Inactivation of Myxoviruses by Lymphoid Cells

By B. ZISMAN AND A. M. DENMAN

Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ

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SUMMARY

Representative myxoviruses were inactivated by cultures of normal human or mouse lymphoid cells even after stimulation with a variety of mitogens. The fractionation of cells by velocity sedimentation indicated that inactivation occurred in the presence of some lymphocyte populations but not macrophages. Pre-treatment with neuraminidase prevented this effect. Cells producing antibody to sheep RBC failed either to adsorb or to inactivate virus. The direct inactivation of myxoviruses by normal lymphoid cells may be one of the mechanisms involved in host resistance against viruses of this group.

INTRODUCTION

Although many viruses replicate in lymphocytes which have been stimulated in vitro by phytohaemagglutinin (PHA) or other mitogens (Merigan & Wheelock, 1971), these cells may not be permissive for viruses of all types. In this paper we describe experiments in which some myxoviruses not only failed to replicate in lymphocytes but appeared to be inactivated by certain lymphocyte populations. The direct inactivation of virus infectivity by lymphocytes may be one form of host defence against infection by some viruses.

METHODS

Mice. These were raised in the Clinical Research Centre breeding unit. Spleens and pooled brachial, axillary, inguinal and aortic lymph nodes were obtained from 6- to 8-week-old CBA mice of either sex. Specific-pathogen-free (SPF) CBA mice were maintained in isolators.

Sendai virus. (Parainfluenza I) was an egg passage of a mouse lung isolate obtained from the World Influenza Centre, Mill Hill. Virus stocks were prepared in the allantoic cavity of 10-day-old embryonated hens' eggs and stored at −70 °C.

Influenza virus. The influenza virus pathogenic to mice was the AO/PR8/34 strain and is referred to as PR8 virus. The human pathogenic influenza virus was the Clone 6 (PR8 × 939) recombinant of AO/PR8[34 and A2/England/939/69 (McCahon & Schild, 1972) and is referred to as human influenza virus.

Both strains of influenza virus were obtained from the MRC Common Cold Unit, Harvard Hospital, Salisbury, and passaged in the allantoic cavity of 10-day-old embryonated eggs.

Vesicular stomatitis virus (VSV, New Jersey). Virus was grown in primary cultures of chick embryo fibroblasts. Supernatant fluids were quick-frozen and stored at −70 °C.

Preparation of lymphocyte suspensions. Mouse spleen cell suspensions were obtained by cutting the capsule, expressing the contents with a probe and dissociating the fragments
with a Pasteur pipette. Mouse lymph node and human spleen cells were forced through a stainless steel mesh of pore size 100 μm. The human spleens were removed from previously healthy individuals following road traffic accidents. Suspensions of single cells were obtained from either spleen or lymph node by filtration through gauze swabs. These were washed twice in buffered salt solution in the cold.

**Preparation of spleen macrophage cultures.** 5 × 10⁷ mouse or human spleen cells in 1-0 ml of medium were placed in the wells of disposable plastic trays (Linbro FB-16-ZY-TC, Linbro catalogue No. 5041, available from Bio-Cult, Paisley, Scotland). After 15 min, non-adherent cells were removed by vigorous washing with medium containing 30% FCS using a Pasteur pipette. Selected cultures were activated with histone (calf thymus type Z, Sigma Chem. Co.), 50 μg/culture, a compound of strongly positive charge which increases pinocytosis 1000-fold (P. Davies, personal communication). For subsequent assay, the adherent macrophages were removed with a rubber policeman.

**Preparation of lymphocyte-free bone marrow cells.** Fragments of mouse bone marrow were flushed out of the femora with a needle and syringe. Human bone marrow was isolated from bone removed in the course of various orthopaedic procedures on patients without abnormalities of the haemopoietic system. After settlement under gravity, rapidly sedimenting cell fractions depleted of lymphocytes were pooled (Amato, Cowan & McCulloch, 1972).

**Human blood.** Lymphocytes were isolated from the heparinized blood of normal donors by treatment with ‘plasmagel’ (Roger Bellon, Neuilly, Seine, France) and sedimentation of the supernatant fluids in Ficoll–Triosil gradients (Böyum, 1968).

**Lymphocyte cultures.** The culture medium was Roswell Park Memorial Institute 1640 (RPMI 1640, Flow Laboratories) with penicillin and streptomycin each 100 i.u./ml, 15 ml/l of 2 mM-L-glutamine, 20 g/l of NaHCO₃, 20 ml/l of HEPES (N-2-hydroxyethylpiperazine-N-2-ethane-sulphonic acid (Flow Laboratories) and 10% foetal calf serum. Both human and murine lymphocytes were cultured in conical-bottomed plastic tubes, containing 2 × 10⁶ viable cells/ml. The cultures were gassed with a mixture of 6% CO₂, 8% O₂ and 86% N₂ and kept in humidified desiccators at 37 °C. Mitogens were added in the following concentrations when the cultures were established: phytohaemagglutinin (PHA) (Burroughs Wellcome) 5 μg/ml; Concanavalin A (Con A) (Calbiochem) 2.5 μg/ml; pokeweed mitogen (PWM) (Gibco) 50 μl/ml. These optimal concentrations for lymphocyte transformation were determined in preliminary experiments.

As a test for lymphocyte survival and transformation, selected cultures of control and mitogen-transformed cells were labelled with 0.08 μCi [¹⁴C]-thymidine of specific activity 62 mCi/m-mol (Radiochemicals, Amersham) after 48 h. After a further 24 h the cell buttons were transferred to Whatman filter pads, washed successively with cold buffered saline, cold trichloroacetic acid and absolute methanol and then placed in vials with scintillation gel before counting for 4 min in a Phillips liquid scintillation counter with internal standards for efficiency and quenching.

**Infection of lymphocyte cultures.** Lymphocyte cultures were centrifuged and resuspended in 1-0 ml of virus diluted in complete medium. All viruses were added at a multiplicity of 10 infective units/cell. Cultures were incubated for 90 min at 37 °C with continuous rotation, after which the cells were washed three times at 4 °C with phosphate buffered saline (PBS) at pH 7.2 to remove non-adsorbed virus. In early experiments the washings were regularly assayed for virus. Of the 10⁷ infective units which were added per 10⁶ cells in 1 ml of medium, fewer than 10 EID₉₀ were recovered. The cells were then restored to the original culture conditions. Duplicate samples were assayed at intervals for virus infectivity and cell viability.
Virus assays

Influenza and Sendai viruses. Samples of 0.1 ml of ten-fold dilutions were assayed for virus infectivity by injection into the allantoic cavity of 10-day-old embryonated hens' eggs. The fluids were tested for haemagglutinating activity after incubation for 2 days at 33 °C for influenza virus or for 3 days at 37 °C for Sendai virus. The lymphocyte suspensions were subjected to one cycle of freezing and thawing before assay. Since virus infectivity in cultures of cells was, within the first 8 h of infection, similar to that in cultures of medium alone, it seemed reasonable to conclude that the assays were measuring the total number of virus particles present and not just infected cells. Virus infectivities are expressed in 50 % egg infective doses (EID50) per 0.1 ml and were calculated by the method of Reed & Meunch (1938). Each sample was assayed in triplicate, usually on two occasions. The s.e. of such repeated assays did not exceed 10^-13 infective units/0.1 ml. Differences in virus infectivity between control and experimental cultures which exceeded 10^-5 infective units/0.1 ml have been regarded as significant (P < 0.001 by Student’s t test).

Vesicular stomatitis virus (VSV, New Jersey). The micro-neutralization technique (De Madrid & Porterfield, 1969) was adopted with some modifications. Monolayers of L-cells were grown in the wells of World Health Organization (WHO) haemagglutination trays and infected with 0.1 ml of serial ten-fold dilutions of each sample. After adsorption for 1 h at 37 °C the plates were overlaid with 0.75% carboxymethylcellulose in L 15 medium with added 10 % tryptose phosphate broth, 1 % glutamine and 3 % foetal calf serum. Two days later the monolayers were stained with crystal violet (1 g/200 ml of 74 % methanol) and the plaques were counted.

Interferon assays

Interferon activity in mouse cultures was assayed in L-cells by reduction of plaque counts. Supernatant fluids from infected mouse lymphocyte cultures were heated at 56 °C for 30 min, acidified to pH 2 by dialysis for 72 h at 4 °C and then restored to neutrality. Interferon titres were recorded as the highest dilution giving a 50 % reduction of the number of VSV plaques compared with the number of plaques in the controls. Interferon activity in cultures of human lymphocytes was not measured.

Titration of anti-Sendai and anti-influenza antibody activity

Sera from normal and immune mice and from human subjects were tested for inhibition of haemagglutination (HA) by Sendai, PR 8 or human influenza virus. Sera were pre-treated with receptor-destroying enzyme (RDE) at 37 °C overnight and later heated for 1 h at 56 °C. Antibody titres were measured as the highest dilution of serum which totally inhibited HA.

Velocity sedimentation. Spleen and lymph node lymphocytes were separated by direct settlement in a ‘buffered step’ gradient of 0.16 to 2.0 % Ficoll in RPMI medium containing 4 % FCS at 4 °C (Miller & Phillips, 1969). The sedimentation apparatus (Grave Glass Co., Solna, Stockholm) incorporated some modifications to previous designs (Denman & Pelton, 1972). Fractions of 20 ml were collected by upward displacement with 10 % sucrose solutions. In some experiments rosettes were separated by sedimentation in a shallow plastic chamber (Edwards, Miller & Phillips, 1971). Cell number and vol. in each fraction were analysed with a Counter model B counter with a ‘J’ plotter attachment.

Immunization against sheep cells and rosette formation. Mice were immunized by intra-peritoneal injection with 2.0 × 10⁸ sheep red cells. Spleen cells were harvested 7 to 21 days later. Rosettes were formed between the antigen and lymphocytes with immune receptors
VSV replication in mouse lymph node cells. $2 \times 10^6$ mouse lymph node cells, with or without PHA stimulation, were cultured in $2 \times 10^6$ ml RPMI 1640 at $37^\circ$C and infected 24 h later with virus at a multiplicity of 10 infective units/cell. ●●, thermal inactivation; ▲▲, PHA-stimulated cells; ■■, unstimulated cells.

Detection of cells with adsorbed virus by rosette formation. Rosettes between lymphoid cells with adsorbed virus and chicken RBC were formed as above.

Neuraminidase treatment. Lymphoid cells were treated at $37^\circ$C for 45 min with 2 units neuraminidase (Hoechst Pharmaceuticals)/$10^6$ cells.

RESULTS

VSV replication in lymphoid cells

As expected (Eustatia & Van der Veen, 1971), VSV replicated in mouse lymph node and spleen cell cultures (Fig. 1).

Loss of myxovirus infectivity in the presence of normal mouse lymphoid cells

In all experiments Sendai virus was inactivated by mouse spleen or lymph node cells whether or not the cultures had been stimulated with PHA. There was a rapid decrease of virus infectivity in the presence of these cells compared with controls in which virus was incubated in medium alone or with frozen and thawed cells. An example of inactivation in the presence of lymph node cells is given in Fig. 2. Similar results were obtained when virus was added at the time the cultures were established or 24, 48 and 72 h later. Variation in cell numbers between $1 \times 10^6$ and $5 \times 10^6$ cells/culture and different input multiplicities between
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0.1 and 1000 EID<sub>50</sub>/cell did not affect the rate of inactivation. Cell viability was not significantly changed by virus infection, whether judged by cell counts of unstimulated cultures or by the response to PHA measured by [<sup>3</sup>H]-thymidine incorporation. After 3 days of culture, uninfected lymph node cells incorporated 799 (± 248 s.d.) cpm/10<sup>6</sup> cells without stimulation, and 17743 (± 1788 s.d.) cpm/10<sup>6</sup> cells after PHA stimulation. The comparable figures in cultures infected with Sendai virus were 721 (± 230 s.d.) cpm/10<sup>6</sup> cells without PHA and 14708 (± 3250 s.d.) cpm/10<sup>6</sup> cells after PHA stimulation. Similarly, Sendai virus had no effect on spleen cell cultures. Furthermore, autoradiographic analysis of infected and control cultures indicated that the virus did not interfere with DNA synthesis in lymphocytes stimulated by PHA.

Under comparable conditions PR 8 virus was inactivated by normal mouse lymphoid cells at a similar rate. Inactivation of this virus by mouse spleen cells is illustrated in Fig. 3.

PR 8 virus did not impair the response to PHA. Infected spleen cultures gave 8420 (± 2049 s.d.) cpm/10<sup>6</sup> cells after PHA stimulation compared with 1730 (± 264 s.d.) cpm/10<sup>6</sup> cells in unstimulated cultures, while the corresponding counts in infected lymph node cultures were 17750 (± 2775 s.d.) cpm/10<sup>6</sup> cells after stimulation and 830 (± 190 s.d.) cpm/10<sup>6</sup> cells without.

When cultures were assayed for virus infectivity at shorter intervals, reductions in infectivity for Sendai and PR 8 virus were first evident after 4 h and were considerable after 24 h (Table 1).
Fig. 3. Inactivation of PR 8 virus by normal mouse spleen cells. Details as for Fig. 1.

### Table 1. Inactivation of PR 8 virus by normal mouse lymph node cells

<table>
<thead>
<tr>
<th>Time after virus infection</th>
<th>Virus infectivity in log (EID₉₀/0.1 ml)</th>
<th>With frozen and thawed cells</th>
<th>With lymph node cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0, 20, 40 min</td>
<td>6.5</td>
<td>6.5</td>
<td>6.0</td>
</tr>
<tr>
<td>60 min</td>
<td>6.5</td>
<td>6.5</td>
<td>5.0</td>
</tr>
<tr>
<td>4 h</td>
<td>6.5</td>
<td>6.5</td>
<td>3.5</td>
</tr>
<tr>
<td>8 h</td>
<td>6.0</td>
<td>6.0</td>
<td>3.5</td>
</tr>
<tr>
<td>24 h</td>
<td>6.0</td>
<td>6.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Cultures of 2 × 10⁴ normal lymph node cells in 2 ml RPMI 1640 were infected at a virus multiplicity of 10 infectious units/cell and incubated at 37 °C. Controls consisted of equivalent numbers of 6-times frozen and thawed cells infected at the same multiplicity or 2 ml medium alone, infected with 2 × 10⁷ infective units in total.

**Effect of specific immunity on in vitro inactivation of myxovirus by mouse lymphoid cells**

The influence of specific immunity on the rate of inactivation of Sendai virus by mouse lymphoid cells was studied by comparing the results of infecting cultures of lymphocytes from immune and non-immune mice. Non-immune lymphocytes were obtained from SPF mice, since no antibody activity to Sendai virus was detected in any of their sera. Antibody was found in the sera of 20 non-SPF mice which were tested, the titres ranging from 1/32 to 1/128; such titres are often a result of endemic infection, which is a common finding in laboratory rodents (Parker, Tennant & Ward, 1966). In addition, 20 mice were infected intranasally with 10³ EID₉₀ of Sendai virus. Spleen and lymph node cultures were established 10 days later when serum antibody titres ranged from 1/128 to 1/512 (mean 1/180). With or without PHA stimulation, lymph node and spleen cells inactivated Sendai virus at the same rate, irrespective of serum antibody titres. No significant differences between cultures from immune and non-immune mice were noted. Similar results were obtained in mice infected with PR 8 virus by intranasal installation.
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Fig. 4. Inactivation of PR8 virus by normal mouse lymph node cells stimulated with different mitogens. Conditions and details as for Fig. 1. O—O—O, Con A stimulated cells; △—△—△, PWM-stimulated cells.

Fig. 5. Inactivation of human influenza virus by lymphocytes from normal human blood. 2·0 x 10⁶ human blood lymphocytes were cultured as described in Fig. 1 and infected 24 h later with virus at a multiplicity of 10 infective units/cell. Key as for Fig. 1.

Effect of other lymphocyte mitogens

Certain mitogens activate different populations of lymphocytes (Janossy & Greaves, 1971) and it is possible that mitogens other than PHA may stimulate lymphocytes in which myxovirus would indeed replicate. However, cultures in which mouse spleen or lymph node cells were stimulated by Con A or PWM inactivated Sendai and PR 8 virus at the same rate as PHA transformed cultures (Fig. 4).

Interferon activity

Supernatant fluids from several mouse lymphocyte cultures infected by myxovirus were tested for interferon and none was detected within the first 96 h of culture.

Inactivation of myxoviruses by human blood lymphocytes

Sendai virus and human influenza virus were also always inactivated in cultures of human peripheral blood lymphocytes whether unstimulated or transformed by mitogens or by allogeneic lymphocytes in mixed lymphocyte reactions. The rate of inactivation in cultures stimulated with PHA is shown in Fig. 5; similar results were observed with the other stimulants. Antibody activity against human influenza and Sendai virus in the sera of the four donors did not exceed 1/10 at the time their lymphocytes were cultured.

Inactivation of heterologous virus by lymphoid cells

Human blood lymphocytes inactivated mouse-adapted PR 8 virus and human influenza virus equally rapidly. Compared with the infectivity of virus incubated at 37 °C in growth medium alone, the infectivity of virus incubated for 24 h in the presence of unstimulated
or PHA-stimulated lymphocytes was regularly reduced to less than 1%. No antibody activity against PR 8 virus was detected in the sera of the lymphocyte donors.

In the converse experiment, mouse spleen cells from SPF donors lacking serum antibody to human influenza virus consistently inactivated the heterologous virus in culture (Fig. 6).

Attempts to characterize lymphoid cells responsible for virus inactivation

In order to characterize the lymphoid cells responsible for myxovirus inactivation, spleen or lymph node cells from SPF mice without evidence of specific immunity were separated by sedimentation rate and virus was added to different fractions. Thus lymphoid cells with differing volumes and properties were separated (Miller & Phillips, 1969). The capacity of three major fractions of mouse spleen or lymph node cells to inactivate Sendai and PR 8 virus was tested. Cultures of $2 \times 10^6$ trypan-blue-excluding cells were established from each fraction. These were at once infected with PR 8 or Sendai virus at a multiplicity of 10 infective units/cell using the procedure described for infecting unseparated lymphoid cells. The infectivity of either virus decreased in the presence of pooled fractions of large and medium-sized spleen cells but not of the most slowly sedimenting small lymphocytes (Fig. 7). Inactivation was delayed compared with virus exposed to unseparated cells, possibly because the separations were performed at 4°C and a latent period preceded the return of normal function. Judged by dye exclusion, the numbers of surviving cells were similar in cultures from the different fractions and ranged from 35 to 40% after 4 days. These results indicated that myxoviruses are inactivated in the presence of some but not all lymphoid cells.

Similar experiments were carried out with human blood mononuclear cells and influenza virus. Cell fractions containing medium and large cells inactivated virus whereas the most slowly sedimenting lymphocytes were ineffective.


![Graph](https://via.placeholder.com/150)

**Fig. 7.** Inactivation of Sendai virus by different cell fractions from normal mouse spleens. Lower half: mouse spleen cells were separated by velocity sedimentation at 19 °C for 4 h. Major pools (1, 2, 3) of the cell fractions were collected as indicated by the vertical lines. Upper half: cultures of $2 \times 10^6$ unstimulated cells in 2.0 ml of medium were established from the cell pools 1, 2 and 3 under the conditions described in Fig. 1 and were infected from the onset at a multiplicity of 10 infective units/cell.

**Failure of cells other than lymphocytes to inactivate myxoviruses**

Neither Sendai virus nor PR 8 virus was inactivated by mouse spleen macrophages, and the infectivity of human influenza virus was similarly unaffected by human spleen macrophages. The results were no different if virus was cultured with macrophages activated by histone. Fractions of marrow cells depleted of lymphocytes but including cells of the granulopoietic and erythropoietic series did not inactivate these viruses.

**Effect of neuraminidase treatment of lymphoid cells**

The ability of Sendai virus and PR 8 virus to adsorb to mouse lymphoid cells was tested using haemadsorption with chicken or guinea pig indicator RBC. At the routine input multiplicity of 10 EID$_{50}$/cell, 3 to 11% of spleen or lymph node cells from SPF mice lacking detectable serum antibody adsorbed either virus. If adsorption to lymphocytes is an essential step in the inactivation of myxoviruses by these cells then neuraminidase-treated cells should have no effect on virus infectivity. Neuraminidase treatment abolished the ability
of mouse spleen and lymph node lymphocytes and human blood lymphocytes to inactivate Sendai virus and the appropriate influenza virus. Whereas the infectivity of Sendai virus and influenza virus exposed to untreated lymphocytes was invariably reduced after 48 h by 2 log units or more compared with virus cultured in medium alone, the loss of virus infectivity in cultures of neuraminidase-treated cells did not differ significantly from that in cultures without lymphocytes. The inactivation of virus in the presence of those lymphocyte fractions which were effective was also abolished by neuraminidase treatment.

Interaction between myxoviruses and antibody-producing cells

Sendai and influenza viruses failed to replicate in lymphocytes stimulated by mitogens or allogeneic lymphocytes, and indeed were inactivated as rapidly in stimulated cultures as by resting lymphocytes. However, these lymphocytes differ from those engaged in antibody synthesis (Roitt et al. 1969) even though they undergo a similar sequence of metabolic events after antigenic challenge. Accordingly, the fate of mouse influenza virus and Sendai virus exposed to antibody-producing cells was investigated.

Spleen cell suspensions were obtained from mice which had been immunized with a single injection of sheep RBC from 5 to 21 days previously (referred to subsequently as RBC-immune lymphocytes). The RBC-immune cells were first exposed to Sendai virus or mouse influenza virus in vitro for 90 min at 37 °C, at an input multiplicity of 10 infective units/cell and then to chicken RBC at 4 °C. In this way rosettes were formed between chicken RBC and those lymphocytes to which virus had adsorbed. These rosettes were readily separated by sedimentation for 4 h since their vol. greatly exceeded that of all other lymphocytes. The fractions enriched for rosettes and those depleted of these aggregates were pooled separately. The chicken RBC were detached by repeated pipetting so that two pools of free lymphocytes were isolated, of which one consisted predominantly of virus-adsorbing cells. Rosette formation with sheep RBC was then attempted with both pools, this time on the basis of immune adherence. At all stages following immunization with sheep RBC, the lymphocytes which had adsorbed virus failed to adsorb sheep RBC, indicating that these cells and the RBC-immune lymphocytes were separate populations. In converse experiments, the spleen cells were exposed first to sheep RBC and the resulting rosettes between the sheep RBC and the RBC-immune lymphocytes were separated by sedimentation. These rosettes were disrupted and the RBC-immune lymphocytes were exposed sequentially to Sendai or PR 8 virus and chicken RBC. These lymphocytes failed completely to bind chicken RBC, whereas the cells in the fractions depleted of RBC-immune lymphocytes formed the expected number of rosettes with chicken RBC after exposure to virus. Furthermore, Sendai or PR 8 virus exposed to fractions consisting predominantly of RBC-immune lymphocytes were inactivated no more rapidly than by medium containing frozen and thawed cells. Finally, spleen cells immune to sheep RBC were exposed in vitro, first to Sendai or PR 8 virus and then to sheep and chicken RBC simultaneously; insignificant numbers of mixed rosettes (< 1/10⁶ cells) were detected. Representative experiments are shown in Table 2. Thus RBC-immune lymphocytes which include the majority of antibody-secreting cells (Wigzell & Anderson, 1969) failed either to adsorb or to inactivate these viruses.

DISCUSSION

These studies have shown that some lymphocytes inactivate certain myxoviruses directly. Macrophages were clearly not responsible for this inactivation. Cell populations from mouse spleen and lymph nodes and from human peripheral blood which were depleted of
Table 2. Failure of Sendai virus to adsorb to RBC-immune mouse spleen cells

<table>
<thead>
<tr>
<th>Fraction pool (sedimentation rate mm/h)</th>
<th>% Cells/pool forming rosettes with chicken RBC = cells with adsorbed virus</th>
<th>% Cells/pool forming rosettes with sheep RBC = RBC-immune cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (3.0 to 4.5)</td>
<td>82 1.6 2.0</td>
<td>2.5 3.0</td>
</tr>
<tr>
<td>2 (4.6 to 6.0)</td>
<td>6.0 0.7 12</td>
<td>0 0</td>
</tr>
<tr>
<td>3 (6.1 to 12.0)</td>
<td>6.4 5.1 79</td>
<td>0 0</td>
</tr>
<tr>
<td>Unseparated cells</td>
<td>100 7.0 7</td>
<td>2.5 2.5</td>
</tr>
<tr>
<td>Exp. 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (3.0 to 4.5)</td>
<td>72 7.1 9.9</td>
<td>0 0</td>
</tr>
<tr>
<td>2 (4.6 to 5.5)</td>
<td>20 1.0 5.0</td>
<td>0.2 1.2</td>
</tr>
<tr>
<td>3 (5.6 to 9.0)</td>
<td>0.8 0 0</td>
<td>0.7 85</td>
</tr>
<tr>
<td>Unseparated cells</td>
<td>100 10 10</td>
<td>1.4 1.4</td>
</tr>
</tbody>
</table>

Spleen cells were obtained from mice immunized with sheep RBC 8 days previously. In experiment 1, 100 x 10^6 cells were infected with Sendai virus and exposed to chicken RBC. Separate pools were made of fractions enriched for or depleted of cells with adsorbed virus which formed rosettes with chicken RBC. After disrupting the rosettes, the pools were assayed for RBC-immune lymphocytes by rosette formation with sheep RBC. In experiment 2, 100 x 10^6 cells were first exposed to sheep RBC and fractions enriched for or depleted of cells forming rosettes with sheep RBC (RBC-immune lymphocytes) were pooled separately. After disrupting the rosettes, the pools were assayed for virus adsorption after infection with Sendai virus and rosette formation with chicken RBC.

Macrophages by sedimentation inactivated both Sendai virus and influenza virus. Furthermore, macrophages even if activated by histone, did not show this effect. Indeed, mouse peritoneal exudate cells support the replication of Sendai virus (B. Zisman & A. C. Allison, unpublished observations). Virus was inactivated only after exposure to certain lymphocyte populations and not after culture with other blood elements or marrow cells depleted of all lymphocytes.

The precise nature of the lymphocytes which did inactivate virus has not been established. Mouse spleen cells with these sedimentation properties include a variety of functional types (Miller & Phillips, 1969; Osoba, 1970), whilst lymphocyte populations of intermediate size from human blood show some response to mitogens and are particularly enriched with cells which are cytotoxic for target cells sensitized with antibody (Denman, 1973). These fractions also contain lymphocytes involved in the antibody response to unrelated antigens, but these are of unlikely relevance since mouse lymphocytes producing antibody to sheep RBC after experimental immunization failed either to adsorb or to inactivate virus. Whether or not the inactivating cells have surface immunoglobulin receptors remains to be determined. Since inactivation of virus is abolished by neuraminidase, it is likely that virus adsorption to sialic acid receptors on these cells is an essential step in the inactivation process. Lymphocyte receptors of this nature for Newcastle disease virus have been described by Woodruff & Woodruff (1972). Absence of surface receptors on antibody-producing cells in DNA synthesis may serve to protect such cells from virus superinfection in a host with disseminated virus infection.

The mechanism by which lymphocytes inactivate Sendai and influenza viruses remains to be explored. Virus could penetrate the cell and undergo an incomplete cycle of replication; such a phenomenon has been described in tumour cells infected by myxovirus (Prince & Ginsburg, 1957). We failed to detect interferon production in our cultures of mouse lymphocytes but its induction in human leucocytes by Sendai virus has been described...
(Tovell & Cantell, 1971) and it is perhaps significant that its synthesis is limited to 8 to 10 h after infection, when virus inactivation is already evident.

The relation of the in vitro phenomenon we have described to in vivo host defence against myxovirus infections is uncertain. The presence of circulating antibody in the donors of mouse spleen and lymph node cells did not affect the rate of inactivation. Similarly, little antibody was detected in the sera of human donors whose lymphocytes inactivated influenza virus. These observations and the ability of lymphocytes from mar and mouse to inactivate homologous influenza virus make it unlikely that the relevant cells were immunologically specific. Nevertheless some lymphocytes have been shown to possess immunoglobulin receptors for bacterial antigens even in non-immunized human subjects with negligible activity of circulating antibody (Dwyer & Mackay, 1970) so that immunological specificity cannot be entirely excluded, particularly since little is known about mechanisms for amplifying specific immunity which may exist in virus-infected cultures.

A variety of observations in tracheal organ cultures infected with myxovirus (Hoorn & Tyrrell, 1969) and mice infected with Sendai virus (Blandford & Heath, 1972) indicate that virus replication is confined primarily to the ciliated epithelium of the respiratory tract and the columnar cells of the bronchial mucosa. Furthermore, Robinson, Cureton & Heath (1968) showed that perivascular and peribronchial infiltration with pyroninophilic mononuclear cells is an early feature in the lungs of mice infected with Sendai virus, preceding the appearance of circulating anti-Sendai antibody. Although it was assumed that the rapidly mobilized cells in the infiltrates containing immunoglobulin were secreting anti-Sendai antibody locally, it is possible that in the early stages only a small proportion of the cells which limit dissemination of virus are specifically immune. Extrapolation to in vivo events can only be indirect but, in studies of direct human clinical relevance, there are few alternatives to in vitro methods.

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