Immunology 1981 **43** 81

Natural killer cells appear to play no role in the recovery of mice from Sindbis virus infection

R. L. HIRSCH *Howard Hughes Medical Institute Laboratories, Department of Neurology, The Johns Hopkins University School of Medicine, Baltimore, Maryland, U.S.A.*

Accepted for publication 11 November 1980

Summary. Previous studies have suggested that non-specific defence mechanisms may be important in the development of age-dependent resistance to fatal Sindbis-virus infection and in the recovery of adult mice from non-fatal infection. In these studies, natural killer (NK) cell induction was studied in 7-day-old susceptible mice and 28–35-day-old resistant mice. It was found that Sindbis virus infection induced NK cells in both the young and older mice, suggesting that NK cells were not important in the acquisition of resistance to fatal Sindbis-virus infection. Transfer of 10^8 lymph node cells from adult mice, at the peak of NK cell activity, did not protect young mice from fatal infections, supporting the *in vitro* findings.

The pathogenesis of Sindbis virus infection in C57BL/6J bg/bg (NK-cell deficient) and bg/+ (NK-cell normal) mice was also studied. Despite a defect in the induction of NK cells by Sindbis virus infection in the bg/bg mice, there were no significant differences in the pathogenesis of either peripheral or intracerebral infection in these strains of mice. These studies suggest that although NK cells are induced, they may not be important in the recovery of mice from Sindbis virus infection.

Abbreviations: NK, natural killer; CEF, chick embryo fibroblasts; BHK, baby hamster kidney; SV, Sindbis virus; CEF-SV, SV grown in CEF; BHK-SV, SV grown in BHK cells; EMCV, encephalomyocarditis virus; LCMV, lymphocytic choriomeningitis virus; bg/bg, beige mutant of the C57BL/6J mouse strain; bg/+, mice heterozygote for the beige mutant of the C57BL/6J strain; CNS, central nervous system; p.f.u., plaque forming units; LNC, lymph node cells; FCS, foetal calf serum; HBSS, Hanks's balanced salt solution; E/T, effector to target cell ratio; i.c., intracerebral; i.p., intraperitoneal; f.p., foot pad; IF, interferon.

Correspondence: Dr R.L. Hirsch, Howard Hughes Medical Institute Laboratories, Department of Neurology, The Johns Hopkins University School of Medicine, Baltimore, Md 21205, U.S.A.

0019-2805/81/0500-0081$02.00

INTRODUCTION

Sindbis virus, an alphavirus of the togavirus group, causes an acute encephalitis in mice in which mortality is age dependent (Johnson, McFarland & Levy, 1972). Mice develop complete resistance to fatal encephalitis between 2 and 3 weeks of age (Griffin, 1976). The mechanisms by which animals acquire resistance with maturation are not understood. Specific immunological responses, including lymphoproliferative and antibody responses, appear to be the same in susceptible and resistant mice (Griffin, 1976)' Some aspects of non-specific defence mechanisms have also been studied. During infections with Sindbis virus it has been shown that interferon levels are higher in the brains of younger, susceptible animals than in older, resistant mice (Vilcek, 1964; Reinarz, Broome, & Sagik, 1971) suggesting that interferon does not play a protective role in the development of age-dependent resistance.

The clearance of virus from the blood stream and its localization in the organs of the reticuloendothelial cell system of susceptible and resistant animals has been studied at one time point after intracardiac inoculation (Hackbarth, Reinarz & Sagik, 1973). the results of this study suggest that virus clearance is more efficient in the adult resistant animals than in the younger susceptible mice but does not explain the development of resistance to both peripheral and intracerebral injection at the same age (Johnson *et al.*, 1972; Griffin, 1976).

Although it is not clear which non-specific factors may be important in the acquisition of age-dependent resistance, studies of Sindbis virus infection of adult, athymic nude mice have shown that the specific cellular immune response, manifest by the mononuclear inflammatory response in the brains of normal mice (McFarland, Griffin & Johnson, 1972; Hirsch & Griffin, 1979) is not necessary for clearance of virus from the central nervous system (Hirsch & Griffin, 1979). These studies in nude mice suggested that either antibody alone and/or non-specific factors might be important in limiting virus growth in the central nervous system (CNS) of adult mice.

Previous studies by others have shown that natural killer (NK) cells are elicited after virus infection (MacFarlan, Burns & White, 1977; Welsh, 1978). In addition, virus-induced NK cells, as well as endogenous NK-cell activity, are at lower levels in suckling mice than in weanling mice (Welsh, 1978; Herberman, Nunn & Lavrin, 1975a). Since the development of age-dependent resistance to fatal Sindbis-virus encephalitis parallels the acquisition of NK-cell function in mice, and since we have shown that athymic, nude mice, which possess high levels of endogenous NK cells (Herberman, Nunn, Holden & Lavrin, 1975b), recover from Sindbis virus infection as well as normal mice, the present studies were performed to evaluate the induction of and role of NK cells in recovery from Sindbis virus infection.

MATERIALS AND METHODS

Animals

BALB/c mice were obtained from Charles River Animal Facilities (Wilmington, Mass.). C3H/HeJ and C57BL/6J bg/+ or bg/bg mice were obtained from Jackson Laboratories (Bar Harbor, Maine). The specific ages of mice used in each particular experiment are indicated in the Results section.

Virus

Sindbis virus (strain AR339) was grown on either primary cultures of chick embryo fibroblasts (CEF) or baby hamster kidney cells (BHK-21). Virus grown in CEF and BHK cells was designated CEF-SV and BHK-SV, respectively. Stock virus preparations contained 10^8 and 10^7 plaque forming units (p.f.u.) per ml for the CEF-SV and BHK-SV, respectively.

Inoculations

In the study of natural-killer-cell activity in draining lymph nodes, animals were inoculated in four foot pads with 10^5 p.f.u. CEF-SV diluted in Hanks's balanced salt solution (HBSS) without Ca++ and MG++. Control animals received foot pad inoculations of HBSS. In the study of the pathogenesis of Sindbis virus infection in the C57BL/6J bg/+ and bg/bg mice, animals received 500 p.f.u. CEF-SV in HBSS containing 5% foetal calf serum (FCS) in each hind foot pad or 1000 p.f.u. BHK-SV in 0·03 ml HBSS containing 5% FCS intracerebrally.

Lymphocyte preparation

Axillary, brachial, and popliteal lymph nodes were removed three days after foot pad inoculation and teased apart with 26 gauge needles. Lymph node cells (LNC) were washed three times with HBSS without Ca++ and Mg++ and resuspended at various concentrations ($1{\cdot}25 \times 10^7$ to $1{\cdot}25 \times 10^5$ cells/ml) in RPMI-1640 containing 5% FCS, 10 mM HEPES buffer, and 30 μg/ml gentamicin.

Modification of effector-cell populations

Cells were resuspended in RPMI containing 20% FCS and adherent cells were removed from LNC by two different methods. Cells were incubated at 37° on plastic culture dishes or passed through columns of Sephadex G-10, and non-adherent and eluted cells were removed by standard techniques (Kumagi, Itoh, Hinuma & Tada, 1979; Ly & Mishell, 1974). Phagocytic cells were removed from LNC by carbonyl iron (GAF Corp., New York,N.Y.)—magnet treatment, as previously described (Golstein & Bolmgren, 1973).

Three different antibody preparations were used to deplete LNC of particular cell populations. A standard AKR anti-C3H Thy-1·2 sera (Miles Laboratories) and an IgM monoclonal anti-Thy-1·2 (New England Nuclear) were used to deplete T cells. B cells were depleted with a rabbit anti-mouse IgG (H & L chain specific) antiserum (Cappel Laboratories). Fresh guinea-pig serum, absorbed with normal BALB/c

lymph node cells, was used as a source of complement. The efficacy of antiserum killing of T-and B-cell populations was determined by assessing PHA (Difco, 10 μg/10^6 cells) or LPS (0:55 BS, Difco, 5 μg/10^6 cells) responsiveness using standard tritiated-thymidine incorporation techniques in lymphocyte transformation assays (Hirsch & Griffin, 1979).

Cytotoxicity assay

Target cells, L-929 (L cells) or 3T3 cells, were plated the day before the assay in ninety-six well flat-bottom microtitre plates (Falcon No. 3042). The next day the target cells ($2{\cdot}5 \times 10^4$/well) were infected with CEF-SV at a virus to cell ratio of 10. After 1 hr at 37°, virus was removed and target cells were labeled with 2 μCi of $Na_2{}^{51}CrO_4$ (Amersham) per well as described previously (Hirsch, Griffin & Johnson, 1979). After labelling, target cells were washed three times with HBSS and 0·2 ml aliquots of effector cells in RPMI-1640 were placed in each well. Four to six replicates were performed for each experiment at each effector to target (E/T) cell ratio employed. The assay was allowed to proceed for 16 hr at which time supernatant fluids were harvested and counted in a Nuclear Chicago gamma spectrometer (Model 1185). The percentage chromium (^{51}Cr) released from target cells was determined as described previously (Hirsch *et al.*, 1979). When the ratio of the spontaneous release to the total isotope incorporated exceeded 0·3 the entire experiment was discarded.

Virus assays

The amount of infectious virus in individual organs or culture supernatants was determined by plaque assay on monolayers of BHK-21 cells as described previously (Hirsch & Griffin, 1979).

Interferon assays

Levels of interferon (IF) in tissue homogenates were determined as previously described (Reinarz *et al.*, 1971) except that encephalomyocarditis virus (EMCV) was used as a challenge virus. IF levels are expressed as the dilution of tissue homogenate which resulted in a 50% reduction in the number of EMCV p.f.u. on L cells.

Histology

Brains of mice were processed for histology (hematoxylin and eosin staining) and the inflammatory response was graded under code, as described previously (Hirsch & Griffin, 1979).

RESULTS

Sindbis virus infection of mice generates NK-cell activity

Recent studies have demonstrated that *in vitro* incubation of normal mouse leukocytes with Sindbis virus-infected L-929 cells generates NK-cell activity (Welsh & Hallenback, 1980). Therefore, preliminary studies were performed to determine if Sindbis virus infection of mice could elicit NK-cell cytotoxicity. After foot pad (f.p.) inoculation of BALB/c or C3H/HeJ mice with Sindbis virus, LNC were obtained and tested for cytotoxic activity against non-infected and Sindbis virus, infected H-2 identical or non-identical target cells. Effector cells lysed both Sindbis virus-infected

Table 1. Cytolytic capacity of LNC obtained 3 days after Sindbis virus infection

		% ^{51}Cr release by targets			
		L cells (H-2^k)		3T3 cells (H-2^d)	
Source of effector cells	Animal treatment	I	NI	I	NI
BALB/c (H-2^d)	CEF-SV	22·5	26·7	13·4	19·8
	control	3·3	5·6	6·6	0·3
C3H/HeJ (H-2^k)	CEF-SV	20·3	18·8	12·9	10·5
	control	0	1·0	1·8	1·3

*Animals were inoculated 3 days previously in four foot pads with CEF-SV or HBSS. LNC were processed as described in Material and Methods. Each value represents mean from six replicate wells. Results are from values obtained at an E/T of 50.

†I and NI designate infected and non-infected targets, respectively.

and non-infected targets. Furthermore, LNC obtained from either strain of mice were cytolytic for both H-2 identical and non-identical target cells (Table 1). LNC did not lyse uninfected Vero cells. Although it appears that LNC from BALB/c mice were less efficient in lysing syngeneic than allogeneic targets, this can be explained by the fact that spontaneous release of ^{51}Cr was higher for the 3T3 target cells. Further studies showed consistent cytotoxic activity present in LNC populations obtained from infected BALB/c mice (Table 2). Thus, LNC from BALB/c mice were used as effector cells throughout the remainder of these studies and non-infected L cells were utilized as target cells.

Studies were performed to determine the requirements for the cytotoxic activity observed, as well as the nature of the effector cells eliciting the cytotoxicity. Experiments were performed to evaluate the possibility that antibody may have mediated the observed cytotoxicity. LNC from infected and control mice were tested for cytotoxicity against non-infected L cells (E/T = 50) in the presence of FCS, FCS absorbed for 2 hr at 4° on monolayers of non-infected L cells, or in the absence of serum. Cytotoxicity in the presence of absorbed FCS (19·8%) was not significantly lower than cytotoxicity in the presence of unabsorbed FCS (22·5%). In the absence of serum cytotoxicity was reduced to 11·8%, possibly reflecting the absence of a nutritional factor provided by serum. Cytotoxicity of LNC from control mice in the presence of FCS, absorbed FCS, and in the absence of FCS was 8·0%, 5·1% and −1 6%, respectively, suggesting that antibody to target cells did not play a significant role in the cytotoxicity. Finally, our previous studies (Hirsch *et al.*, 1979) showed that addition of exogenous mouse anti-Sindbis-virus antibody did not enhance cytotoxicity by sensitized or normal spleen cells or LNC.

Table 2. Cytotoxic activity of LNC obtained from BALB/c mice*

	% ^{51}Cr Release		
E/T	Infected mice	Control mice	$P <$ †
100	20·5 ± 4·2 (8)	10·6 ± 5·4 (8)	0·05
50	26·7 ± 4·2 (13)	10·4 ± 4·0 (13)	0·005
10	11·9 ± 5·4 (8)	4·2 ± 3·6 (8)	NS

*BALB/c mice were infected 3 days previously with 10^5 p.f.u. CEF-SV in HBSS. Control mice received HBSS only. Cytotoxic activity was measured against non-infected L-cell targets. Values represent mean ± standard error. Numbers in parentheses indicate number of individual paired experiments.

†Differences between the cytotoxic activity generated by LNC from infected and control mice were compared by Student's *t* test.

Supernatants obtained after the incubation of 3-day LNC on L cells for 16 hr were not cytotoxic. Furthermore, virus was not detectable in these supernatants and thus the cytotoxicity observed could not be due to carry over of virus by infected lymph node cells with subsequent infection of susceptible target cells.

The majority of the cytotoxic activity was associated with the non-adherent and non-phagocytic cell fraction, was non-Ig bearing and, was not depleted by treatment with a monoclonal anti-Thy-1·2 antibody reagent (Table 3). However, consistent with previous studies on endogenous NK cells in BALB/c mice (Herberman *et al.*, 1975b; Mattes Sharrow, Herberman & Holden, 1979), a standard anti-Thy-1·2 antisera removed some of the cytotoxic activity. These results suggest that Sindbis virus infection of mice, like other toga viruses (MacFarland *et al.*, 1979), induces NK-cell activity.

NK-cell activity in susceptible and resistant mice

The above studies strongly suggested that Sindbis virus infection induced NK-cell activity in adult BALB/c mice. To determine whether the induction of these early defence cells might play a role in the age-dependent acquisition of resistance to fatal Sindbis-virus infection, NK-cell induction in 7-day-old susceptible mice was compared with that in 35-day-old resistant mice after foot pad infection with Sindbis virus (Table 4). Young as well as older animals produced significant levels of NK-cell activity after infection with Sindbis virus. On a cell-to-cell basis LNC from young mice may have even been more effective in killing of targets than LNC obtained from adult mice. However, since there are fewer LNC available in the younger mice, it is possible that an individual young mouse infected with Sindbis virus may not have sufficient NK cells present to kill the infected cells and subsequently eliminate the infection early enough to prevent fatal encephalitis. Thus, transfer studies were performed in which LNC (10^8) obtained from adult mice at the peak of NK-cell activity, 3 days after f.p. inoculation, were given to 7-day-old mice one day after subcutaneous inocula-

Table 3. Effect of selective removal of LNC populations of cytotoxicity*

Cell treatment	% ^{51}Cr release	
	Before treatment	After treatment
Anti-Thy-1·2 (1/10) + GPS†	29·0	18·9
Anti-Thy-1·2 (1/1000) + GPS‡	25·3	32·5
Anti-IgG + GPS§	29·0	30·2
GPS only (1/10)	25·3	28
GPS only (1/10)	29·0	31·6
Adherence to plastic	24·6	23·4
Adherence to sephadex G-10	36·7	29·8
Carbonyl Iron-Magnet	44·0	33·0
Carageenan	16·0	15·8

*LNC were obtained from 4-week-old BALB/c mice 3 days after f.p. inoculation. For antiserum treatment cells, (1×10^7/ml in HBSS), were incubated for one hr at 4° with the indicated antibody. The cells were washed and GPS, absorbed previously with normal LNC, was added for 45 min at 37°. The cells were washed, cell concentration corrected for dead cells, and tested for cytotoxicity against non-infected L-cell targets at an E/T of 50. the other cell treatments were performed as described in Materials and Methods. Control LNC cytotoxicity was 9·0% or less in each experiment.

†Commercial (Miles) AKR/J anti C3H/HeJ Thy-1·2 at 1/10 final dilution.

‡Commercial (New England Nuclear) monoclonal IgM anti-C3H/HeJ Thy-1·2 at 1/1000 final dilution.

§Commercial (Cappel) rabbit anti-mouse IgG (H and L chain) at 1/10th final dilution.

Table 4. Natural cytotoxicity in suckling and weanling BALB/c mice*

E/T	% ^{51}Cr release			
	Adult		Young	
	SV-infected mice†	control mice	SV-infected mice	control mice
100/1	22·2	6·5	40·0	7·5
50/1	18·1	4·3	27·1	3·9
10/1	9·0	0·2	11·6	5·1
1/1	1·5	1·4	1·3	−3·1

*Animals were inoculated in four foot pads with CEF-SV at 7 days (young) or 35 days (adult) of age and NK activity assessed 3 days later against non-infected L-cell target. The data shown is representative of three experiments.

†Interferon levels (dilution causing 50% inhibition of EMCV p.f.u.) in pooled foot homogenates were 1/6 and 1/18 for adult and young mice, respectively.

tion with 1000 p.f.u. CEF-SV. Seventy-eight percent (7/9) of control mice receiving virus only died. One hundred percent (8/8) of mice receiving CEF-SV sensitized LNC plus virus and 71% (5/7) of mice receiving normal LNC plus virus died. Thus, transfer of these cell populations did not protect mice from fatal infection.

Role of NK cells in recovery of adult mice from Sindbis virus infection

The previous studies suggested that NK cells were not important in the acquisition of age-dependent resistance to fatal Sindbis-virus encephalitis of mice. However, it could not be determined from these studies if NK cells were important in the recovery from infection in the adult mouse. Since our previous studies showed that adult nude mice, animals possessing high levels of endogenous NK cells, were resistant to fatal Sindbis-virus infection, in the absence of lymphoproliferative and IgG-antibody responses to Sindbis virus, we studied the importance of NK cells in recovery from infection in the adult animal. In these studies the beige (bg/bg) mutation of the C57BL/6J mice was used. This animal has been shown previously to have impaired endogenous NK-cell activity (Roder, 1979). Furthermore, NK cell activity in these animals is poorly stimulated by interferon or interferon inducers *in vivo* or *in vitro* (Roder, 1979).

Preliminary studies were performed to determine whether Sindbis virus infection induced NK-cell activity in bg/bg mice. LNC obtained from bg/bg mice 3 days after f.p. inoculation demonstrated cytotoxic activity against non-infected L-cell targets (11·6% E/T = 50), however control LNC exhibited a similar degree of cytotoxicity (13·3%). LNC from inoculated bg/+ mice (32·5%) exhibited significantly greater cytotoxicity than LNC from uninoculated bg/+ mice (12·4%). Since infection did not induce NK cells in bg/bg mice, the pathogenesis of infection after both peripheral and intracerebral inoculation was compared in bg/bg and bg/+ mice.

After footpad inoculation of virus there was no difference in the local growth of virus in the foot of the bg/bg mouse and the heterozygote, NK-cell normal, bg/+ mouse. Similarly virus in the blood was detected only on day 1 after inoculation and virus appeared transiently in the brain, but was cleared by day 10 in both the homozygote bg/bg mouse and the heterozygote bg/+ normal mouse (Fig. 1).

These studies suggested that NK cells may not play a role in the control of local virus replication in the muscle. However, since the viraemia was low in magnitude and only small quantities of virus were detectable in the brain, further studies were performed to determine the effect of the NK-cell defect on growth of virus in the CNS. Mice of each strain were inoculated intracerebrally with 10^3 p.f.u. BHK-SV and separate groups of animals were used to assess morbidity and mortality and virus growth in the brain. The i.c. infection was avirulent in both groups of animals and no differences in virus growth were observed in the brains of bg/bg and bg/+ mice (Fig. 2). Furthermore, there were no differences in the mononuclear inflammatory response in the brains 4, 6, and 8 days after infection.

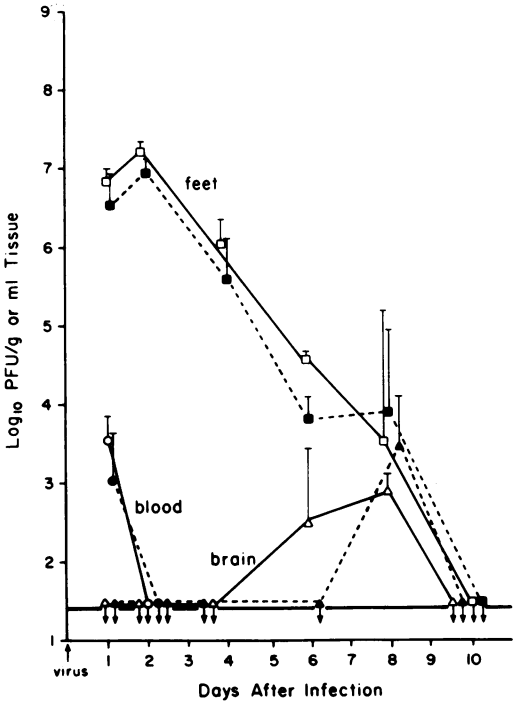

Figure 1. Levels of virus in the blood, feet and brains of C57BL/6J-bg/bg (●,■,▲) and C57BL/6J-bg/+ (○,□,△) mice after f.p. inoculation. Animals were inoculated with 500 PFU CEF-SV in each hind foot pad. At the indicated times, blood, feet, and brains from three mice in each group were assayed individually for virus content. Although not shown on the graph, virus was not detected in the blood after day 1. The solid line at 1·4 represents the lower limit of virus detection and inverted arrows represent the inability to detect virus at the indicated time. Vertical bars represent the standard deviation.

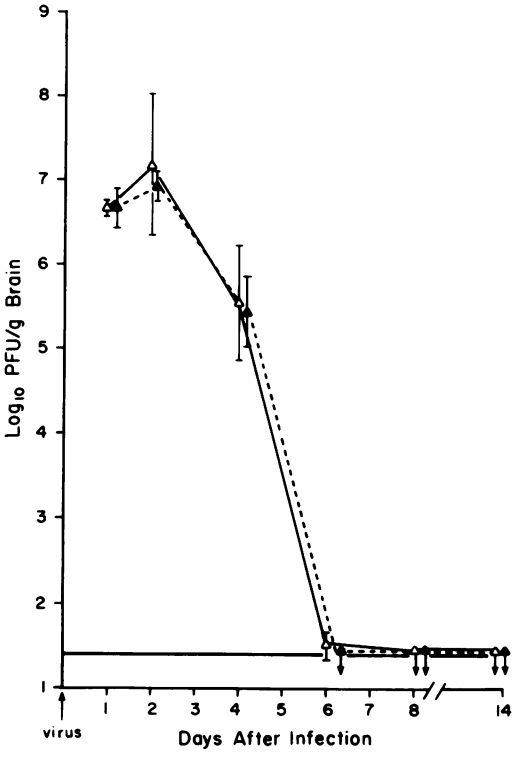

Figure 2. Levels of virus in the brains of C57BL/6J- bg/bg (▲–▲) and C57BL/6J-bg/+ (▽–△) mice after i.c. inoculation. Animals were inoculated with 1000 PFU BHK-SV and levels of virus in the brains of individual mice was assessed at the indicated times. The solid line at 1·4 represent the lower limit of virus detection and inverted arrows represent the inability to detect virus. Vertical bars represent the standard deviation.

DISCUSSION

These studies have shown that NK cells are induced by Sindbis virus infection but do not appear to play a significant role in the development of age-dependent resistance or normal recovery from SV infection in adult mice. It was found that the properties of the cytotoxic cells elicited 3 days after Sindbis virus infection were similar to the properties of NK cells induced after other virus infections of mice, including infections with Semliki forest or Kunjin virus (MacFarlane *et al.*, 1977), two other viruses of the togavirus group. The cells responsible for the cytotoxic activity were non-Ig bearing and the majority of the cytotoxic activity was present in the non-Thy-1·2 antigen-bearing cell population. As judged by two methods, the NK-cell activity was present in the non-adherent cell fraction. A portion of the cytotoxic activity, however, may have been due to the presence of phagocytic cells. This is consistent with a previous report on cytotoxic cells elicited after Semliki forest virus infection in mice (Rodda & White, 1976). It is also possible that the carbonyl-iron treatment may have non-specifically removed cells binding, but not phagocytosing, the iron. Removal of non-phagocytic cells has been reported to be a problem associated with this technique (Golstein & Blomgren, 1973).

Since the acquisition of resistance to fatal Sindbis-virus infection parallels the development of endogenous NK-cell activity, it was hypothesized that NK cells may be an important early defence mechanism in determining resistance. To evaluate this hypothesis NK-cell activity was assessed in young, susceptible mice and older, resistant mice. These studies showed that Sindbis virus infection induced NK-cell activity in both young and adult mice, and that NK-cell activity of LNC from young mice was equal to or greater than that in adult mice. Studies on the induction of NK cells during lymphocytic choriomeningitis virus (LCMV) infection of mice also showed that younger animals developed NK-cell activity, but it was approximately 30% lower than that of the 5-week-old mice (Welsh, 1978). However, since LCMV is known to infect lymphocytes, while Sindbis virus does not, this may interfere with NK cell induction in the younger animal. In addition, the fact that LCMV induces much greater NK-cell activity (Welsh, 1978) may also account for the differences between LCMV and Sindbis virus induction of NK-cell activity in young mice. The induction of NK-cell activity in young mice has not been studied in other virus infections.

Although endogenous NK cells have recently been shown to be involved in tumour rejection *in vivo* (Talmadge, Meyers, Prieur, & Starkey, 1980; Karre, Klein, Kiessling, Klein & Roder, 1980) the role that virus-induced NK cells play *in vivo* is not well understood. Welsh & Kiessling (1980) have recently shown that LCMV growth in the spleens of bg/+ and bg/bg mice is not significantly different. The present studies show that NK cells probably do not participate in recovery from Sindbis virus infection. This was inferred in three ways. First, as noted, above, both young, susceptible animals and older, resistant animals developed NK-cell activity after Sindbis virus infection. Secondly, transfer of 10^8 LNC obtained from 4-week-old mice at the peak of NK activity, did not alter the course of fatal Sindbis-virus encephalitis

in 1-week-old mice. It should be noted however, that positive controls for protection are not included in these studies and therefore, the conclusion that NK cells are not important in the development of age-dependent resistance must be considered tentative.

Studies of Sindbis virus infection in the bg/+ and bg/bg mutant of the C57Bl/6J mouse, showed no differences in the pathogenesis of the infection after s.c. inoculation. Similarly, the intracerebral infection was non-lethal in both strains of mice and no differences in growth of the virus in the CNS were observed. Thus, recovery from both a low-virus-dose challenge of the CNS, as a result of s.c. infection, and a relatively high-virus-dose challenge (i.c. infection) did not depend upon the induced NK-cell response. Finally, *in vitro* studies showed that unlike the bg/+ mouse, Sindbis virus infection did not result in increased NK-cell activity in the bg/bg mouse. This result is similar to that observed for LCMV infection, which showed a deficient LCMV induced NK-cell response in bg/bg mice when L cells, rather than tumour cells, were employed as targets (Welsh & Kiessling, 1980; Talmadge *et al.*, 1980). Thus, it would appear, that for Sindbis virus infection of mice, NK cells are not critical for recovery from infection. The role that NK cells may play in the defence against other virus infections is not known.

ACKNOWLEDGMENTS

I wish to thank Drs Diane Griffin and Richard Johnson for helpful discussions and critical review of this manuscript. I also thank Norman Karasanyi for technical assistance and Linda Kelly for preparation of the manuscript.

RLH is a Research Associate of the Howard Hughes Medical Institute. This work was supported in part by USPHS grant No. NS 15749.

REFERENCES

GOLSTEIN P. & BLOMGREN, H. (1973) Further evidence for autonomy of T cells mediating specific *in vitro* cytotoxicity: efficiency of very small amounts of highly purified T cells. *Cell. Immunol.* **9,** 127.

GRIFFIN D.E. (1976) Role of the immune response in age-dependent resistance of mice to encephalitis due to Sindbis virus. *J. Infect. Dis.* **133,** 456.

HACKBARTH S.A., REINARZ A.B.G. & SAGIK B.P. (1973) Age-dependent resistance of mice to Sindbis virus infection: reticuloendothelial role. *J. Reticuloendothel. Soc.* **14,** 405.

HERBERMAN R.B., NUNN M.E. & LAVRIN D.H. (1975a) Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. I. Distribution of reactivity and specificity. *Int. J. Cancer,* **16,** 216.

HERBERMAN R.B., NUNN M.E., HOLDEN H.T. & LAVRIN D.H. (1975b) Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumours. II. Characterization of effector cells. *Int. J. Cancer,* **16,** 230.

HIRSCH R.L. & GRIFFIN D.E. (1979) The pathogenesis of Sindbis virus infection in athymic nude mice. *J. Immunol.* **123,** 1215.

HIRSCH R.L., GRIFFIN D.E. & JOHNSON R.T. (1979) Interactions between immune cells and antibody in protection from fatal Sindbis virus encephalitis. *Infect. Immunity,* **23,** 320.

JOHNSON R.T., MCFARLAND H.F. & LEVY S.E. (1972) Age-dependent resistance to viral encephalitis: studies of infections due to Sindbis virus in mice. *J. infect. Dis.* **125,** 257.

KARRE K., KLEIN G.O., KIESSLING R., KLEIN G. & RODER J.C. (1980) Low natural in vivo resistance to syngenic leukaemias in natural killer-deficient mice. *Nature,* **284,** 624.

KUMAGI K., ITOH K., HINUMA S & TADA M. (1979) Pretreatment of plastic petri dishes with fetal calf serum. A simple method for macrophage isolation. *J. immunol. Meth.* **29,** 17.

LY I.A. & MISHELL R.I. (1974) Separation of mouse spleen cells by passage through columns of Sephadex-G10. *J. immunol. Meth.* **5,** 239.

MACFARLAN R.I., BURNS W.H. & WHITE D.C. (1977) Two cytotoxic cells in peritoneal cavity of virus infected mice: Antibody dependent macrophages and nonspecific killer cells. *J. Immunol.* **119,** 1569.

MCFARLAND H.F., GRIFFIN D.E. & JOHNSON R.T. (1972) Specificity of the inflammatory response in viral encephalitis. I. Adoptive immunization of immunosuppressed mice infected with Sindbis virus. *J. exp. Med.* **136,** 216.

MATTES J.M., SHARROW S.O., HERBERMAN R.B. & HOLDEN H.T. (1979) Identification and separation of Thy-1 positive mouse spleen cells active in natural cytotoxicity and antibody dependent cell-mediated cytotoxicity. *J. Immunol.* **123,** 2851.

REINARZ A.B.G., BROOME M.G. & SAGIK B.P. (1971) Age-dependent resistance of mice to Sindbis virus infection: viral replication as a function of host age. *Infect. Immunity,* **3,** 268.

RODDA S.J. & WHITE D.O. (1976) Cytotoxic macrophages: A rapid-non-specific response to viral infection. *J. Immunol.* **117,** 2069.

RODER J.C. (1979) The beige mutation in the mouse. I. a stem cell predetermined impairment in natural killer cell function. *J. Immunol.* **123,** 2168.

TALMADGE J.E., MEYERS K.M., PRIEUR D.J. & STARKEY J.R. (1980) Role of NK cells in tumour growth and metastasis in beige mice. *Nature (Lond.),* **284:** 622.

VILCEK J. (1964) Production of interferon by newborn and

adult mice infected with Sindbis virus. *Virology*, **22,** 631.

WELSH R.M. (1978) Cytotoxic cells induced during lymphocytic chriomeningitis virus infection of mice. I. Characterization of natural killer cell induction. *J. exp. Med.* **148,** 163.

WELSH R.M. & HALLENBECK L.A. (1980) Effect of virus infections on target cell susceptibility to natural killer cell-mediated lysis. *J. Immunol.* **124,** 2491.

WELSH R.M. & KIESSLING R.W. (1980) Natural killer cell response to lymphocytic choriomeningitis virus in beige mice. *Scand. J. Immunol.* **11,** 363.