LEUKOCYTES AND INTERFERON IN THE HOST RESPONSE TO VIRAL INFECTIONS

I. Mouse Leukocytes and Leukocyte-Produced Interferon in Vaccinia Virus Infection In Vitro

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The contribution of leukocytes, macrophages, or other phagocytic cell elements of the reticuloendothelial system to host defense in viral infections has never been fully defined. Viruses have been found associated with, and isolated from, peripheral white blood cells during infection (1–9). Other workers have noted either the failure of leukocytes or macrophages to exert a clearly defined viricidal effect or have demonstrated virus replication in these cells (5, 9–21). On the basis of these data it has been postulated that leukocytes may actually contribute to the dissemination of viruses in the host (5, 9–13, 22).

Contrasting evidence, on the other hand, has been presented in a number of studies which have failed to demonstrate viral replication in phagocytic cells (16, 22–25) or have correlated host resistance with resistance to viral multiplication of macrophages or reticuloendothelial system both *in vivo* and *in vitro* (26–29).

Recent reports of interferon production in human leukocytes by Gresser (30) and in mouse macrophages by Glasgow and Habel (31) suggest a possible mechanism by which these cells may contribute to host defenses in the absence of a direct viricidal action.

The present studies were initiated to develop a model to study the role of leukocytes in the host response to viral infections by using an *in vitro* system which eliminates the host immune mechanisms. It was hoped that such a model would allow us to examine the two components of the leukocytic response, namely, interferon production and phagocytosis.

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Materials and Methods

Virus.—A calf lymph strain of vaccinia virus maintained by passage in monkey kidney cultures was originally obtained from Dr. Joel Warren. The virus pool was prepared in HeLa cell cultures and titered 8×10^6 plaque-forming units (PFU) per ml.

Vesicular stomatitis virus (VSV), Indiana strain, was obtained from the American Type Culture Collection, Rockville, Maryland. Virus pools were grown in primary mouse embryo fibroblast cultures (MEF) and titered 4×10^6 PFU per ml on assay in ME-29 cells (31).

Encephalomyocarditis virus (EMC) was a large plaque-forming mutant, EMC r, obtained from Dr. K. K. Takemoto, National Institutes of Health. Virus pools were prepared in L cells and titered approximately 8×10^6 PFU per 0.2 ml in primary MEF.

Cells.—Primary mouse embryo (MEF) cells used in these experiments were prepared by trypsinization of 13- to 16-day-old embryos from random bred mice obtained from the Western New York Animal Resources, Webster, New York (strain MLM-1 derived from a ICR strain).

Leukocytes were obtained from 30 to 45 gm ex-breeder CD-1 mice obtained from the Charles River Mouse Farms, Wilmington, Massachusetts. Animals were inoculated intraperitoneally with 2 ml of nutrient broth. White cell preparations containing 1 to 2×10^6 cells per ml were obtained 18 to 24 hours later by flushing the peritoneal cavity with 10 ml of Eagle's medium. Differential counts of the peritoneal exudate showed 8 to 30 per cent polymorphonuclear and 70 to 92 per cent mononuclear cells.

ME-29 is an established mouse embryo fibroblast tissue culture line which has been described previously (31).

Media.—All tissue cells were maintained in Eagle's MEM medium with 5 to 10 per cent calf serum (Hyland Laboratories, Los Angeles, California). The media used for plaque titrations was Eagle's MEM with Difco noble agar and 5 per cent calf serum.

Virus Titration.—Vaccinia virus was assayed by a plaque method in HeLa cells. Virus samples were serially diluted, adsorbed for 3 hours, and overlaid with 5 ml of agar medium. A second overlay containing a final concentration of 1:20,000 neutral red was made on the 4th day and plaques were counted on the 5th, 6th, and 7th days.

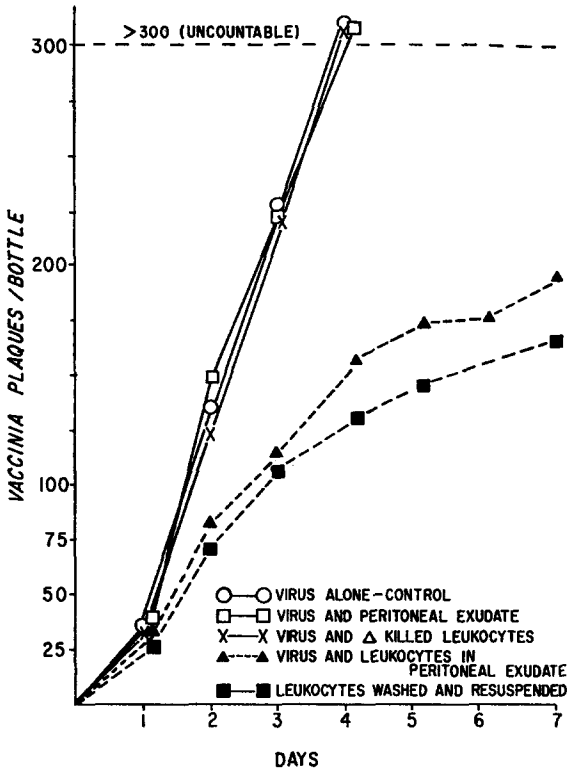
Interferon Assay.—Interferon preparations were cleared of cellular debris by centrifugation, acid-treated for 20 to 24 hours to destroy infectious particles as previously described (24), and assayed by the plaque reduction technique in ME-29 or primary MEF cultures utilizing VSV or EMC r as the challenge virus.

Interferon preparations were characterized by: (*a*) failure to inhibit plaque formation by Sindbis or vaccinia virus in chick embryo cultures; (*b*) trypsin sensitivity; (*c*) stability at 56°C for 30 minutes; and (*d*) failure to be sedimented at 100,000 *g* for 1 hour.

RESULTS

Primary mouse embryo fibroblast cultures (MEF) are susceptible to vaccinia virus infection following initiation of infection with a low inoculum, 50 to 200 PFU. Circumscribed plaque-like foci of cell destruction develop which are visible in the gross and may be counted. The MEF cultures were less susceptible than HeLa cells to vaccinia virus infection as evidenced by the reduced plaquing efficiency; 50 to 200 PFU (HeLa) initiated only 5 to 40 plaques in MEF. Progression of the infection is manifested by the increase in size of these foci of cytopathic effect (CPE), presumably by cell to cell transmission of virus, and by the development of new foci or plaques by the spread of virus through the culture medium. This vaccinia virus–MEF system provided an

excellent model for this study because: (*a*) the progression of the infection was slow, requiring 5 to 14 days for complete destruction of the culture; (*b*) in previously reported studies we demonstrated interferon production in mouse embryo tissue (31) and mouse leukocytes (24), and presented data suggesting that interferon production was a significant factor in the resistance of a continuous line of mouse embryo fibroblasts (3-B) to vaccinia virus infection;



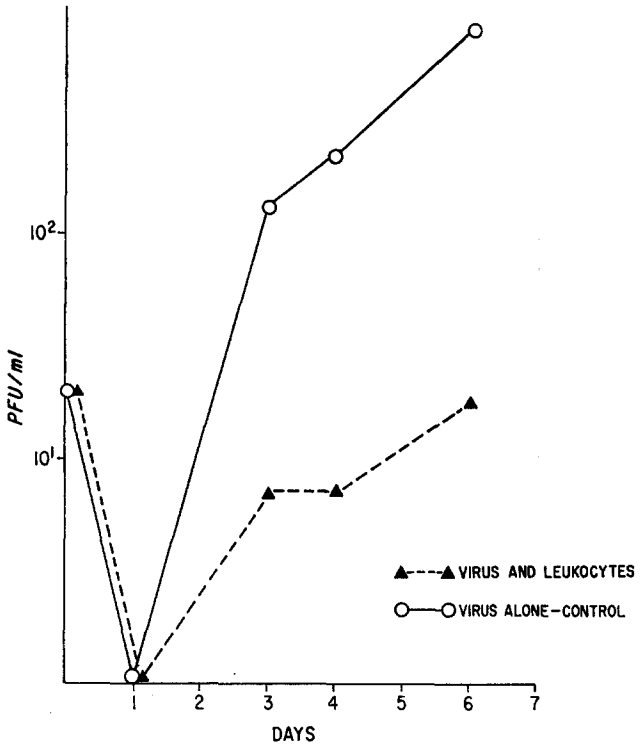
TEXT-FIG. 1. Protective effect of leukocytes on a vaccinia virus infection in primary MEF cultures, experiment 2-A. Progression of the infection is plotted as the number of plaque-like foci per bottle. Each point represents the average of two bottles.

and finally (*c*) the course of infection could be quantitated both by counting the gross plaques and by assaying the supernatant fluid for virus.

Protective Effect of Leukocytes.—Replicate cultures of primary MEF in 12-ounce prescription bottles were infected with 50 to 200 PFU of vaccinia virus in 0.5 ml of Eagle's MEM. After 1½ hours' adsorption, 1 to 2 ml of the mouse leukocyte preparation containing 1½ to 3 × 10^6 leukocytes was added to the culture and then the volume of medium was brought up to 15 ml by the addi-

tion of Eagle's MEM. The progression of the infection was quantitated by daily counts of the plaque-like foci of infection visible in the gross and by harvesting samples for virus assay.

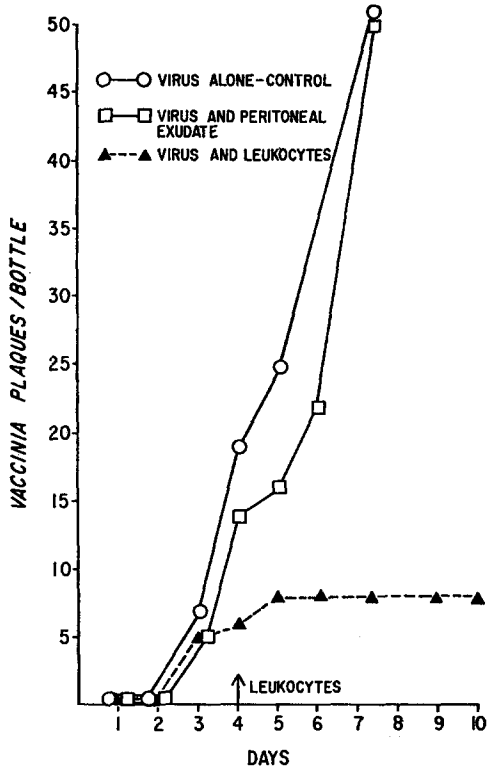
Data from 1 experiment using an inoculum of 200 PFU are summarized in Text-fig. 1. Each point represents the average daily plaque count from two



TEXT-FIG. 2. The effect of leukocytes in suppressing the progression of vaccinia virus infection in primary MEF cultures. Vaccinia virus titers in supernatant fluids of control cultures and cultures to which leukocytes have been added are expressed as PFU per ml.

cultures handled in an identical fashion. All cultures had approximately the same number of plaques by day 1, which indicated that most of the inoculum had adsorbed to susceptible cells prior to the addition of leukocytes and that the leukocytes affected only the progress of the infection after cell penetration. The results of this experiment demonstrate that addition of leukocytes produced a significant suppression of viral proliferation and also of spread of CPE as evidenced by inhibition of the development of new foci of infection. In addition slower enlargement of the existing plaques occurred in the cultures treated with leukocytes. This protective effect was observed in cultures where

a peritoneal-leukocyte mixture was used or where the leukocytes were washed prior to their addition to the cultures. The effect of leukocytes on the progress of virus infection was confirmed by virus assays on aliquots of the culture medium sampled periodically during the course of the experiment, Text-fig. 2.



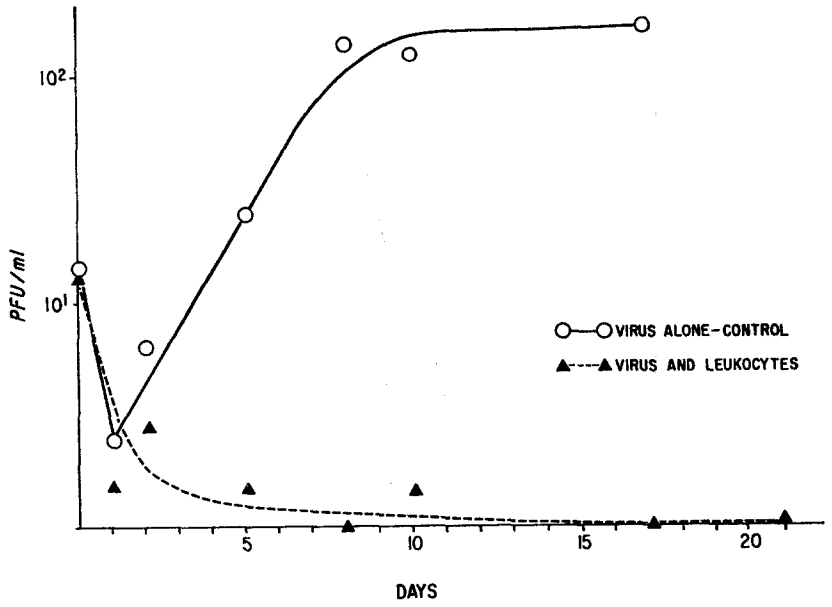
TEXT-FIG. 3. Control of vaccinia virus infection in primary MEF by addition of peritonea leukocytes. Progression of infection in control cultures (O——O) (□——□) is compared with replicate cultures to which leukocytes have been added. Progression of the infection is quantitated by gross plaque counts. Each point represents the average of three bottles.

Fluids harvested from the leukocyte-MEM group contained only approximately 2 per cent as much virus as was found in similar samples from control cultures.

Other replicate sets of cultures received an equal volume of peritoneal exudate with leukocytes removed by centrifugation to control for possible virus inhibitors present in the peritoneal fluid. In 4 of the 30 experiments which comprised these studies a moderate degree of inhibition was exhibited by peritoneal fluid controls. In no instance was this of the magnitude of the protective effect of the leukocytes.

Another set of replicate cultures received an equal volume of peritoneal exudate in which the leukocytes were killed by heating at 56°C for 10 minutes. The failure to observe protection suggested that the protective effect was a function of living cells.

During the course of the 30 experiments on which this report is based, a variation was noted in the protective effect of the leukocytes. The experiment presented in Text-fig. 1 represented one in which a low degree of protection



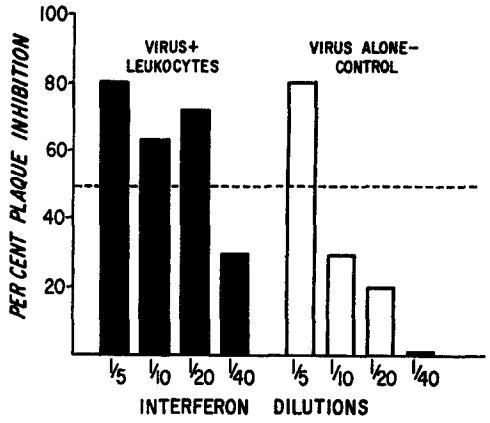
TEXT-FIG. 4. *In vitro* recovery from vaccinia virus infection in primary MEF cultures, following addition of peritoneal leukocytes. Vaccinia virus titers of supernatant fluids from control cultures and cultures to which leukocytes were added are expressed as PFU per ml.

was observed. In Text-fig. 3 the results of an experiment in which a striking protective effect was noted are summarized. The protocol differs in two ways from that illustrated in Text-fig. 1; the initial viral inoculum contained approximately 40 to 50 PFU as opposed to 200 PFU and a second addition of leukocytes was made on the 4th day. It was noted during these studies that the leukocytes provided a greater degree of protection when the inoculum was low and that with increasing multiplicity of infection, the leukocytes' defense could be overcome. In this experiment the vaccinia virus infection was controlled and after 5 to 7 days, regrowth of cells into the areas of CPE occurred. Over the subsequent 2-week period, the plaques gradually disappeared and were replaced by a normal healthy monolayer of cells. Samples

taken of the culture fluid during the course of this experiment were assayed for virus and the results are presented in Text-fig. 4. Vaccinia virus was cleared from the leukocyte-MEF culture; an *in vitro* recovery had occurred.

A series of photomicrographs (Figs. 1 to 3) illustrate the progression of CPE from a single focus of infection to wide-spread destruction in a control culture. These may be compared with the recovery process illustrated in a leukocyte-MEF culture (Figs. 4 to 6).

Production of Interferon.—The effect of leukocytes which was observed is explicable in terms of at least two possible mechanisms: (*a*) a mechanical effect



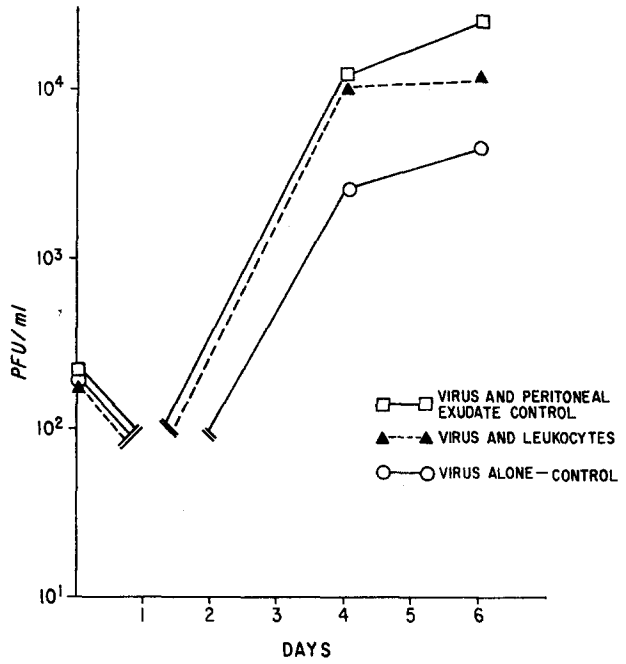
TEXT-FIG. 5. Interferon activity, expressed as per cent plaque reduction, in supernatant fluids of vaccinia virus infected primary MEF cultures. Higher levels of interferon are demonstrated in cultures to which leukocytes were added in comparison with replicate control cultures to which leukocytes were not added.

of phagocytosis with inability of phagocytosed particles to replicate within the leukocytes; or (*b*) interferon production by leukocytes which had phagocytosed or become infected with vaccinia virus. We have previously reported interferon production by vaccinia virus–infected mouse leukocytes (24) and it seemed probable that interferon production was a factor in the results described above.

Samples of medium from control and leukocyte-MEF cultures from 1 experiment were harvested, cleared of cell debris by centrifugation, and acid-treated as described in Materials and Methods. Serial dilutions of the resultant preparations were assayed for interferon activity in ME-29 or primary MEF cultures by the plaque reduction technique.

The interferon assays from this experiment are presented in Text-fig. 5. Interferon was demonstrated in both control and leukocyte-cultures, however, a significantly greater titer was present in the culture to which leukocytes were

added. Interferon production by vaccinia virus–mouse tissue systems is relatively low. The data presented in Text-fig. 5 are from an experiment in which a relatively high inoculum of vaccinia virus was used and in which a moderate degree of CPE was present in both groups of cultures. In fluids harvested from experiments in which a low virus inoculum was utilized, interferon could be demonstrated only with undiluted fluids or not at all, and significant differences in interferon production between the groups were not obtainable. These low interferon titers would be expected since probably 98 per cent or more of



TEXT-FIG. 6. Lack of protective effect of mouse peritoneal leukocytes in vaccinia virus–infected chick embryo tissue. Vaccinia virus titers in supernatant fluids from control MEF and leukocyte-MEF cultures, assayed in HeLa cells, are plotted as PFU per ml.

the total cell population in cultures infected with a low inoculum remain uninfected by vaccinia virus and hence are able to absorb the interferon produced by the small number of infected interferon-producing cells.

Species Specificity of Leukocyte Protection.—Further experiments were designed to delineate the relative contribution of phagocytosis and interferon production to the observed protective effect. The species specificity of mouse interferon has recently been definitively established with highly purified preparations (32); mouse interferon has no protective effect in chick tissue. Identical experiments were carried out, therefore, with vaccinia virus infections

in chick embryo tissue. The course of viral infection in control cultures was compared with that in cultures to which mouse leukocytes were added. The foci of infection were considerably less distinct in chick embryo tissue and gross plaque counts were not possible. CPE, however, appeared to progress at similar rates in both control and mouse leukocyte-chick embryo cultures with eventual destruction of all cultures. The results of virus assays on culture fluid samples from a representative experiment (Text-fig. 6) confirm the impression that viral infection was not affected by the addition of mouse leukocytes. Virus titers in all fluids rose in a logarithmic fashion until cultures were destroyed. There was no demonstrable protective effect of mouse leukocytes in chick embryo cell cultures.

These data have been interpreted to indicate that phagocytosis of extracellular viral particles plays a relatively minor role in the suppression of viral spread and proliferation and suggest that interferon is the significant factor in the observed suppressive effect of leukocytes on vaccinia virus infection *in vitro*.

TABLE I

Failure of Mouse Leukocytes to Inhibit Vaccinia Virus Plaque Formation in Chick Embryo Tissue

Experimental group	No. of plaques		
	Exp. 1	Exp. 2	Exp. 3
Virus alone	78	30	52
Virus + leukocytes at time 0*	69	34	53
Virus + leukocytes at 1 hr.*	70	36	61
Virus + leukocytes at 2 hrs.*	68	32	62
Virus + leukocytes at 3 hrs.*	66		

* Virus inoculum was added to all groups at time 0 and leukocytes at the time intervals indicated.

To further define the relative roles of phagocytosis and interferon production in the observed protective effect, a series of experiments was designed to utilize further the species specificity of interferon to differentiate these two mechanisms. In preliminary experiments it was demonstrated that addition of mouse leukocytes simultaneously with the vaccinia virus inoculum to mouse tissue cultures decreased the number of foci of infection which were initiated. It was postulated that phagocytosis of virus particles by leukocytes might be responsible for this inhibitory effect on primary plaque formation. If this hypothesis were true then primary, but *not* secondary, plaque formation should be inhibited in heterologous tissue. Data from a series of experiments in chick embryo cultures are summarized in Table I. Cultures of chick-embryo fibroblasts were

inoculated with 0.5 ml of vaccinia virus containing approximately 50 PFU. One set of 5 plates received 0.5 ml of MEM containing 6×10^5 to 10^6 mouse peritoneal leukocytes simultaneously with the virus inoculum while similar leukocyte preparations were added to other groups at 1, 2, and 3 hour intervals. The dilution effect of the addition of 0.5 ml of medium was controlled by adding 0.5 ml of MEM to two additional control plates at each time interval. This volume increase had no effect on plaquing efficiency and so these controls have not been included in the summary of results in Tables I and II. Liquid media containing the added leukocytes was removed after 12 to 18 hours and plates were overlaid with agar-plaque media, plates were stained

TABLE II

Inhibition of Vaccinia Plaque Formation in HeLa Cell Cultures By Addition of Mouse Leukocytes

Experimental group	No. of plaques	
	Exp. 1	Exp. 2
Virus alone	41	200
Virus + leukocytes at time 0*	27	32
Virus + leukocytes at 2 hrs.*	—	91
Virus + leukocytes at 3 hrs.*	45	125

* Virus inoculum was added to all groups at time 0 and leukocytes at the time intervals indicated.

with neutral red and plaques counted on the 3rd and 4th days. Since the majority of the vaccinia virus inoculum should be attached to cells by 3 hours, it was thought that phagocytosis of virus particles, if it occurred, would be manifested primarily in plates to which leukocytes were added at time 0 or at 1 hour. The failure to detect any reduction in primary plaque formation, therefore, was unexpected. The data presented represents 3 of 5 experiments in which the results were reproduced. The results suggest that under the conditions of these experiments, mouse leukocytes failed to compete successfully for virus particles with the monolayer of chick fibroblasts and that phagocytosis of vaccinia virus by leukocytes was not a significant factor in preventing the initiation of infection.

To extend these studies an identical protocol, with the exception of the substitution of HeLa cells for chick embryo fibroblasts, was utilized in the following series of experiments. Again, an unexpected result was obtained. In contrast to the lack of inhibitory effect in chick tissue, mouse leukocytes significantly reduced the formation of primary plaques in human cells. In a previous paper (31) we had noted that in contrast to the species specificity in mouse-chicken system, a low degree of antiviral activity by mouse interferon was demonstrable in HeLa cells.

In order to determine whether interferon produced by mouse leukocytes could be effective in human tissue under our present experimental conditions and could explain the plaque inhibition in HeLa cells, assays of mouse derived interferon were compared in cell cultures derived from the three species. Results are summarized in Table III. Interferon of mouse origin was again shown to exert a significant, though reduced, inhibitory effect in HeLa cultures. It is thought, therefore, that these data are consistent with the hypothesis that leukocyte-produced interferon was the primary factor in the *in vitro* control of vaccinia virus infection.

Interferon Elimination By Trypsin.—In order to define further the role of interferon in this *in vitro* model we attempted to alter a single variable by elimi-

TABLE III

The antiviral effect of interferon produced in cultures of mouse fibroblast is compared in mouse, HeLa and chick cells using a vaccinia virus plaque inhibition assay. Interferon activity in a heterologous species is demonstrated in HeLa but not chick cells.

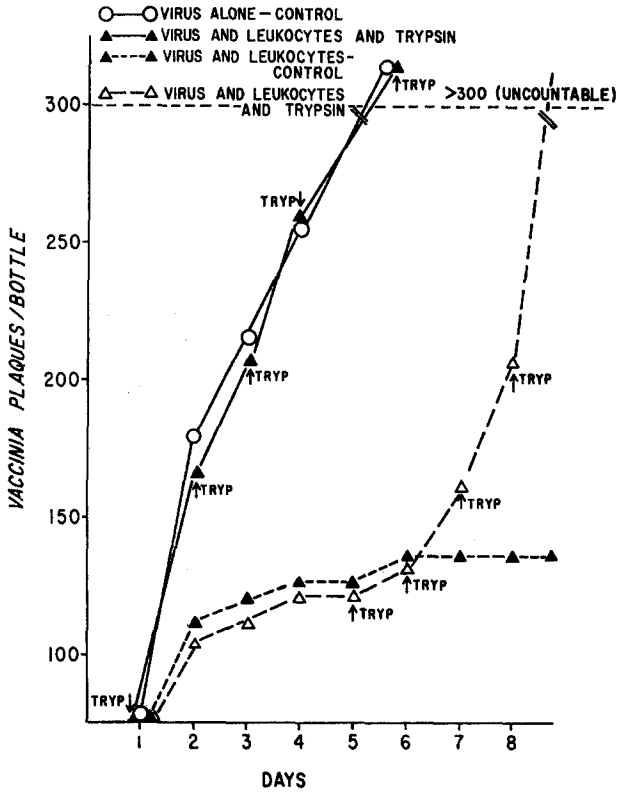
Interferon dilution	Plaque reduction		
	HeLa	Chick	Mouse
	per cent	*per cent*	*per cent*
1/2	55	0	100
1/10	38	0	100
1/20	26	0	100
1/100	—	0	96
1/200	0	0	85

nating interferon activity. In this series of experiments we utilized the sensitivity of interferon to the action of proteolytic enzymes. Again a similar protocol was followed except that in one leukocyte-MEF group trypsin was added daily (0.1 to 0.2 ml of a 1 to 200 dilution of trypsin) from day 0, (the time of the addition of vaccinia virus and leukocytes) while in replicate leukocyte cultures the progression of the infection was followed until control was established and then trypsin was added daily.

The results of one such experiment are represented in Text-fig. 7. The addition of trypsin from day 0 eliminated the protective effect of added leukocytes. Similarly when trypsin was added to cultures after the infection had been controlled, progression was again noted and new foci of infection appeared at a more rapid rate than in virus-infected leukocyte-MEF cultures which were not treated with trypsin. Not only did new plaques appear, but existing plaques were observed to be up to three times larger in the trypsin-treated cultures as compared to the untreated control cultures. Although in all experiments our data were consistent, it should be noted that it was difficult to maintain trypsin-

treated cultures satisfactorily to the completion of the experiment, as the cell sheets in these bottles peeled much more readily than in other cultures and were frequently lost or the plaques became difficult to count.

The data from this group of experiments are also compatible with the hy-



TEXT-FIG. 7. Elimination of the protective effect of leukocytes by the addition of trypsin (TRYP). No protective effect is demonstrable in the culture to which trypsin and leukocytes are added from day 1 (▲——▲). The addition of trypsin to a leukocyte-MEF culture after the protective action of leukocytes is already manifested (△---△) results in the elimination of that protective effect. The course of the infection is expressed as plaques per bottle. Each point represents the count from 1 bottle.

pothesis that interferon produced by leukocytes was responsible for the observed protective effect. The results, however, must be interpreted conservatively for the following reasons: (*a*) trypsin tends to release infected cells from the areas of cell destruction, and thus may enhance the initiation of new foci of infection; (*b*) trypsin may disperse clumps of virus particles hence increasing the possibility of initiation of a larger number of new plaques; or (*c*) trypsin could have a toxic effect on the leukocytes. All of these mechanisms may be

operative and the observed effect on leukocyte protection could be independent of any proteolytic activity on interferon.

DISCUSSION

The present study was initiated to investigate the possible contribution of leukocytes to host defenses in viral infections. In previous studies we demonstrated that vaccinia virus was phagocytosed by, or penetrated, leukocytes and induced interferon production in the absence of virus replication (24). This failure of vaccinia virus multiplication in mouse leukocytes or macrophage cultures has been reported by Nishmi and Bernkopf (23) and again more recently by Nishmi and Niecikowski (25). The presence within a host of a population of cells which takes up virus, does not permit viral replication, but releases interferon into the surrounding environment might be expected to enhance the resistance of that host. An *in vitro* model that would support such a hypothesis was reported by Gresser and Enders (33). They demonstrated protection of a population of susceptible cells against Sindbis virus infection by the introduction into the culture of cells of a virus resistant line which responded to virus infection with interferon production. It would appear reasonable, therefore, to postulate that leukocytes or reticuloendothelial system macrophages might function in a similar fashion either *in vitro* or *in vivo*. In a recent review of a series of studies on the role of the reticuloendothelial system in viral infections *in vivo*, Mims (29) presents evidence that in certain virus infections the virus-phagocytic cell interaction may be a critical determinant in the subsequent course of the disease. The reticuloendothelial system has been shown to participate in the clearing of virus particles from the circulation during the course of a viremia (29, 34). Interferon production by organs rich in reticuloendothelial cells has been demonstrated in a rabbit system recently reported by Kono and Ho (35) and confirmed in mice in experiments in our laboratory. Mouse spleen and liver tissue removed within 3 hours of an experimental viremia and grown in tissue culture were shown to produce significant titers of interferon. That interferon is also produced *in vivo* during these early phases of the host response to virus infection has been demonstrated by Baron and Buckler (36) who presented evidence of peak interferon levels occurring in mouse serum within 4 hours following an experimental viremia in mice. Although it remains to be shown that the reticuloendothelial system makes a significant contribution to the early interferon response and that this interferon in turn is a significant factor in host resistance, these preliminary data support the developing concept that interferon, and more specifically leukocyte or reticuloendothelial system–produced interferon, may play a role in the host defense against some viral infections.

A review of the literature concerning vaccinia virus and leukocytes or macrophages reveals a number of conflicting reports. The association or fixation of

virus by peripheral leukocytes and peritoneal macrophages has been demonstrated repeatedly, but with varied interpretation. Fairbrother (37) noted a protective effect of leukocytes when combined with immune serum and inoculated with vaccinia virus intracerebrally. The small number of animals utilized in these studies, however, makes evaluation of the work difficult. Douglas and Smith (7) in addition to the demonstration of virus fixation by leukocytes, presented data supporting an antiviral activity of cells from immune animals. In contrast, isolation of infectious particles from white blood cells when no free virus was demonstrable in whole blood and the failure of leukocyte cultures to inactivate vaccinia virus *in vitro* led Sabin (5) to conclude that these cells were not viricidal for vaccinia. Beard and Rous (19), in an extensive series of experiments, demonstrated that phagocytic cells of the reticuloendothelial system (Kupffer's cells) did exert a suppressive effect on the pathogenicity of vaccinia virus when a virus-cell mixture was inoculated intradermally. In similar experiments, however, peripheral leukocytes and peritoneal macrophages failed to alter the normal host response to vaccinia virus infection. In opposition to their *in vivo* studies, these authors also presented evidence of virus multiplication in the same liver macrophages maintained in tissue culture. In 1941 Florman and Enders (10) corroborated these *in vitro* studies utilizing peripheral mononuclear cells and interpreted their data to indicate that phagocytic cells may contribute to the dissemination of virus *in vivo*.

It is interesting to note that the studies indicating either failure of leukocytes to protect *in vivo* or of multiplication of virus *in vitro* have all been carried out in rabbit tissue or in rabbits; an animal which is relatively susceptible to vaccinia virus infections. In contrast, the mouse whose leukocytes or macrophages appear resistant to vaccinia virus replication in tissue culture is relatively resistant to infection by this virus *in vivo*. This proposed correlation is suggested by studies of Bang and Warwick (26), Goodman and Koprowski (27, 28), and by work reviewed by Mims (29). Mouse hepatitis virus was observed to multiply in liver explant macrophages from genetically susceptible mice but not in cultures from a resistant strain (26). A similar situation obtained in a group B arbovirus system in which the natural resistance *in vivo* of a mouse strain is reflected *in vitro* by the failure of its peritoneal macrophages grown in tissue culture to support multiplication of virus (27).

To extrapolate from an *in vitro* model to man is always hazardous, however the question of the possible role of human leukocytes in vaccinia virus infection is an important one. Kempe (38) has reported the case of a one-year-old child who developed progressive vaccinia which failed to respond to hyperimmune gamma globulin. Control of the infection was finally obtained when leukocytes from immune donors were injected along the leading edge of the lesion and a lymph node cell suspension, also from immune donors, was inoculated both intramuscularly and intravenously. In view of the data presented that peri-

toneal leukocytes and leukocyte-produced interferon may be a significant factor in limiting the spread of vaccinia virus infection *in vitro* it is tempting to speculate that a similar situation obtained *in vivo*; and that interferon production by the donor leukocytes may have contributed to the control of the vaccinia virus infection in this child. Furthermore, if we consider the proposed correlation of *in vitro* resistance of leukocytes with *in vivo* resistance of the host, man's degree of susceptibility to vaccinia virus is probably more closely analogous to that of the mouse in contrast to the rabbit which readily supports vaccinia virus replication in a number of organ systems *in vivo* as well as in leukocytes and macrophages *in vitro*.

Utilizing the *in vitro* model described, studies currently in progress in this laboratory comparing mouse, rabbit, and human leukocytes suggest that for human leukocytes a similar protective effect is demonstrable (39, 40). It is proposed that while leukocytes may not be the single determining factor in host resistance, in some virus infections interferon production by leukocytes may play a significant role in host defense mechanism and contribute to the recovery process.

SUMMARY

1. Investigation of the role of leukocytes in vaccinia virus infection is reported in an *in vitro* model, in the absence of an immune response.

2. Mouse leukocytes were shown to be capable of inhibiting the progression of vaccinia virus infection in primary mouse embryo fibroblast cultures. The degree of protection varied from slowing of spread of infection to complete control of the infection with eventual elimination of detectable virus and recovery of the culture.

3. Interferon production by leukocytes is thought to be an important factor in the observed protective effect.

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EXPLANATION OF PLATE 81

FIG. 1. Plaque-like focus of vaccinia virus infection in primary MEF cultures, day 1 to 2. × 50.

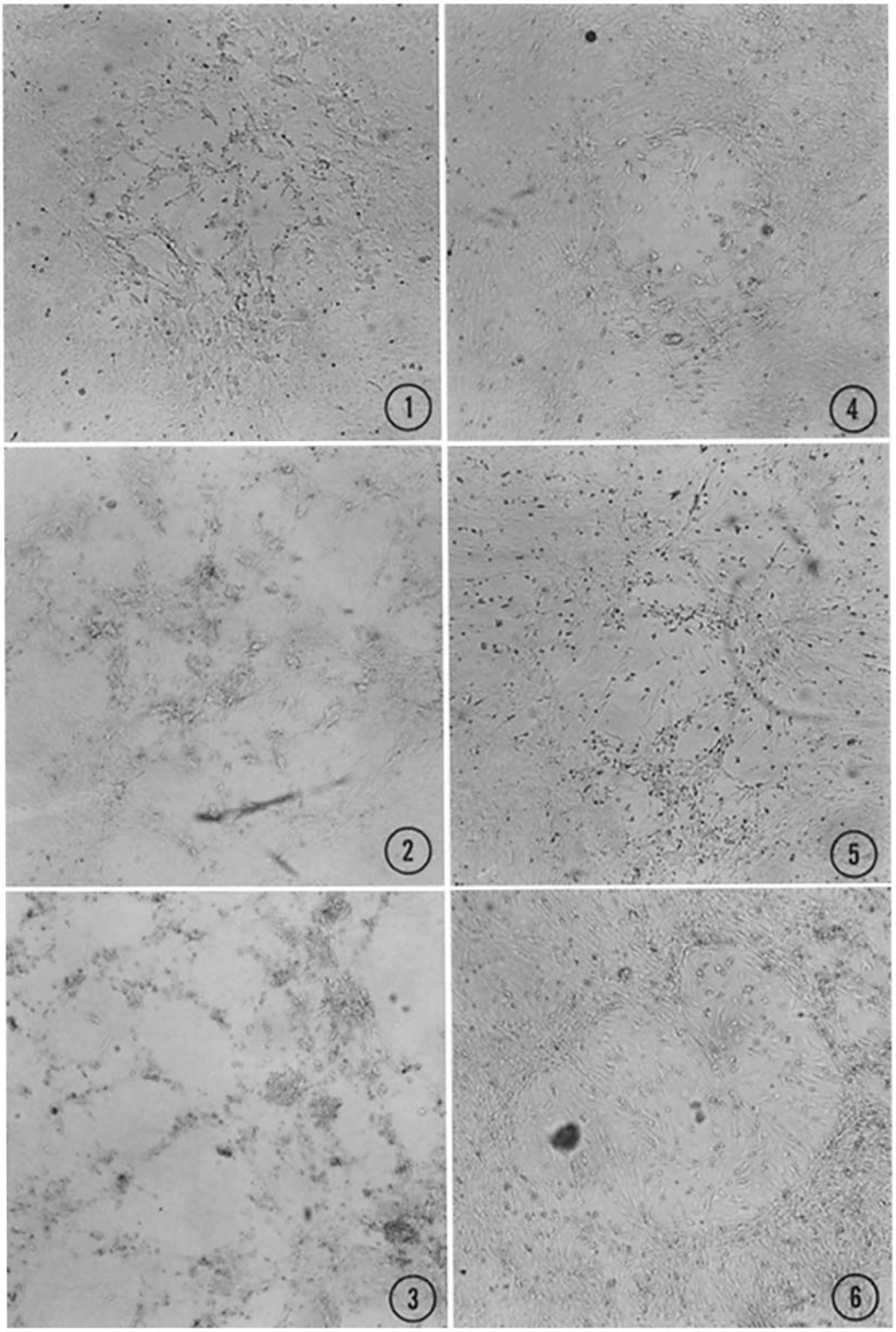
FIG. 2. Progression of the focus of infection in control culture, day 3 to 4. × 50.

FIG. 3. Ultimate wide-spread vaccinia virus CPE in unprotected controls, day 5 to 7. × 50.

FIG. 4. Vaccinia virus CPE in primary MEF culture to which peritoneal leukocytes have been added, day 1 to 2. × 50.

FIG. 5. Failure of progression of the viral lesion in leukocyte-MEF culture. New cell growth in the area of CPE may be noted. × 50.

FIG. 6. *In vitro* recovery in a leukocyte-MEF culture with disappearance of viral CPE and formation of new healthy cell monolayer. × 50.



(Glasgow: Leukocytes and interferon. I)