INFECTION AND IMMUNITY, Dec. 1970, p. 783-790

Vol. 2, No. 6
Printed in U.S.A.

In Vitro Assessment of Cellular Immunity to Vaccinia Virus: Contribution of Lymphocytes and Macrophages

W. A. F. TOMPKINS,[1] J. M. ZARLING, AND W. E. RAWLS[2]
Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas 77025

Received for publication 16 July 1970

The possible contributions of lymphocytes and macrophages to immunity to vaccinia virus were examined in vitro by determination of antigen-specific inhibition of migration of macrophages and the replication of the virus in purified macrophages. Lymphocytes from various anatomic sites of vaccinia virus-immune rabbits mixed with macrophages from nonimmune rabbits resulted in antigen-specific inhibition of macrophage migration; purified lymphocytes of peritoneal exudates were found to be the most potent inhibitors. Macrophages from peritoneal exudates of immune rabbits purified to contain less than 1% lymphocytes were sensitive to inhibition of migration by vaccinia antigens. Virus grew in macrophages from nonimmune rabbits, but failed to replicate in macrophages from the peritoneal cavity of immune rabbits. Alveolar maracrophages from immune rabbits were not inhibited from migrating in the presence of the viral antigen, and the virus replicated in these cells. The data suggest, but do not prove, that cellular immunity to vaccinia virus in rabbits may be mediated both by lymphocytes and by macrophages.

There is increasing evidence that cellular immunity may play an important role in the defense of the host against certain viral infections (2, 8, 22, 25). Lymphocytes and macrophages constitute the major cell types contributing to the cellular immune responses, and it is currently thought that lymphocytes possess the information of immunological specificity (6, 10). However, several studies have suggested that macrophages are involved in determining the outcome of some viral infections (20, 22). Furthermore, viruses appear to adsorb to and replicate poorly in mature lymphocytes (14, 17, 28).

The present study was, therefore, undertaken to investigate whether immunological specificity may be associated with macrophages. This was approached in two ways. One approach was to use an in vitro test, the macrophage migration inhibition test, which is known to correlate with the delayed hypersensitivity reaction (11). Secondly, the replication of vaccinia virus was compared in macrophages obtained from immune and nonimmune rabbits.

[1] Present address: Center for Zoonoses and Comparative Medicine, University of Illinois School of Veterinary Medicine, Urbana, Ill. 61801.

[2] Public Health Service Research Career Development Awardee (5-K3-AI-25,943 from the National Institute of Allergy Infectious Diseases).

MATERIALS AND METHODS

Tissue culture cells and media. Primary rabbit kidney cells were prepared by trypsinization of kidneys from 3-week-old rabbits. Eagle's medium supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), sodium bicarbonate (0.075 g/liter), and 10% fetal bovine serum was used to grow the rabbit kidney cells. The same medium containing an additional 5% normal rabbit serum was used in the migration chambers.

Preparation of viruses and viral antigens. The study was performed with the WR strain of vaccinia virus. Virus stocks were prepared in primary rabbit kidney cells grown in 16-oz (473-ml) prescription bottles. The cell monolayers were inoculated with 5×10^7 plaque-forming units (PFU) of the virus. The virus was allowed to adsorb for 2 hr at 37 C, after which 10 ml of medium was added to the cultures. After incubation of the cultures for 48 hr at 37 C, the virus was harvested by three cycles of freeze-thawing and 1 min of sonic treatment at 10 kc. The virus was assayed by the plaque-counting method in monolayers of primary rabbit kidney cells with the use of an overlay consisting of Eagle's medium with 1% agar. Titers were expressed as PFU per milliliter. Stocks of the Patuxent strain of fibroma virus were prepared and assayed by a method previously described (26). Viral antigens were prepared by infecting the cells as described above; however, the antigens were harvested by removing the medium, washing the cell monolayers three times with Hanks balanced salt solution (HBSS), and scraping the cells into 5 ml of HBSS with the aid of a rubber

policeman. The cells were then sonically disrupted. Viruses and viral antigens were stored at −20 C. The vaccinia virus antigen preparations contained 10^8 PFU/ml; before being used in the macrophage migration inhibition test, the virus was inactivated by ultraviolet (UV) irradiation. Control antigens consisted of fibroma virus or uninfected rabbit kidney cells prepared in the same manner.

Immunization of rabbits. Adult New Zealand white rabbits were immunized by inoculating 0.1 ml (5×10^6 PFU) of vaccinia virus intradermally at four separate sites on their shaved backs. In certain instances, rabbits were given a second similar injection 3 to 4 weeks after the initial inoculation. A delayed cutaneous response to an intradermal inoculation of UV-irradiated vaccinia virus was taken as evidence that the rabbits had been sensitized.

Isolation and purification of macrophages and lymphocytes. Cells were obtained from sensitized rabbits 3 to 6 weeks after the primary inoculation of vaccinia virus. Peritoneal exudate cells were induced with paraffin oil as previously described (25), and contained about 80% macrophages, 8 to 10% lymphocytes, and 10 to 12% polymorphonuclear cells as determined by differential counts of at least 200 cells. Purified macrophages were obtained by placing 5×10^7 peritoneal exudate cells (in 10 ml of medium) into a 32-oz (946 ml) prescription bottle and allowing the cells to adhere to the glass surface during a 30-min period at 37 C in a 5% CO_2 in air atmosphere. The unattached cells were decanted, and the cells adhering to the glass were washed three times with HBSS. The adherent cells were then scraped from the glass into medium with a rubber policeman, and the cells were allowed to adhere to the surface of a second 32-oz bottle. They were again washed and finally resuspended in medium. This purification procedure resulted in a population of cells containing greater than 99% macrophages.

Alveolar cells were obtained as described by Myrvick et al. (21). Rabbits were exsanguinated, the trachea was clamped, and the lungs were removed. The exterior of the lungs was washed repeatedly with HBSS containing 0.5 units of heparin per ml. HBSS (30 ml) was injected into the bronchus of each lung, which was then subjected to gentle squeezing. The fluid was removed from the lungs. By repeating the process three times, a total of 10^7 to 2×10^7 cells were collected from each rabbit. Differential counts of at least 200 cells revealed that these preparations consisted of 90 to 95% well-differentiated macrophages.

To obtain lymphocytes from blood, the blood was collected by cardiac puncture in heparin (5 units/ml). The erythrocytes were allowed to settle for 1 hr at 37 C, after which the leukocyte-rich plasma was removed and diluted to 50% fetal bovine serum. Lymphocytes were purified from the leukocyte-rich plasma or from peritoneal exudate cells by passing the cell mixtures through columns of unsiliconized glass beads (soda lime glass microbeads class IV, Microbead Div., Cataphote Corp., Jackson, Miss.). Glass columns 2.5 cm in diameter were packed with 38 cm of glass beads. The columns were incubated for 30 min at 37 C with 150 ml of Eagle's medium containing 10% fetal bovine serum. The medium was drained, and the leukocyte suspensions in medium with 50% fetal bovine serum were added to the columns. After incubation at 37 C for 30 min, the cells were eluted at a rate of 5 ml/min. Removal of the initial eluate was followed by the addition of 40 ml of medium to the column. This fluid was eluted from the column at the same rate and added to the first eluate. The cells were then pelleted at 1,000 rev/min for 10 min, washed twice with medium, and resuspended to the desired concentration. The procedure yielded preparations containing 80 to 90% lymphocytes from the peripheral blood, and the preparations from peritoneal exudates contained at least 90% lymphocytes. The viability of the lymphocytes ranged from 93 to 97% as determined by trypan blue exclusion.

Lymph node cells were obtained from inguinal and axillary lymph nodes. The lymph nodes were excised, placed in HBSS, debrided of fat, and gently teased apart to release lymphocytes. Crude spleen cells were collected by making several superficial incisions in the spleen capsule and applying gentle pressure over the spleen to force cells into the surrounding HBSS.

Macrophage migration inhibition test. The techniques of the migration test have been previously described (25). The cells to be used for inhibition of migration were pelleted in capillary tubes (20 x Micro-pipettes, Drummond Scientific Co.), and the capillary tubes were placed in MacKaness-type migration chambers. The chambers were filled with Eagle's medium containing 10% fetal bovine serum, 5% normal rabbit serum, 1.5% $NaHCO_3$/liter, and antigen. The area of migration was measured after 24 hr, as previously described (25). Inhibition of the area of migration of the macrophages by 15% or greater was considered significant.

Replication of vaccinia virus in macrophages. Peritoneal exudate cells and alveolar macrophages were obtained from immune and nonimmune rabbits. The peritoneal exudate macrophages were purified by adherence to glass. The suspensions of macrophages were inoculated with vaccinia virus at a multiplicity of 0.05 PFU/cell, and the virus was allowed to adsorb to the cells for 90 min at 4 C. Excess virus was removed by two washes with medium, and the cells were resuspended in medium to a concentration of 10^6 cells/ml. Samples (1 ml) of the cell suspension were placed in culture tubes which were incubated at 37 C. Duplicate tubes were harvested at the beginning of the experiment and at daily intervals for 3 days. The cells were sonically disrupted, the contents of the duplicate tubes were pooled, and the quantity of vaccinia virus present was assayed on monolayers of primary rabbit kidney cells. The effect of peritoneal exudate lymphocytes from immune rabbits on the replication of vaccinia virus in macrophages from nonimmune animals was examined by adding 5% purified lymphocytes to the macrophage preparations prior to inoculation of the virus.

RESULTS

Antigen-induced inhibition of migration of peritoneal macrophages from rabbits immune to vaccinia virus. Peritoneal exudate cells from im-

TABLE 1. *Inhibition of migration of peritoneal exudate cells from vaccinia-virus immune and nonimmune rabbits by various amounts of vaccinia virus antigen*

Source of peritoneal exudate cells	No. of experiments	Antigen	Per cent inhibition of migration[a]				
			1:10[b]	1:20	1:40	1:80	1:160
Vaccinia-sensitized rabbits	5	Vaccinia	83.1 ± 3.9	71.3 ± 11.7	56.6 ± 16.1	38.1 ± 13.5	21.2 ± 7.0
	1	Fibroma	—	6.5	2.5	—	—
	4	PRK[c]	—	10.2 ± 5.1	6.0 ± 3.3	2.8 ± 2.3	—
Nonsensitized rabbits	5	Vaccinia	19.9 ± 8.4	10.5 ± 5.8	8.4 ± 4.1	0.5 ± 4.2	1.4 ± 2.8
	1	Fibroma	—	0.5	6.0	—	—
	4	PRK	—	12.5 ± 4.2	4.6 ± 3.1	—	—

[a] Per cent inhibition of migration = [1 − (area of migration with antigen/area of migration without antigen)] × 100.
[b] Dilution of antigen; undiluted vaccinia virus antigen equivalent to 10^8 PFU/ml before irradiation.
[c] Primary rabbit kidney control antigen.

TABLE 2. *Ability of peritoneal exudate cells and lymphocytes from different sources to inhibit migration of macrophages when mixed with peritoneal exudate cells from nonimmune rabbits*

Source of immune cells which were added to nonimmune macrophages	No. of experiments	Per cent inhibition of migration by vaccinia[a]					
		100[b]	20	10	5	1	0
Peritoneal exudate cells	5	63.2 ± 9.1	59.8 ± 11.7	54.0 ± 8.3	35.8 ± 7.4	12.6 ± 9.7	5.0 ± 4.6
Purified peritoneal lymphocytes	3	NT	60.3 ± 5.5	NT	43.7 ± 10.1	32.4[c]	—
Lymph node cells	4	NT	27.5 ± 7.3	NT	17.3 ± 16.5	6.2 ± 6.1	—
Peripheral blood lymphocytes	4	NT	42.4 ± 12.5	NT	27.1 ± 7.4	8.8 ± 6.3	—
Spleen cells	2	NT	30.0[c]	NT	NT	NT	—

[a] Vaccinia virus antigen diluted to a concentration equivalent to 2.5×10^6 PFU/ml before irradiation. Per cent inhibition calculated as shown in Table 1. NT = not tested.
[b] Percentage of cells from sensitized rabbits. Mixed migration test performed by mixing a known number of cells from sensitized animals with a known number of peritoneal exudate cells from nonimmune rabbits.
[c] Mean of two experiments only.

mune animals were inhibited from migrating in the presence of vaccinia virus antigens. Peritoneal exudate cells from nonimmune rabbits were inhibited by 19.9% at the highest concentration of virus antigen used, a concentration which inhibited the migration of immune peritoneal exudate cells by 83.1% (Table 1). There was a positive correlation between the degree of inhibition of migration of immune peritoneal exudate cells and the concentration of antigen used. A control antigen consisting of sonically treated noninfected rabbit kidney cells failed to inhibit the migration of macrophages obtained from both immune and nonimmune animals. Fibroma virus antigen, at a concentration known to inhibit strongly the migration of peritoneal exudate cells from rabbits sensitized to fibroma virus (25) also failed to inhibit the migration of cells from rabbits immune to vaccinia virus.

Ability of lymphocytes from different sources of immune rabbits to confirm antigen-induced inhibition of migration of macrophages. The effect of lymphocytes from immune rabbits on the migration of macrophages from nonimmune rabbits was examined by mixing lymphocytes from different anatomical sites obtained from immune animals with peritoneal exudate cells from nonimmune animals in the presence of a standard concentration of vaccinia virus antigen. The results presented in Table 2 indicate that macrophages from nonimmune rabbits can be inhibited from migrating by the addition of peritoneal exudate cells or lymphocytes from various sources of the immune rabbits. The degree of inhibition produced by the addition of equal numbers of cells from different anatomical sites varied. The least effective cells in conferring sensitivity were those obtained from the lymph nodes, followed by

TABLE 3. *Inhibition of migration of partially purified peritoneal exudate macrophages byvaccinia virus*

Migrating cells	Immune status of rabbits donating cells	Per cent inhibition of migration by vaccinia virus[a]
Crude exudate cells	Nonimmune	11.5 ± 5.5
Crude exudate cells	Immune	71.5 ± 7.3
Purified macrophages[b]	Nonimmune	9.5 ± 8.1
Purified macrophages[b]	Immune	62.6 ± 13.0
Crude exudate cells (80%) + Purified macrophages (20%)	Nonimmune	25.4 ± 5.1
	Immune	

[a] Vaccinia virus antigen diluted to a concentration equivalent to 2.5×10^6 PFU/ml before irradiation. Per cent inhibition calculated as shown in Table 1. Each result is based on three experiments.

[b] Peritoneal macrophages purified by two cycles of adherence to glass. These preparations contained less than 1% lymphocytes.

spleen cells, blood lymphocytes, and crude peritoneal exudate cells. Purified lymphocytes from peritoneal exudates were the most efficient in causing the inhibition of migration of peritoneal exudate cells from nonimmune rabbits (Table 2). Furthermore, when only 1% immune lymphocytes was added, inhibition of migration of normal peritoneal exudate cells was observed only when the lymphocytes were derived from the peritoneal cavity.

Immune specificity of macrophages in the migration inhibition test. The above experiments revealed that 1% purified lymphocytes from peritoneal exudates from immune rabbits produced approximately half (32%) of the maximal inhibition of migration (60.3%) conferred when greater numbers of lymphocytes were mixed with peritoneal exudates from nonimmune rabbits (Table 2). Peritoneal exudate macrophages were, therefore, purified to contain less than 1% lymphocytes and examined for their ability to migrate in the presence of vaccinia antigen. As shown in Table 3, removal of lymphocytes from the peritoneal exudates did not significantly alter the inhibition of migration of the macrophages. In the presence of vaccinia antigen, the migration of crude peritoneal exudate cells and purified macrophages was inhibited by 72 and 63%, respectively. In addition, some inhibition of migration was observed when 20% purified peritoneal exudate macrophages from immune rabbits were added to peritoneal exudate cells of nonimmune rabbits, although the degree of inhibition was much less than that caused by the addition of purified peritoneal exudate lymphocytes.

The observed inhibition of migration of purified macrophages by vaccinia virus antigens could have been accounted for by the presence of globulins similar to the globulins described by Heise et al. (18). These authors have been able to elute a globulin from the surface of sensitized macrophages which could inhibit the migration of normal macrophages. However, heat eluates from peritoneal exudate macrophages prepared according to the method of Heise et al. (18) and lysates of the cells prepared by sonic treatment of the macrophages at 10 kc for 2 min failed to produce significant inhibition of migration of macrophages from nonimmune rabbits when these cells were preincubated with the eluate or lysate. In addition, incubation of macrophages from nonimmune rabbits with hyperimmune rabbit serum for 30 min at 37 C did not render the cells sensitive to inhibition of migration upon subsequent exposure to the viral antigen.

Failure to inhibit the migration of alveolar macrophages. The results of the previous experiments suggested that, like lymphocytes, peritoneal macrophages from vaccinia-sensitized rabbits contributed specifically to the inhibition of migration reactions. Another source of macrophages was therefore tested. Alveolar macrophage preparations were examined for their migration characteristics in the presence of vaccinia virus antigens. Unlike the peritoneal exudate cells, the alveolar macrophages from sensitized animals were not inhibited from migrating in the presence of the viral antigen (Fig. 1). However, the addition of 20% lymph node cells or peritoneal exudate cells from immune animals resulted in significant inhibition of migration of alveolar macrophages in the presence of the antigen. These data suggest that the alveolar macrophages possessed a degree of sensitivity to lymphocyte-mediated inhibition similar to that of the peritoneal macrophages; however, they were not specifically inhibited by the viral antigen in the absence of added immune lymphocytes.

Replication of vaccinia virus in macrophages. Evidence for specific immunity of macrophages to the virus was sought by examining the replication of vaccinia virus in macrophages from immune and nonimmune rabbits. Whereas a 200-fold increase in infectious virus was observed in purified peritoneal macrophages from nonimmune animals after 3 days of incubation, there was no increase of vaccinia virus in the purified peritoneal macrophages from immune rabbits (Fig. 2). The replication of vaccinia virus in alveolar macro-

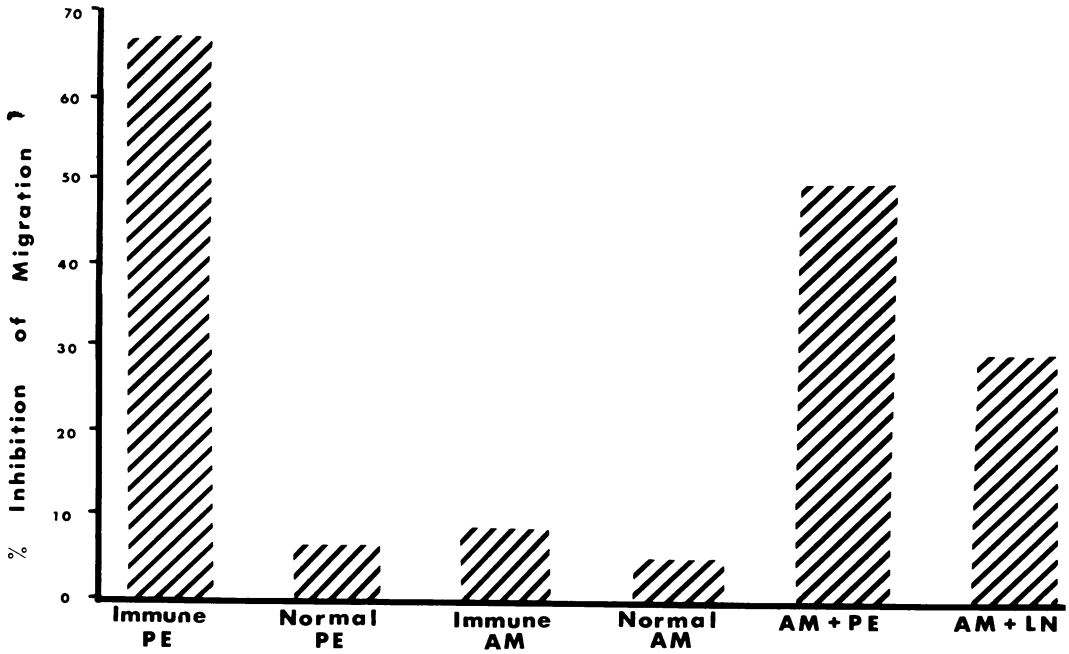

FIG. 1. *Inhibition of migration of peritoneal exudate cells (PE) and alveolar macrophages (AM) from immune and nonimmune rabbits by vaccinia virus antigen. The migration of alveolar macrophages from immune rabbits was not inhibited by the antigen. The addition of 20% peritoneal exudate cells (AM + PE) or lymph node cells (AM + LN) from immune animals resulted in reduced migration of the alveolar macrophages.*

phages was distinctly different from virus replication in peritoneal macrophages. The virus titers increased more rapidly and the yield of virus was greater. The virus replicated equally well in alveolar macrophages from immune and nonimmune rabbits (Fig. 2).

The effect of the addition of immune lymphocytes on virus replication in peritoneal macrophages was examined by adding purified lymphocytes from immune rabbits to macrophages from nonimmune rabbits. The addition of 5% purified peritoneal lymphocytes from immune rabbits caused inhibition of migration, but lymphocytes from the same rabbits did not impair the replication of vaccinia virus in the macrophages from nonimmune rabbits. The suppression of replication of the virus in the cultures of macrophages from immune rabbits did not, therefore, appear to be due to the presence of residual lymphocytes.

No evidence for residual antibody associated with the cell preparations could be obtained. Heat eluates designed to remove cytophilic antibody (18) and lysates of the macrophages from immune animals were prepared. No virus-neutralizing activity could be demonstrated at a 1:2 dilution of either the heat eluates or cell lysates, although the serum from the rabbits from which these cells were obtained neutralized vaccinia virus at a dilution of 1:128 in the same plaque-reduction test.

To determine whether the inhibition of virus replication in the immune peritoneal macrophages could be due to enhanced production of interferon, macrophages from immune and nonimmune rabbits were inoculated with vaccinia virus. The cultures were harvested at daily intervals and assayed for interferon-like activity by previously described methods (12). Inoculation of cells with infectious virus at a multiplicity of 0.05 PFU per cell resulted in interferon-like activity at a dilution of 1:5 only. Inoculation of the cells with UV-irradiated virus resulted in higher levels of interferon-like activity; however, the titer of the activity was never greater in cultures containing macrophages from immune rabbits than in cultures containing macrophages from nonimmune rabbits.

DISCUSSION

There is evidence to suggest that delayed-type hypersensitivity reactions are of importance in immunity to poxvirus (1–3, 8, 22, 25). The major difficulty in assessing the relative contributions of humoral and cell-mediated immunological mecha-

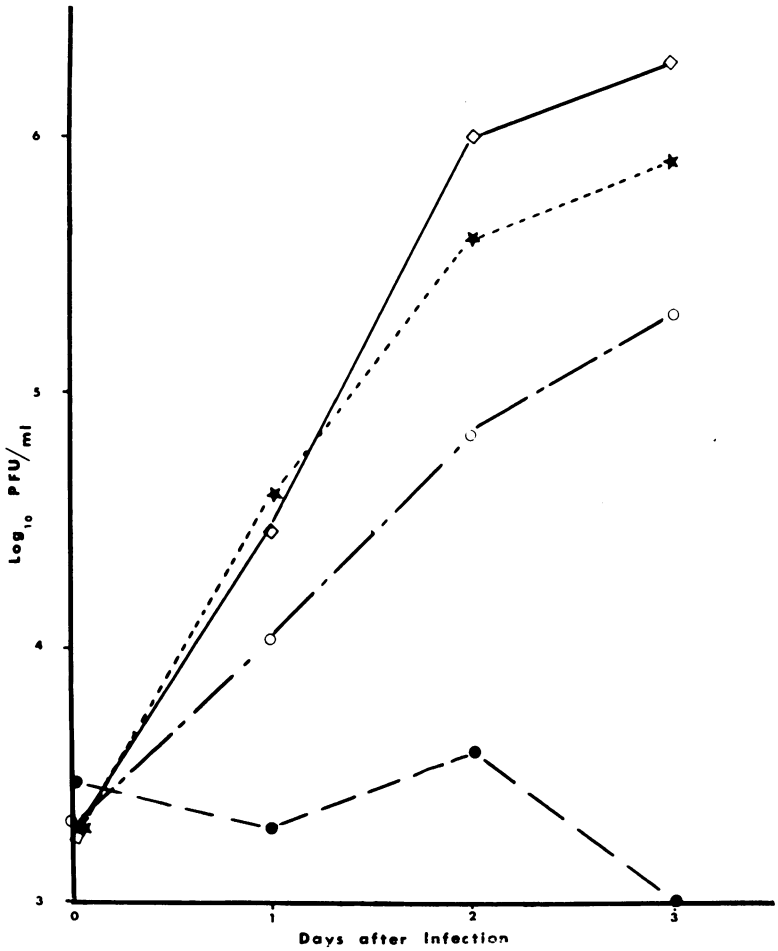

FIG. 2. *Replication of vaccinia virus in purified peritoneal macrophages from immune* (●) *and nonimmune* (○) *rabbits and in alveolar macrophages from immune* (□) *and nonimmune* (★) *rabbits.*

nisms to resistance to viral infections has been the inability to examine these two types of responses separately. With the recent development of in vitro methods which correlate with delayed hypersensitivity, it is now possible to measure cellular immunity, independent of humoral immunity, to a number of antigens, including viruses (15, 25, 27). Macrophage migration inhibition appears to result from a specific interaction between antigen and sensitized lymphocytes (6, 10). The results presented in this paper provide suggestive evidence that cellular immunity to vaccinia virus may involve both lymphocytes and macrophages.

Vaccinia virus antigens inhibited the migration of peritoneal exudate cells obtained from rabbits who demonstrated a delayed hypersensitivity to the virus. Therefore, it appears that with vaccinia virus the macrophage migration inhibition test is an in vitro correlate of delayed hypersensitivity. Peritoneal exudate cells were inhibited from migration only when vaccinia antigen was present. The addition of fibroma antigen or normal rabbit kidney antigen to peritoneal exudate cells from vaccinia-immune rabbits resulted in negligible inhibition of migration. Furthermore, vaccinia antigen at concentrations inhibiting the migration of sensitized macrophages by 70% did not significantly inhibit the migration of peritoneal exudate cells from nonimmune rabbits.

One factor involved in causing inhibition of migration of macrophages is a protein released by sensitized lymphocytes upon interaction with the sensitizing antigen (6). As reported by others, we also observed that lymphocytes from immune animals are effective in inhibiting the migration of macrophages from nonimmune animals in the presence of the sensitizing antigen. Lymphocytes obtained from the peritoneal exudate of rabbits

immune to vaccinia virus are more effective than lymphocytes obtained from the blood and cells from lymph nodes or spleen in inhibiting the migration of macrophages obtained from nonimmune rabbits. These results are in accordance with those of other workers who reported that small numbers of purified peritoneal exudate lymphocytes are capable of affecting the inhibition of migration of nonimmune peritoneal exudate cells and that lymphocytes from peritoneal exudates are more effective than lymphocytes from other sources in mediating migration inhibition (13), target cell destruction (19), and tumor rejection (5).

In contrast to the findings of Bloom and Bennett (7) and Dumonde (13), who observed no inhibition of migration of purified peritoneal macrophages, it was found in the present study that preparations of immune peritoneal macrophages containing less than 1% lymphocytes were only slightly less inhibited than were the crude peritoneal exudate cells. Inhibition of the partially purified peritoneal macrophages could not be explained solely by the small number of lymphocytes remaining, since 20% purified peritoneal lymphocytes from the same animal was required to produce the same level of inhibition of migration of peritoneal exudate cells from nonimmune rabbits. Addition of 1% purified lymphocytes resulted in only 32.4% inhibition of nonimmune peritoneal exudate cells, whereas immune peritoneal exudate cells containing $< 1\%$ lymphocytes were inhibited by 62.6%. The discrepancy in the observations reported by others (7, 13) and those presented here may rest in the differences in methods of obtaining purified macrophages or in the antigen used.

Heise et al. (18) demonstrated that alveolar macrophages from guinea pigs sensitized to tuberculin were inhibited from migrating in the presence of the antigen by virtue of an interaction between the antigen and cytophilic antibody on the surface of the macrophages. The possibility that peritoneal macrophages from vaccinia-immune rabbits might be bound by cytophilic antibody at the cell surface or that such cells release antibody which can react with vaccinia virus antigens was examined. However, we were unable to detect a factor from heat eluates or whole-cell lysates of macrophages from immune rabbits which could mediate the inhibition of migration of macrophages from nonimmune rabbits in the presence of antigen. The possibility cannot be excluded that such cells possess a cell-bound antibody, but under the conditions used no such factor was obtained.

The suppression of the replication of vaccinia virus in purified peritoneal macrophages from immune rabbits supports the contention that immune specificity also resides within macrophages. There have been previous reports indicating that macrophages from virus-immune animals may contain specific antiviral activity. Steinberger and Rights (23) showed that freshly explanted spleen cells from rabbits immunized against vaccinia virus were more resistant to challenge in vitro with this virus than comparable spleen cells from nonimmunized animals. These authors were also unable to detect antibody in their cultures. An antiviral activity associated with macrophages and specific for the immunizing virus has also been reported by other workers (*see* 22). On the other hand, some investigators have failed to detect a difference in susceptibility of macrophages from immune and nonimmune animals to these viruses (22).

Results of experiments reported here clearly demonstrate that vaccinia virus is capable of replicating in vitro in peritoneal macrophages from nonimmune rabbits but not in peritoneal macrophages from immune animals. The suppression of virus replication did not appear to be due to residual lymphocytes, because the addition of peritoneal exudate lymphocytes from immune rabbits to macrophages from nonimmune rabbits did not interfere significantly with growth of the virus, whereas these cells did inhibit the migration of normal macrophages in the presence of antigen. The possibility of cell-bound antibody to vaccinia virus also appears to be an unlikely explanation for the inhibition of vaccinia replication, since no vaccinia neutralizing activity was found associated with either heat eluates or whole-cell extracts of sensitized macrophages.

Glasgow (16) reported that macrophages from mice immunized with Chikungunya virus produced more interferon upon exposure to this virus than did macrophages from nonimmune mice. Furthermore, the enhanced production of interferon resulted only when cells were challenged with Chikungunya virus, suggesting that macrophages may possess a specific "immunological memory." Enhanced production of an interferon-like substance by immune macrophages seems not to be the cause of suppression of vaccinia virus replication in our system, as we have been able to detect only low levels of interferon-like activity in our cultures, and in no instance have we observed a difference between titers of interferon-like activity from normal or immune cells infected with vaccinia. These results are consistent with those of Subrahmanyan and Mims (24), who were unable to induce interferon with vaccinia virus in macrophages from either immune or nonimmune mice.

It is of interest that the macrophage-associated immunity was characteristic of peritoneal macro-

phages and was not observed with alveolar macrophages. Vaccinia virus replicated readily in alveolar macrophages from both immune and nonimmune rabbits. Furthermore, alveolar macrophages were not inhibited from migration in the presence of antigen. These results could reflect the route of immunization or could represent basic differences in cells form the two sources. Whereas the peritoneal macrophages appear to represent circulating nonfixed cells, the alveolar macrophages are thought to originate within the lung interstitium (9), where they develop biochemical characteristics that distinguish them from other types of monocytes or macrophages.

The results of the present study imply that, in addition to lymphocytes, macrophages may have an active role in the immune response to vaccinia virus. Passive transfer of contact sensitivity in the mouse has been accomplished with macrophages which had presumably acquired cytophilic antibody from lymphocytes (4). Whether or not the specificity of the reaction of the macrophages with vaccinia virus, as measured by inhibition of migration and suppression of virus replication in the present study, represents undetected cytophilic antibody or some other mechanism remains to be determined.

ACKNOWLEDGMENTS

We are grateful for the superb technical assistance of Doris Petronelli.

This study was supported by Public Health Service research grants CA 04600 from the National Cancer Institute and HE 05435 from the National Heart and Lung Institute, and by training grant 5-T1-AI-74 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. Allison, A. C. 1966. Immune responses to Shope fibroma virus in adult and newborn rabbits. J. Nat. Cancer Inst. **36**:869–876.
2. Allison, A. C. 1967. Cell-mediated immune responses to virus infections and virus-induced tumours. Brit. Med. Bull. **23**: 60–65.
3. Allison, A. C., and R. M. Freidman. 1966. Effects of immunosuppressants on Shope rabbit fibroma. J. Nat. Cancer Inst. **36**:859–868.
4. Asherson, G. L., and M. Zembala. 1970. Contact sensitivity in the mouse. IV. The role of lymphocytes and macrophages in passive transfer and the mechanism of their interaction. J. Exp. Med. **132**:1–15.
5. Bennett, B. 1965. Specific suppression of tumor growth by isolated peritoneal macrophages from immunized mice. J. Immunol. **95**:656–664.
6. Bennett, B., and B. R. Bloom. 1967. Studies on the migration inhibitory factor associated with delayed-type hypersensitivity: cytodynamics and specificity. Transplantation **5**:996–1000.
7. Bloom, B. R., and B. Bennett. 1966. Mechanism of a reaction in vitro associated with delayed-type hypersensitivity. Science **153**:80–82.
8. Boulter, E. A. 1969. Protection against poxviruses. Proc. Roy. Soc. Med. **62**:295–297.
9. Bowden, D. H., I. Y. R. Adamson, G. W. Grantham, and J. P. Wyatt. 1969. Origin of the lung macrophage, evidence derived from radiation injury. Arch. Pathol. **88**:540–546.
10. David, J. R. 1966. Delayed hypersensitivity in vitro: its mediation by cell-free substances formed by lymphoid cell-antigen interaction. Proc. Nat. Acad. Sci. U.S.A. **56**:72–77.
11. David, J. R., S. Al-Askari, H. S. Lawrence, and L. Thomas. 1964. The specificity of inhibition of cell migration by antigens. J. Immunol. **93**:264–273.
12. Desmyter, J., W. E. Rawls, and J. L. Melnick. 1968. A human interferon that crosses the species line. Proc. Nat. Acad. Sci. U.S.A. **59**:69–76.
13. Dumonde, D. C. 1967. The role of the macrophage in delayed hypersensitivity. Brit. Med. Bull. **23**:9–14.
14. Edelman, R., and E. F. Wheelock. 1968. Specific role of each human leukocyte type in viral infections. II. Phytohemagglutinin-treated lymphocytes as host cells for vesicular stomatitis virus replication in vitro. J. Virol. **2**:440–448.
15. Feinstone, S. M., E. H. Beachey, and M. W. Rytel. 1969. Induction of delayed hypersensitivity to influenza and mumps viruses in mice. J. Immunol. **103**:844–849.
16. Glasgow, L. A. 1966. Leukocytes and interferon in the host response to viral infections. II. Enhanced interferon response of leukocytes from immune animals. J. Bacteriol. **91**:2185–2191.
17. Gresser, I., and D. L. Lang. 1966. Relationships between viruses and leucocytes. Progr. Med. Virol. **8**:62–130.
18. Heise, E. R., S. Han, and R. S. Weiser. 1968. In vitro studies on the mechanism of macrophage migration inhibition in tuberculin sensitivity. J. Immunol. **101**:1004–1015.
19. Klein, G., and H. F. Oettgen. 1969. Immunologic factors involved in the growth of primary tumors in human or animal hosts. Cancer Res. **29**:1741–1746.
20. Mims, C. A. 1964. Aspects of the pathogenesis of virus diseases. Bacteriol. Rev. **28**:30–71.
21. Myrvik, Q. N., E. S. Leake, and B. Fariss. 1961. Studies of pulmonary alveolar macrophages from the normal rabbit: a technique to procure them in a high state of purity. J. Immunol. **86**:128–132.
22. Silverstein, S. 1970. Macrophages and viral immunity. Seminars Hematol. **7**:185–214.
23. Steinberger, A., and I. L. Rights. 1963. Effect of immunization on tissue susceptibility to vaccinia in vitro. Virology **21**: 402–410.
24. Subrahmanyan, T. P., and C. A. Mims. 1970. Interferon production by mouse peritoneal cells. J. Reticuloendothel. Soc. **7**:32–42.
25. Tompkins, W. A. F., C. Adams, and W. E. Rawls. 1970. An in vitro measure of cellular immunity to fibroma virus. J. Immunol. **104**:502–510.
26. Tompkins, W. A. F., D. L. Walker, and H. C. Hinze. 1969. Cellular deoxyribonucleic acid synthesis and loss of contact inhibition in irradiated and contact-inhibited cell cultures infected with fibroma virus. J. Virol. **4**:603–609.
27. Tremonti, L. P., and G. G. Jackson. 1969. Study of delayed hypersensitivity to myxoviruses induced by vaccines. Appl. Microbiol. **17**:577–583.
28. Willems, F. T. C., J. L. Melnick, and W. E. Rawls. 1969. Replication of poliovirus in phytohemagglutinin-stimulated human lymphocytes. J. Virol. **3**:451–457.