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# Humoral and Cell-Mediated Immune Mechanisms in the Production of Pathology in Avirulent Semliki Forest Virus Encephalitis

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Seven days after peripheral inoculation with an avirulent strain of Semliki Forest virus, the brains of CBA and nude mice exhibited a mononuclear inflammation and spongiform degeneration. Mice that had received cyclophosphamide (150 mg/kg) 24 h after infection showed no pathology until day 11. However, immunofluorescence studies of the brains of immunosuppressed, infected mice demonstrated viral antigen within the soma and processes of neurons at earlier periods. The brain lesions could be reconstituted on day 7 in immunosuppressed, infected recipients with 6-day immune spleen cells. Immune spleen cells depleted of T lymphocytes, the non-immunoglobulin-bearing population deficient in B lymphocytes, or immune sera plus nonimmune bone marrow cells could also reconstitute the lesions. However, inflammation and spongiform changes were reduced when donor immune cells were depleted of either T or B lymphocytes. When both T and B lymphocytes were removed from the donor immune population, recipient brains did not show pathology. The results demonstrate that either antibody or immune T cells can trigger pathology, but there is also participation of nonimmune bone marrow-derived mononuclear cells, probably of the monocyte-macrophage lineage.

Immune responses can play a dual role in viral pathogenesis. One function is to reduce viral titers in tissue and thus decrease viral cytolytic activity. However, these same virus-specific responses can also have deleterious effects on tissue. The immune-mediated damage may be so extensive as to dominate the pathological expression of the infectious process. This is illustrated in several experimental virus infections of rodents, including lymphocytic choriomeningitis virus (14), parainfluenza type 1 virus (15), and Theiler's murine encephalomyelitis virus (20). Postinfectious encephalomyelitis, acute hemorrhagic leukoencephalitis, and Landry-Guillain-Barre syndrome are examples of human disorders where immune-mediated damage to the nervous system may occur subsequent to a viral infection (1, 25).

Semliki Forest virus (SFV) is an alphavirus that produces a central nervous system (CNS) infection in experimental animals. Unlike some virulent strains of SFV which produce a fatal encephalitis in adult mice, the A7 strain and its derivatives replicate in the brain for a limited period without causing clinical signs or death (5). Even though there is a subclinical infection, the brains exhibit an inflammatory spongiform degeneration. Previous studies using an immunosuppressive agent suggest that the brain lesions are immunopathological (27).

There are several possible immunological mechanisms of tissue injury which include: immediate hypersensitivity; direct lysis of cells by antibody and complement; the recruitment of inflammatory cells by antigen-antibody complexes; the direct lysis of cells by T lymphocytes; the recruitment of inflammatory cells by T lymphocytes; and the antibody-dependent cellular cytotoxicity reaction whereby cell lysis occurs through the specific interaction between target cell-bound antibody and K cells. To define the specific mechanisms involved in SFV encephalitis, passive transfer experiments were performed. The results show that both humoral and cellular immune mechanisms are involved in the full expression of the pathological process.

## MATERIALS AND METHODS

**Animals.** Female CBA/H mice were used at 4 to 6 weeks of age. Nude mice (nu/nu) were derived from (BALB/c × CBA)$F_1$ hybrids and were used at 6 to 7 weeks of age. All strains were bred at the Animal Breeding Establishment of the John Curtin School of Medical Research.

**Virus.** The avirulent strain of SFV was provided by H. Smith, Department of Microbiology, University of Birmingham, Birmingham, England. The virus has been passaged eight times in infant mice and twice in chicken embryo cells. The working stock virus preparation is a 10% (wt/vol) suspension of infected infant mouse brain and has a titer of $10^{8.0}$ plaque-forming

units (PFU) per ml. The virus was titrated by a plaque assay on Vero cell monolayers, as previously described (16), except that infected monolayers were incubated for 2 days.

Stocks of avirulent Hampstead egg strain ectromelia virus (13) were obtained from the infected chorioallantoic membranes of 14-day-old chicken embryos. The working stock virus preparation has a titer of $10^{8.7}$ PFU per ml. The virus was titrated by a plaque assay on L929 cells, as previously described (3).

**Immunofluorescence.** Brains were immediately frozen by immersion in liquid $N_2$. Sections of 6 $\mu$m were cut on a cryostat, thoroughly dried, and fixed in acetone for 10 min. After further drying, sections were stained for 30 min with fluorescein-conjugated mouse immunoglobulins specific for SFV antigens. The specimens were then washed for 20 min with phosphate-buffered saline and mounted in neutral glycerol for examination and photography.

**Medium.** Eagle minimal essential medium (MEM) with nonessential amino acids (GIBCO Laboratories, Grand Island, New York, catalog no. F15) containing 10% fetal calf serum (FCS) (Commonwealth Serum Laboratories, Melbourne, Australia), referred to as MEM 10% FCS, was used in preparation of spleen cell suspensions.

**Preparation of immune spleen cells.** Spleens were removed from CBA/H mice which had been infected intraperitoneally (i.p.) 6 days previously with $10^{6.0}$ PFU of SFV. Spleens were placed in MEM 10% FCS and then pressed through stainless-steel sieves. After dissociation of clumps by pipetting, the cells were washed twice in MEM 10% FCS. Viability of the final suspensions as assessed by trypan blue (0.5%) exclusion was usually between 75 and 90%. The yield from one spleen was from $1 \times 10^8$ to $2 \times 10^8$ viable cells. Multiple spleens were assayed for infectious virus and none could be detected. Spleen cells from normal mice were used as controls.

**Antitheta serum.** Antithy-1.2 F7DS monoclonal immunoglobulin M (IgM) antibody was kindly provided by Phil Lake, University College, London, England. Microcytotoxicity tests against spleen cells showed a titer of 1:2,560 for the preparation. Six-day donor immune spleen cells were suspended in a 1:500 dilution of antitheta antibody and incubated on ice for 30 min. The cells were then washed twice in MEM, resuspended in a 1:2 dilution of guinea pig complement in MEM, and incubated at 37°C for 15 min.

**Separation of $Ig^+$ and $Ig^-$ lymphocytes.** Immunoglobulin-bearing cells ($Ig^+$) were separated from non-immunoglobulin-bearing ($Ig^-$) cells by the method of Parish et al. (22). Essentially, mouse spleen cells were rosetted with sheep erythrocytes coated, via $CrCl_3$, with sheep IgG which was specific for mouse immunoglobulin. The cells were centrifuged on Isopaque-Ficoll, and the $Ig^+$ rosettes were treated with 0.87% $NH_4Cl$ to remove erythrocytes. Between $2 \times 10^7$ and $2.5 \times 10^7$ $Ig^-$ cells per spleen were recovered, and 2% of the apparently $Ig^-$ population were found to possess immunoglobulin receptors as determined by retesting the number of immunoglobulin-rosetting cells. The recovery of $Ig^+$ cells per spleen was between $4 \times 10^7$ and $5 \times 10^7$ cells, and 5% of these were theta positive as tested by their susceptibility to lysis by antitheta serum plus complement.

**Adoptive transfer experiments.** Donors of immune spleen cells were immunized i.p. with $10^6$ PFU of SFV, and the spleens were harvested 6 days later. The cells were then fractionated into various populations. The number of donor cells of each fraction transferred represented the approximate number of cells of that category recovered per spleen after fractionation.

Recipients were infected i.p. with $10^{4.7}$ PFU of SFV on day 0 and given a single i.p. injection of cyclophosphamide (150 mg/kg) on day 1 postinfection. They then received selected populations of immune spleen cells intravenously (i.v.) on day 3. All animals were then sacrificed on day 7. Control groups always included mice which received SFV on day 0 and cyclophosphamide on day 1. Adoptive transfers using other experimental designs are described in their appropriate sections.

**Anti-SFV serum.** CBA/H mice were given three i.p. injections of $10^6$ PFU of SFV. The second injection was given 2 weeks after the first, and the third injection was given 1 week later. All animals were bled 7 days after the last injection; the sera were pooled, heat inactivated at 56°C for 30 min, and stored in portions at −20°C. In repeated assays, the SFV neutralization titer was 1:2,560.

**Cyclophosphamide.** Cyclophosphamide (Endoxan-Asta), obtained from Bristol Laboratories, Crows Nest, N.S.W., Australia, was given to mice i.p. at a dose of 150 mg per kg of body weight.

**Sampling techniques.** Mice were anesthetized with chloroform and exsanguinated. The sera were collected, heat inactivated at 56°C for 30 min, and stored at −20°C for antibody determination. The brain was then removed and cut midsagittally. One half of the brain was stored at −70°C for virus assay; the other half was fixed in 10% formalin saline for histological examination.

**Hemagglutination-inhibiting (HI) antibody titration.** The Takatsy microtechnique (26) was adapted to the general methods of Clarke and Casals (9). SFV hemagglutinins were prepared by sucrose-acetone extraction of infected infant mouse brain. Pooled sera were extracted with acetone-ether and absorbed with gander erythrocytes before testing for HI antibodies. Endpoints were taken at the level of 100% agglutination.

**Histology.** Brains were embedded in paraffin, sectioned sagittally at four levels, and stained with hematoxylin and eosin. The slides were coded and examined in a blind fashion.

**Statistical methods.** Virus titers are expressed as mean ± standard error of mean, $\log_{10}$ for the groups of mice. Because the lower limit of sensitivity of the plaque assay was 50 PFU per g of brain, all individual values of zero were calculated at 40 PFU per g of brain or $10^{1.6}$. Groups were compared by the Student $t$ test.

## RESULTS

**Kinetics of viral replication and pathology in the brains of normal, nude, and cyclophosphamide-treated mice after primary avirulent SFV infection.** Nude and CBA mice were inoculated i.p. with $10^{5.0}$ PFU of SFV. Half of the CBA mice were then given a

single dose of cyclophosphamide (150 mg/kg) i.p. 24 h postinfection. On days 1, 3, 5, 7 ... 21, 28, and 35 postinfection, four mice from each group were sacrificed; the brains were removed and cut midsagittally. One half of the brain was used for viral titration, and the other was used for histopathology. For nude mice, the study was carried out to only 9 days postinfection.

No clinical signs or mortality were noted in any mouse throughout the study. In all three groups the brain virus titers rose rapidly within 24 h postinfection, reaching higher maximal titers in the nude and cyclophosphamide-treated mice than in normal mice (Fig. 1). In both normal and nude mice, viral titers began to decline on day 5. In normal mice, they fell to undetectable levels by day 7. However, virus titers in nude mice tapered off on day 7 without complete clearing by day 9. Viral clearance was delayed in the cyclophosphamide-treated group until day 11, falling to undetectable levels by day 15.

Pathology in the infected normal mice was first noted on day 5 postinfection and was characterized by a mononuclear perivascular inflammation as well as focal accumulations of mononuclear cells within the brain parenchyma. By 7 days postinfection, cystic lesions appeared within the brain in association with mononuclear inflammatory cells and reactive astrocytes (Fig. 2A). These lesions, representing a spongiform degeneration, were associated with minimal neuronal degeneration and could be found throughout the brain, although they occurred most frequently in the brain stem and cerebellum (Fig. 2B, C). The cystic areas in the white matter were at times confluent, appearing as large foci (Fig. 2D). The inflammatory spongiform degeneration was maximal between 7 and 21 days postinfection and had resolved over the ensuing 2 weeks.

The initial changes in the brains of infected nude mice were found on day 5 with focal accumulations of mononuclear inflammatory cells, followed on day 7 by an inflammatory spongiform degeneration (Fig. 3). However, the intensity of the perivascular cuffing, focal mononuclear infiltrates, and associated spongiform changes were less prominent than lesions in normal, infected mice. Inflammation did not appear in the brains of cyclophosphamide-treated, infected mice until day 9, with spongiform lesions developing by day 11. When the lesions appeared in these mice, the inflammation and cystic changes were more intense than in normal or nude mice.

**Immunofluorescent study of the brains of normal and immunosuppressed mice after primary infection with SFV.** One group of CBA mice received an i.p. inoculation of $10^{6.0}$ PFU of SFV. A second group was given an i.p. inoculation of $10^{6.0}$ PFU of SFV followed 24 h later by a single i.p. dose of cyclophosphamide (150 mg/kg). Two mice were sacrificed on days 1, 3, 5, and 7 postinfection. The brains were removed and cut midsagittally; one half of each brain was frozen in liquid $N_2$ for immunofluorescent study, and the other half was fixed in formalin for histopathology.

The normal, infected mice failed to exhibit viral antigens in their brains at any time throughout the study. However, pathology was present which was identical both in temporal sequence and in appearance to that described previously.

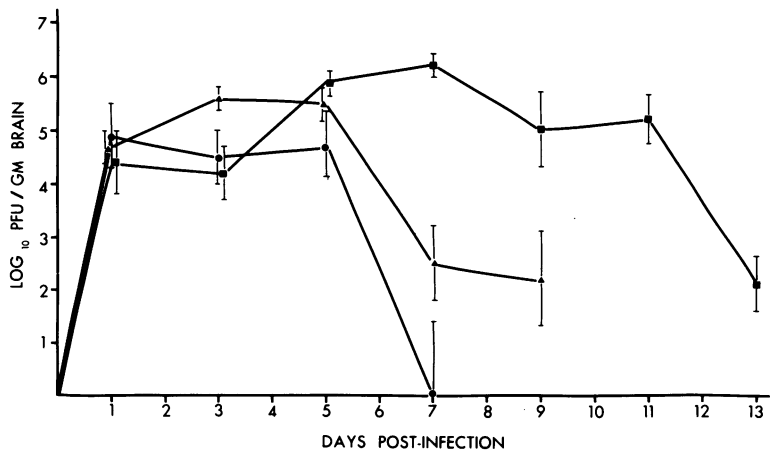


FIG. 1. *Infectivity of mouse brains after i.p. inoculation of $10^{5.0}$ PFU of SFV in CBA (●), nude (▲), and CBA mice given cyclophosphamide (150 mg/kg) i.p. 24 h after infection (■). Each point represents the mean ± standard deviation from four mice.*

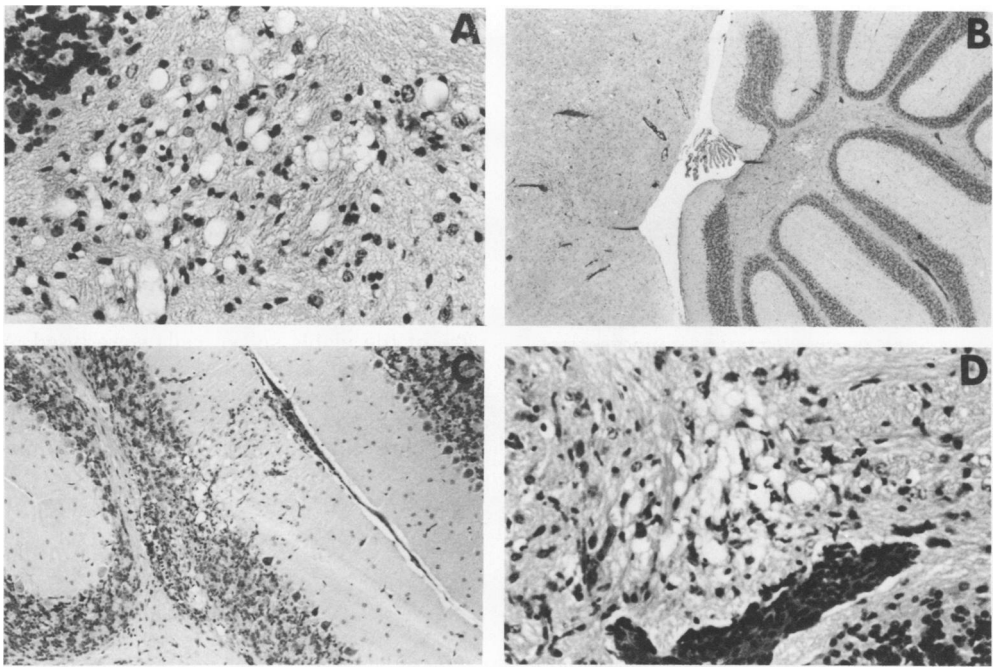


FIG. 2. *Sections of mouse brain taken 7 days after i.p. inoculation with $10^{5.0}$ PFU of SFV. (A) Spongiform lesions in cerebellar white matter associated with mononuclear inflammatory cells and reactive astrocytes (×250); (B) focal cystic lesions in cerebellum and perivascular inflammation within the adjacent brain stem (×32); (C) inflammatory spongiform lesions involving molecular, Purkinje, and granule cell layer of cerebellum (×125); (D) large confluent cystic lesion in cerebellum (×250).*

Fluorescent foci were first seen in the brains of immunosuppressed, infected mice beginning on day 5. The foci consisted of small groups of neurons which could be found within the cerebral hemispheres, brain stem, and Purkinje layer of the cerebellum. Viral antigen was not limited to the cytoplasm of infected cells but could be demonstrated on the dendrites and myelinated axons as well (Fig. 4). Fluorescence was never observed in the meninges, choroid plexus, ependyma, or glial cells. The pattern of fluorescence on day 7 was similar to that on day 5, with only small groups of neurons and their processes exhibiting viral antigen. Pathology was absent in the brains of the immunosuppressed, infected mice throughout the 7 days of observation.

In the previous section, it was found that brain virus titers reached a higher maximum in cyclophosphamide-treated mice than in normals. In some arbovirus encephalitides, it has been shown that the amount of viral antigen in the brain is directly proportional to the virus titer (8, 10). Therefore, the demonstration of fluorescent foci only in the brains of cyclophosphamide-treated mice is probably a reflection of the greater amount of viral antigen present.

**Effect of transfer of immune spleen cells on brain pathology in SFV encephalitis.** Recipient mice were given $10^{4.7}$ PFU SFV on day 0, cyclophosphamide (150 mg/kg) i.p. on day 1, and either $10^8$ immune cells or $10^8$ normal cells on day 3. All mice were sacrificed on day 7.

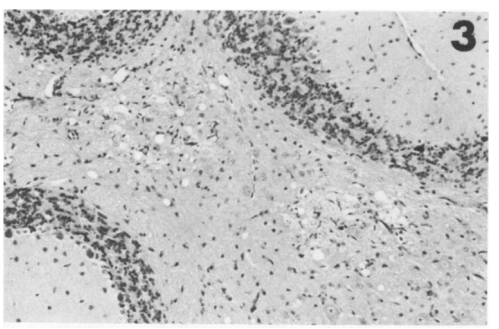


FIG. 3. *Section of nude mouse cerebellum taken 7 days after i.p. inoculation with $10^{5.0}$ PFU of SFV. The typical inflammatory spongiform lesions are present in association with degenerating neurons (×125).*

The characteristic inflammatory spongiform degeneration could be found within the brains of immune-cell recipients. Antiviral antibody was present in their serum, and significantly lower titers of virus were present in their brains

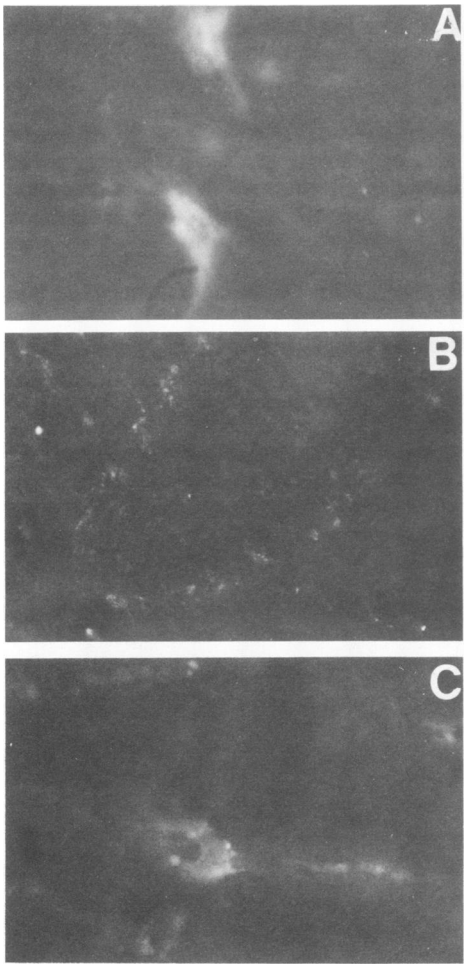


FIG. 4. *Sections of brain from CBA mice on day 5 after having received $10^6$ PFU of SFV on day 0 and cyclophosphamide (150 mg/kg) i.p. on day 1. The preparations were stained with fluorescein-conjugated mouse anti-SFV globulin. (A) Fluorescent (infected) neuron and its process within the cerebral hemisphere (×625); (B) some fluorescent Purkinje cells within the cerebellum (×100); (C) single Purkinje cell with fluorescence of the dendrite within the molecular layer (right), the cytoplasm of the cell body, and the myelinated axon projecting into the granule cell layer (left) (×625).*

than in recipients of normal cells (Table 1). The latter group had no brain pathology or detectable serum antibody. The degree of inflammation and spongiform changes in immune cell recipients was identical to that of the lesions in infected, normal controls.

**Effect of transfer of immune sera.** Recipient mice were infected i.p. with $10^{4.7}$ PFU of SFV on day 0 and given cyclophosphamide (150 mg/kg) i.p. on day 1. They then received 0.3 ml i.v. of either immune or normal sera on day 3. All mice were sacrificed on day 7.

Only immune sera reduced the brain virus titers in immunosuppressed, infected mice (Table 2). Even though there was significant antiviral activity, the brains of immune-serum recipients did not show lesions.

**Effect of transfer of immune spleen cells depleted of T lymphocytes.** Treatment of immune spleen cells with antitheta serum plus guinea pig complement results in the specific lysis of T lymphocytes (24). With this treatment, 30% of the viable spleen cells were lysed. As a control, equal numbers of immune spleen cells were treated with normal mouse serum (NMS) plus complement.

Recipient mice were infected with SFV on day 0 and given cyclophosphamide on day 1. They

TABLE 1. *Pathology, virus titers, and serum antibody in recipients[a] of immune spleen cells*

| Treatment | No. of mice | Brain virus titer[b] | Serum HI antibody titer[c] | Brain pathology[d] |
|---|---|---|---|---|
| Control | | | | |
| SFV only | 8 | 1.7 ± 0.1 | 1:160 | 7 |
| SFV + cyclophosphamide | 8 | 5.6 ± 0.2 | <1:10 | 0 |
| Cells | | | | |
| Immune cells | 10 | 2.6 ± 0.3[e] | 1:80 | 7 |
| Normal cells | 8 | 5.0 ± 0.3 | <1:10 | 0 |

[a] Recipients were given SFV i.p. on day 0, cyclophosphamide i.p. on day 1, and cell transfer i.v. on day 3; they were killed on day 7.

[b] Expressed as mean $\log_{10}$ PFU per gram of brain ± standard error of the mean.

[c] Antibody titer determined from pooled sera.

[d] Number of mice with focal inflammatory spongiform lesions.

[e] Significantly less than control group receiving virus plus cyclophosphamide ($P < 0.001$).

TABLE 2. *Pathology, virus titers, and serum antibody in recipients[a] of immune sera*

| Treatment | No. of mice | Brain virus titers[b] | Serum HI antibody titer[c] | Brain pathology[d] |
|---|---|---|---|---|
| Control | | | | |
| SFV only | 5 | 1.6 ± 0.0 | 1:160 | 5 |
| SFV + cyclophosphamide | 4 | 4.9 ± 0.3 | <1:10 | 0 |
| Serum transfer | | | | |
| Immune sera | 6 | 1.9 ± 0.4[e] | 1:80 | 0 |
| Normal sera | 6 | 5.6 ± 0.3 | <1:10 | 0 |

[a-e] See Table 1.

were then given either $7 \times 10^7$ immune spleen cells that had been treated with either antitheta serum or NMS, plus complement.

The brains of recipient mice developed inflammatory spongiform lesions whether or not the immune cells were depleted of T lymphocytes (Table 3). However, the inflammation and cystic changes were greater in the recipients of NMS-treated immune cells and infected, normal controls. Both recipient groups also had reduced brain virus titers, although the antiviral activity was greater in the donor immune cell population that contained T lymphocytes. Serum antiviral antibody titers were lower in recipients that had received immune cells depleted of T lymphocytes. This finding could be attributed to a reduction in helper T-lymphocyte activity in the donor cell population (7).

**Effect of $Ig^-$ and $Ig^+$ immune cells on the pathological process.** Immune mouse spleen populations were separated into $Ig^+$ cells and $Ig^-$ cells. The $Ig^+$ cell population consists mainly of B lymphocytes, whereas the $Ig^-$ population is comprised of T lymphocytes, macrophages, polymorphs, and stem cells (22). Recipient mice were given SFV on day 0, cyclophosphamide on day 1, and either $3 \times 10^7$ immune $Ig^-$ cells or $3 \times 10^7$ $Ig^+$ cells i.v. on day 3. All animals were sacrificed on day 7.

Recipients of immune $Ig^-$ cells showed inflammatory spongiform lesions as well as reduced viral titers within their brains (Table 4). These pathological changes were less intense than in infected, normal controls. The absence of serum antiviral antibody is consistent with B-lymphocyte depletion from the donor population. The $Ig^+$ donor cells did not produce lesions, nor did they significantly reduce brain virus titers. The reduced amount of serum antiviral antibody in recipient mice could again be due to reduced helper T-lymphocyte activity in the $Ig^+$ donor population.

**Effect of transfer of immune spleen cells depleted of both T and B lymphocytes.** The experimental design consisted of dividing equal numbers of 6-day immune spleen cells into three groups. One group of immune cells was separated into $Ig^-$ and $Ig^+$ fractions. Twenty-five percent of the viable cells were recovered in the $Ig^-$ fraction consisting of immune cells depleted of B lymphocytes only. Another group of immune cells was pretreated with antitheta antibody in the absence of complement and then separated into $Ig^-$ and $Ig^+$ fractions. The antitheta antibody is a monoclonal IgM antibody which binds to theta antigen on T lymphocytes, and thus provides T lymphocytes with a surface immunoglobulin. Therefore, like the B lymphocyte, the T lymphocyte becomes an $Ig^+$ cell. The IgM antibody did not bind to macrophages since complement was not present in the incubation mixture (19). Nine percent of the viable cells were recovered in the $Ig^-$ fraction which was comprised of immune cells depleted of both T and B lymphocytes. A third group included an

TABLE 4. *Pathology, virus titers, and serum antibody in recipients[a] of $Ig^-$ or $Ig^+$ immune spleen populations*

| Treatment | No. of mice | Brain virus titers[b] | Serum HI antibody titer[c] | Brain pathology[d] |
|---|---|---|---|---|
| Control | | | | |
| SFV only | 4 | 1.6 ± 0.0 | 1:160 | 4 |
| SFV + cyclophosphamide | 4 | 5.9 ± 0.3 | <1:10 | 0 |
| Cell transfer | | | | |
| $Ig^-$ immune cells | 7 | 2.6 ± 6[e] | <1:10 | 5 |
| $Ig^+$ immune cells | 6 | 4.8 ± 0.3 | 1:20 | 0 |

[a-d] See Table 1.

[e] Significantly less than control group receiving virus plus cyclophosphamide ($P < 0.01$).

TABLE 3. *Pathology, virus titers, and serum antibody in recipients[a] of immune spleen cells depleted of T lymphocytes*

| Treatment | No. of mice | Brain virus titers[b] | Serum HI antibody titer[c] | Brain pathology[d] |
|---|---|---|---|---|
| Controls | | | | |
| SFV only | 4 | 1.6 ± 0.0 | 1:160 | 4 |
| SFV + cyclophosphamide | 4 | 5.8 ± 0.1 | <1:10 | 0 |
| Cell transfer | | | | |
| Immune cells treated with antitheta antibody + complement | 4 | 4.2 ± 0.4[e] | 1:40 | 3 |
| Immune cells treated with NMS + complement | 3 | 2.0 ± 0.3[f] | 1:160 | 3 |

[a-d] See Table 1.

[e] Significantly less than control groups receiving virus plus cyclophosphamide ($P < 0.01$).

[f] Significantly less than control groups receiving virus plus cyclophosphamide ($P < 0.001$).

unfractionated population of immune cells. The number of donor cells transferred from each group represented the number of cells recovered per spleen after fractionation.

Recipient mice, given SFV on day 0 and cyclophosphamide on day 1, received either $10^8$ unfractionated immune spleen cells (equivalent to one spleen), $2.5 \times 10^7$ immune cells depleted of B lymphocytes only, or $10^7$ immune cells depleted of both T and B lymphocytes i.v. on day 3. All mice were sacrificed on day 7.

Recipients of whole immune cells or immune cells depleted of B lymphocytes only had brain pathology and reduced virus titers (Table 5). Immune cells depleted of both T and B lymphocytes did not reconstitute lesions or clear virus from the brain.

One explanation for the negative findings after transfer of immune cells depleted of both T and B lymphocytes could be a dilution effect. That is, the number of cells transferred was too low to have an effect within the brain. In this study, it was shown that $2.5 \times 10^7$ $Ig^-$ immune spleen cells could reconstitute pathology. In preliminary studies, treatment with antitheta serum plus complement demonstrated that $1.5 \times 10^7$ of these cells were T lymphocytes, and $10^7$ cells were not. Therefore, if it is assumed that immune cells depleted of T and B lymphocytes could alone produce pathology and the antiviral activity, then $10^7$ of these cells must have been responsible for the brain lesions in recipients of immune cells depleted of B lymphocytes only. From the results of the present experiment (Table 5), this does not appear to be the case. A more probable explanation for the findings is that either T or B lymphocytes must be present in the donor cell population in order to produce the pathology.

**Transfer studies with immune sera plus BM cells.** Bone marrow (BM) cells were aspirated from the femurs of normal, uninfected CBA mice. The cells were then treated with antitheta antibody plus complement to remove T lymphocytes.

Recipient mice were infected i.p. with SFV on day 0 and given cyclophosphamide on day 1. They then received $4 \times 10^7$ normal BM cells i.v. on day 4 followed 8 h later by 0.3 ml i.v. of immune sera. All mice were sacrificed on day 7. Control groups included infected immunosuppressed recipients receiving BM cells plus normal sera, BM cells only, or immune sera only.

Recipients receiving immune sera had reduced brain virus titers, but only the group receiving BM cells in addition to immune sera demonstrated the mononuclear inflammation and spongiform degeneration (Table 6). The pathological changes were less marked than in infected, normal controls.

**Effect of transfer of SFV-immune cells on CNS pathology in ectromelia-infected recipients.** To determine the specificity of the CNS pathology induced by SFV-immune cells, recipients were infected with an unrelated neurotropic virus. Intracerebral (i.c.) infection of mice with the Hampstead egg strain of ectromelia virus, a poxvirus, results in a CNS infection. The pathological changes are primarily immune-mediated (17).

Recipient mice were infected with either $10^5$ PFU of ectromelia virus i.c. or $10^5$ PFU of SFV i.p. on day 0. Cyclophosphamide was given on day 1, and both groups received $10^8$ SFV immune spleen cells on day 3. All mice were sacrificed on day 7.

The brains from mice infected 7 days previously with SFV demonstrated focal inflammatory spongiform lesions. They occurred primarily in the cerebellum and brain stem. Ectromelia-infected brains showed a mononuclear inflammation involving the meninges, superficial cortical layers, choroid plexus, ependyma, and subependymal areas. Focal spongiform lesions were absent. Treatment with cyclophosphamide 24 h after virus inoculation prevented pathology in both infections (Table 7). Reconstitution of immunosuppressed, SFV-infected mice with SFV immune cells produced inflammatory spongiform lesions. However, immunosuppressed ec-

TABLE 5. *Pathology, virus titers, and serum antibody in recipients[a] of immune spleen cells depleted of T and B lymphocytes*

| Treatment | | Brain pathology[d] | | |
|---|---|---|---|---|
| Controls | | | | |
| SFV only | 5 | 1.9 ± 0.3 | 1:80 | 4 |
| SFV + cyclophosphamide | 4 | 5.0 ± 003 | <1:10 | 0 |
| Cell transfer | | | | |
| Whole immune cells | 5 | 2.6 ± 0.3[e] | <1:80 | 4 |
| Immune cells depleted of B lymphocytes | 4 | 3.2 ± 0.3[f] | <1:10 | 3 |
| Immune cells depleted of T and B lymphocytes | 5 | 4.5 ± 0.4 | <1:10 | 0 |

[a-e] See Table 1.

[f] Significantly less than control group receiving virus plus cyclophosphamide ($P < 0.01$)

TABLE 6. *Pathology, virus titers, and serum antibody in recipients[a] of immune sera plus BM cells*

| Treatment | No. of mice | Brain virus titer[b] | Serum HI antibody titer[c] | Brain pathology[d] |
|---|---|---|---|---|
| Control | | | | |
| SFV only | 4 | 2.3 ± 0.3 | 1:160 | 4 |
| SFV + cyclophosphamide | 6 | 6.0 ± 0.2 | <1:10 | 0 |
| Passive transfer | | | | |
| BM only | 4 | 5.7 ± 0.1 | <1:10 | 0 |
| Immune sera | 5 | 2.2 ± 0.3[e] | 1:160 | 0 |
| Immune sera + BM | 6 | 2.5 ± 0.4[e] | 1:80 | 4 |
| Normal sera + BM | 4 | 5.6 ± 0.4 | <1:10 | 0 |

[a-e] See Table 1.

tromelia-infected mice reconstituted with SFV immune cells lacked pathology. The reduction in brain virus titers by the immune cells was also virus specific (Table 7).

## DISCUSSION

Inflammatory spongiform lesions appeared in the brains of CBA mice 7 days after i.p. inoculation of SFV. When the CBA mice were given a single dose of cyclophosphamide i.p. 24 h postinfection, there was a delay in the appearance of lesions until day 11. Immune-mediated damage was suggested by the appearance of lesions in normal CBA and cyclophosphamide-treated mice at a time when virus was being cleared from the brain (Fig. 1). Furthermore, on day 7, brain virus titers were 4 logs higher in cyclophosphamide-treated mice than normal CBA, yet lesions did not appear in the former until day 11. Immunofluorescence studies did demonstrate viral antigen within the soma and processes of neurons in the cyclophosphamide-treated mice on day 7. Thus, direct viral cytolysis does not play an important role in the production of pathology.

The reconstitution of brain pathology in immunosuppressed, infected recipients on day 7 postinfection with immune spleen cells established the immunological nature of the lesions. Furthermore, tissue damage did not occur when ectromelia-infected recipients were reconstituted with SFV-immune cells. This suggests that at least the initiation of the immunopathology is virus specific.

Inflammatory spongiform lesions appeared in the brains of nude mice 7 days after i.p. inoculation of SFV. Nude mice are lacking in functional T lymphocytes (18). However, the early synthesis of IgM is unimpaired and is maximal on day 7 after SFV infection (6). Thus, an antibody-mediated process independent of T cells did produce pathology. This is further supported by the reconstitution of lesions in immunosuppressed, infected mice with immune cells deficient in T lymphocytes. Adoptive transfers with either the $Ig^+$ immune fraction (an enriched population of B lymphocytes) or antibody alone were unsuccessful. When normal BM cells were injected along with antibody, mononuclear inflammation with associated spongiform degeneration appeared in recipient brains. The mononuclear cells or their precursors must reside in the spleen and bone marrow as well as in nude mice. These cells are most likely to be of the monocyte-macrophage lineage. They can be recruited into infected tissue through the formation of antibody-antigen complexes and activation of the complement pathway. Ectromelia and chronic lymphocytic choriomeningitis virus infections in mice are examples where antibody, independent of T cells, can induce mononuclear inflammatory lesions which are histologically similar to T-cell-mediated lesions (4, 21). Another possibility is that some of the mononuclear infiltrate is K cells. An antibody-dependent cellular cytotoxicity reaction is more commonly mediated by IgG binding to the target cells (23).

TABLE 7. *Effect of passive transfer of SFV-immune cells on pathology and virus titers in SFV-infected and ectromelia-infected recipients*

| Treatment[a] | Brain virus titer[b] | Brain pathology (no. of mice) |
|---|---|---|
| Ectromelia-infected recipients | | |
| Ectromelia only | 3.5 ± 0.2 | 4[c] |
| Ectromelia + cyclophosphamide | 5.5 ± 0.3 | 0 |
| Ectromelia + cyclophosphamide + SFV-immune cells | 5.6 ± 0.3 | 0 |
| SFV-infected recipients | | |
| SFV only | 1.6 ± 0.0 | 4[d] |
| SFV + cyclophosphamide | 5.8 ± 0.3 | 0 |
| SFV + cyclophosphamide + SFV-immune cells | 2.8 ± 4[e] | 3[d] |

[a] Recipients (four mice per group) were given either ectromelia i.c. or SFV i.p. on day 0 and killed on day 7. Cyclophosphamide was given i.p. on day 1 and SFV-immune cells were given on day 3.

[b] Expressed as mean $\log_{10}$ PFU per gram of brain ± standard error of the mean.

[c] Characterized by mononuclear inflammation of meninges, superficial cortical layers, choroid plexus, ependyma, and subependymal areas.

[d] Characterized by focal inflammatory spongiform lesions in brain parenchyma.

[e] Significantly less than control group receiving SFV plus cyclophosphamide ($P < 0.02$).

Therefore, this mechanism would be less likely to occur in nude mice, where IgM presumably initiates the pathological process.

Conversely, adoptive transfer studies have demonstrated a role for T lymphocytes in the production of pathology independent of antibody production. Recipients of $Ig^-$ immune spleen cells, a fraction depleted of B lymphocytes, had inflammatory spongiform lesions without detectable serum antiviral antibody. The T lymphocytes could directly lyse infected tissue or recruit circulating monocytes into the infected areas through the secretion of lymphokines.

The findings discussed here suggest that humoral and cellular immune responses can independently produce qualitatively similar brain lesions during the course of SFV infection, probably through the recruitment of a common effector cell (monocyte-macrophage) into infected brain. Monocyte recruitment is immunologically mediated because adoptive transfer with spleen cells depleted of both T and B lymphocytes did not reconstitute lesions. If monocytes became activated macrophages within infected brain, they could produce spongiform necrosis by direct lysis of infected cells or indirectly through the release of hydrolytic enzymes (11, 12). There remains the possibility that other cells, such as K cells or cytotoxic T lymphocytes, could act as effector cells in the immunopathogenesis.

The findings presented here are not unique. For example, in studies on experimental autoimmune thyroiditis, adoptive transfer and *H-2* restriction of thyroid damage have demonstrated that either T lymphocytes or antibody can induce lesions (2, 28). Similarly, the present study has demonstrated that both humoral and cellular immunological mechanisms may play a role in the evolution of CNS pathology that occurs during the course of avirulent SFV infection in mice.

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