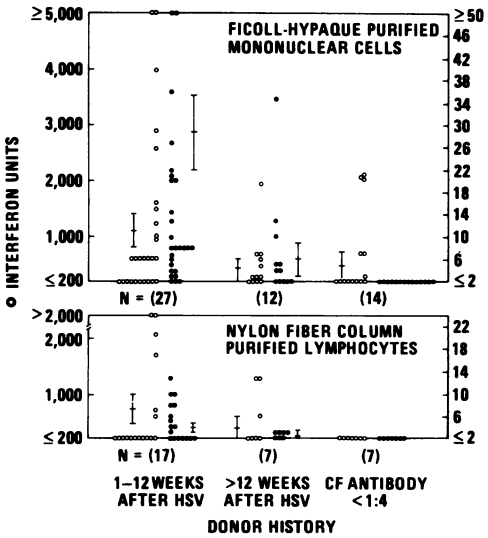

FIG. 5. *Comparison of in vitro interferon production (○) and transformation index (●) in human donors with various categories of HSV disease history. In vitro responses were studied in Ficoll-Hypaque-purified mononuclear cells (upper half) and nylon fiber column-purified lymphocytes (lower half). Mean ± standard error values are shown by solid vertical lines.*

TABLE 2. *Characteristics of interferon produced in different types of mononuclear cell cultures*

Cell culture	No. of Assays	Neutralization with leukocyte interferon antiserum	pH 2.0 stable for 24 h	Heat labile for 1 h at 56 C
M + L	3	−	−	+
Lymphocytes	3	+	+	−
F-H	8	+	+	−

transformation in F-H and lymphocyte microcultures. In this same connection, the greater blastogenesis we have observed with purified antigen may relate to its poor interferon induction. These speculations are based on the finding that exogenous interferon can inhibit blastogenesis stimulated by various mitogens (3, 7, 11). Additionally, association of viruses with membrane fragments in crude but not purified antigen preparations may facilitate blastogenesis.

Finally, there are differences in the relationship of HSV immune status to interferon production, according to the cell culture used for study. Interferon produced by mixtures of monocytes and T and B lymphocytes appears to be immunologically nonspecific. This finding is in agreement with the report of Jordan and Merigan (6) using varicella-zoster antigen. As in that study and also observed elsewhere (19), the interferon produced in F-H microcultures is type I. The interferon produced by T lymphocytes alone is immunologically specific, since it is detectable only in those with history of HSV disease. The basis for the difference between interferon production in seronegative donors according to the cell culture type is not understood. One possibility is that T lymphocytes produce less interferon in the absence of B cells and monocytes, a supposition supported by the slightly higher mean titers in mixtures of mononuclear cell types as compared with T lymphocytes alone (Fig. 5). The lack of immunological specificity for interferon production is probably not the result of using a crude antigen preparation, since gradient-purified HSV antigen also produced interferon in cultures prepared on F-H gradients from seronegative donors.

The populations of reactive cells in the mononuclear cell cultures, that is, relative proportions of T lymphocytes, B lymphocytes, monocytes, and macrophages, undoubtedly influence the nature of lymphokine production as well as the duration and magnitude of these responses. Studies to identify the necessary interactions among cell populations that control the type of interferon produced and the extent of blastogenesis are now in progress.

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LITERATURE CITED

1. **Armstrong, J. A.** 1971. Semi-micro, dye-binding assay for rabbit interferon. Appl. Microbiol. 21:723–725.
2. **Boyum, A.** 1968. Separation of leucocytes from blood and bone marrow. Scand. J. Clin. Lab. Invest. 21(Suppl. 97).
3. **Brodeur, B., and T. C. Merigan.** 1975. Mechanism of the suppressive effect of interferon on antibody synthesis *in vivo*. J. Immunol. 114:1323–1328.
4. **Epstein, L., M. J. Cline, and T. C. Merigan.** 1971. The interaction of human macrophages and lymphocytes in the phytohemagglutinin-stimulated production of interferon. J. Clin. Invest. 50:744–753.
5. **Fujibayashi, T., J. Hooks, and A. Notkins.** 1975. Production of interferon by immune lymphocytes exposed to herpes simplex virus-antibody complexes. J. Immunol. 115:1191–1193.
6. **Jordan, G. W., and T. C. Merigan.** 1974. Cell mediated immunity to varicella-zoster virus: *in vitro* lymphocyte responses. J. Infect. Dis. 130:495–501.
7. **Lindahl-Magnusson, P., P. Leary, and I. Gresser.** 1972. Interferon inhibits DNA synthesis induced in mouse lymphocyte suspensions by phytohaemagglutinin or by allogeneic cells. Nature (London) New Biol.

237:120-121.
8. **Merigan, T. C., D. F. Gregory, and J. K. Petralli.** 1966. Physical properties of human interferon prepared *in vitro* and *in vivo*. Virology 29:515-522.
9. **Rasmussen, L. E., G. W. Jordan, D. A. Stevens, and T. C. Merigan.** 1974. Lymphocyte interferon production and transformation after herpes simplex infections in humans. J. Immunol. 112:728-736.
10. **Rosenberg, G. L., R. Snyderman, and A. L. Notkins.** 1974. Production of chemotactic factor and lymphotoxin by human leukocytes stimulated with herpes simplex virus. Infect. Immun. 10:111-115.
11. **Rozee, K. R., S. H. S. Lee, and J. Ngan.** 1973. Effect of priming on interferon inhibition of Con A induced spleen cell blastogenesis. Nature (London) New Biol. 245:16-18.
12. **Russell, A. S.** 1973. Cell-mediated immunity to herpes simplex virus in man. Am. J. Clin. Pathol. 60:826-830.
13. **Schmidt, N. J., E. H. Lennette, and C. W. Shor.** 1960. A complement-fixing antigen for herpes simplex derived from chick-embryo tissue cultures. Am. J. Hyg. 72:59-72.
14. **Spear, P. G., and B. Roizman.** 1972. Proteins specified by herpes simplex virus. V. Purification and structural proteins of the herpesvirion. J. Virol. 9:143-159.
15. **Starr, S., S. A. Karatela, S. L. Shore, A. Duffey, and A. J. Nahmias.** 1975. Stimulation of human lymphocytes by herpes simplex viral antigens. Infect. Immun. 11:109-112.
16. **Steele, R. W., M. M. Vincent, S. A. Hensen, D. A. Fuccillo, I. A. Chapa, and L. Canales.** 1975. Cellular immune responses to herpes simplex virus type I in recurrent herpes labiales: *in vitro* blastogenesis and cytotoxicity to infected cell lines. J. Infect. Dis. 131:528-534.
17. **Thong, Y. H., M. M. Vincent, S. A. Hensen, D. A. Fuccillo, M. Rola-Rleszczynski, and J. A. Bellanti.** 1975. Depressed specific cell-mediated immunity to herpes simplex virus type I in patients with recurrent herpes labialis. Infect. Immun. 12:76-80.
18. **Valle, M. J., A. M. Bobrove, S. Strober, and T. C. Merigan.** 1975. Immune specific production of interferon by human T cells in combined macrophage-lymphocyte cultures in response to herpes simplex antigen. J. Immunol. 114:435-451.
19. **Valle, M. J., G. W. Jordan, S. Haahr, and T. C. Merigan.** 1975. Characteristics of immune interferon produced by human lymphocyte cultures compared to other human interferons. J. Immunol. 115:230-233.
20. **Youngner, J. and S. Salvin.** 1973. Production and properties of migration inhibitory factor and interferon in the circulation of mice with delayed hypersensitivity. J. Immunol. 111:1914-1922.