# Host Defense Mechanisms Against Murine Cytomegalovirus Infection Induced by Poly I:C in Severe Combined Immune Deficient (SCID) Mice (43806)

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*Abstract.* **The role of host defense mechanism against murine cytomegalovirus (MCMV) infection in mice with severe combined immunodeficiency (SCID) was investigated using polyinosinic:polycytidylic acid (poly I:C) as a nonspecific stimulator of the immune system. When administered ip at doses of 3.7 or 15 mg/kg 18 hr prior to infection of SCID mice with $10^3$ or $10^4$ plaque-forming units of MCMV, poly I:C significantly increased the animals' life span. Poly I:C enhanced, in a dose-dependent manner (0.01–1 mg/kg), the peritoneal natural killer (NK)-cell activity and macrophage activity of SCID mice. When SCID mice were pretreated with anti-asialo $GM_1$ antibody (against NK cells) or anti-$Mac_1$ antibody (against macrophages), poly I:C failed to stimulate the activity of NK cells and macrophages. Intraperitoneal administration of poly I:C also induced both early (2 hr) and late (18 hr) type interferon (IFN) in the peritoneal fluid and blood. The IFN-inducing activity of polyI:C was not affected by pretreatment of the mice with anti-asialo $GM_1$ or anti-$Mac_1$ antibody. Poly I:C also caused a significant but less pronounced increase in the life span of MCMV-infected SCID mice in which the NK cells or macrophages had been depleted by treatment with anti-asialo $GM_1$ or anti-$Mac_1$ antibody, respectively. These results suggest that poly I:C-induced interferon as well as activation of NK cell and macrophages contribute to the host defense mechanism against MCMV infection in SCID mice.**

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Human cytomegalovirus (HCMV) causes serious infections in immunocompromised hosts such as Acquired Immuno Deficiency Syndrome (AIDS) patients, transplant recipients, and cancer patients under immunosuppressive therapy (1–3). In these patients, the lymphocyte, macrophage, and natural killer (NK) cell counts decrease, resulting in a higher susceptibility to various infectious agents. HCMV also leads to severe clinical manifestations in the fetus or neonate (4, 5). Congenitally athymic-nude mice, NK cell-defective mice, newborn mice, and acquired immunosuppressed mice (which have been treated with cyclophosphamide) are more susceptible to MCMV infection than their heterozygous littermates or nonimmunosuppressed adult mice (6–9). We recently developed a model for MCMV infection in severe combined immune deficient (SCID) mice (10). This model mimics CMV disease progression in the immunocompromised host more closely than the commonly used animal models. Since SCID mice lack functional B- and T-cell immunity, it may be useful to unravel the nonspecific defense mechanisms of the host against MCMV infection.

A number of immunomodulating substances that offer nonspecific protection against a variety of infectious agents including viruses, bacteria, and fungi have been described (11–15). One such adjuvant, the dou-

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ble-stranded (ds) RNA polyinosinic:polycytidylic acid (poly I:C) is a potent inducer of IFN (16, 17). Modulatory effects of synthetic dsRNA on both humoral and cellular immune response have also been reported (18).

Kern *et al.* (19) have demonstrated that poly I:C and poly I:C/poly lysine complex (poly I:C/LC) elicit prophylactic activity against MCMV infection in adult Swiss Webster mice. Recently, Adams *et al.* (20) reported protective activity of polyadenylic:polyuridylic acid (polyA:U) against acute MCMV infection in BALB/c mice. However, the mechanism by which this occurs has not been clarified. In general, NK cells are considered to play a key role in the early defense mechanism, and cytotoxic T cells in the late defense mechanism, against acute MCMV infection (21–24).

The present study was aimed at investigating the early defense mechanism against MCMV infection in SCID (BALB/c scid/scid) mice, following treatment of these mice with polyI:C.

## Materials and Methods

**Animals.** The animals used were 5- to 6-week-old SCID mice (BALB/c CB-17 scid/scid inbred strain); males and females were used at random. The mice were bred at the Rega Institute under germ-free conditions and were housed under specific pathogen-free conditions during the experiments.

**Virus.** Stocks of MCMV (ATCC VR 194) were prepared from 10% homogenates of salivary glands of NMRI mice that had been infected with a sublethal dose of MCMV. Virus was titrated on primary murine embryo fibroblast cells (MEF) and stored at −80°C until used.

**Agents and Antisera.** Polyinosinic (poly I) and polycytidylic acid (poly C) were obtained from P-L Biochemicals Inc. (Milwaukee, WI). Polymerization of poly I and poly C (poly I:C) was carried out according to the method described previously (25). Rabbit anti-asialo $GM_1$ serum and rat monoclonal anti-$Mac_1$ antibody were purchased from Wako Chemical Ltd. (Osaka, Japan) and Serotec (Oxford, UK), respectively.

**Determination of Protective Activity Against MCMV Infection.** Protective activity against MCMV was assessed by scoring the survival of the mice (10). Poly I:C was administered ip at the doses indicated in the tables. Mice were infected 18 hr later with $10^3$ or $10^4$ plaque-forming units (PFU) of MCMV per 0.2 ml Eagle's Minimum Essential Medium (MEM). The mice were observed daily and the results are expressed as the mean day of death (MDD) ± standard deviation (SD) for five mice per group.

**Determination of NK Cell Activity.** Peritoneal cells obtained from SCID mice which had been given an ip injection of poly I:C 18 hr earlier were incubated for 4 hr at 37°C with [$^{51}$Cr] (sodium chromate, 3.7MBq; Amersham, England)-labeled YAC-1 cells in an effector:target ratio of 25:1 (26). Assays were run in triplicate. To determine spontaneous release of radioactivity, 100-μl aliquots of the culture medium were added to cultures without effector cells. A complete release of the radioactivity was measured by lysing target cells alone with 100-μl aliquots of 0.1% Nonidet P40. The radioactivity released in supernatants was measured by an autogamma scintillator. The specific release of radioactivity (specific lysis) was calculated according to the following formula, where cpm are counts per minute: specific lysis (%) = [(cpm of tested groups − cpm of the spontaneous release/(cpm of the complete release − cpm of the spontaneous release)] × 100. Results are expressed as mean percent lysis ± SD of triplicate cultures.

**Determination of Macrophage Activity.** Phagocytic activity of macrophages was determined as described in a previous paper (26). Briefly, peritoneal cells obtained from SCID mice which had received poly I:C ip 18 hr earlier were incubated for 2 hr at a concentration of $5 \times 10^5$ cells/well in 24-well plates. After washing the wells twice with MEM, nonadherent cells were removed and the resulting adherent cells were used as macrophage source. [$^{51}$Cr]-labeled and antibody-sensitized sheep erythrocytes ([$^{51}$Cr]-labeled EA) suspended in RPMI-1640 medium containing 10% FCS were added to each well ($2 \times 10^7$ EA/0.5 ml/well). After incubation for 1 hr at 37°C, cells were treated with 0.85% ammonium chloride Tris-HCl buffer (pH 7.6) to lyse [$^{51}$Cr]-labeled EA attached on the surface of macrophages. The radioactivity of [$^{51}$Cr]-labeled EA taken into macrophages was then determined. Phagocytic activity is expressed as mean cpm ± SD of triplicate cultures.

**Determination of IFN-Inducing Activity.** IFN-Inducing activity was assessed by measuring IFN titers in peritoneal fluid and serum, expressed as reciprocals of the highest dilution of peritoneal fluid and sera that reduced vesicular stomatitis virus-induced cytopathic effect in L-929 cells by 50% as described previously (27). Briefly, groups of three SCID mice were injected ip with poly I:C at doses ranging from 0.01 to 10 mg/kg. Peritoneal fluids and blood were taken 2 hr and 18 hr after induction. IFN titers are expressed as international reference units (IU) using standard IFN-α/β (kindly donated by Dr. H. Heremans [Rega Institute, Belgium]) and the results are represented as the mean of two experiments carried out independently.

**Treatment with Antibody.** To deplete NK cells or macrophages in SCID mice, 0.2 ml of anti-asialo $GM_1$ (1:30) or anti-$Mac_1$ (1:30) antibody was administered ip to the mice (28). One day after treatment with antibody, poly I:C (1 mg/kg) was administered. Eigh-

teen hours after administration of poly I:C, peritoneal NK cell and macrophage activities were determined. Protective tests against virus infection were performed using mice which had been treated with antibody (48 hr and 2 hr before infection) and poly I:C (18 hr before infection).

**Statistics.** All results are expressed as the mean ± SD, and differences between control and treated groups were evaluated by the two-tailed Student's *t* test.

## Results

**Protective Activity of Poly I:C Against MCMV-Induced Mortality in SCID Mice.** When SCID mice were challenged ip with $1 \times 10^3$ PFU or $1 \times 10^4$ PFU of MCMV, the mice developed a wasting syndrome (with ruffled fur) within 10–11 days ($10^3$ PFU) or 4–5 days ($10^4$ PFU) after infection, and died a few days later (mean day of death [MDD]: 16.0 ± 0.6 [$10^3$ PFU] and 9.4 ± 1.8 [$10^4$ PFU]) (Table I). The life span of MCMV-infected mice was significantly increased when they had been treated ip with poly I:C at 3.7 or 15 mg/kg at 18 hr before virus challenge. No adverse effects due to the poly I:C treatment were noted the first 10–12 days postinfection. Thereafter the animals became sick, however, due to progression of the MCMV disease.

**Activation of NK Cell by Poly I:C in SCID Mice.** NK cells are considered to play a key role in the early defense against MCMV infection (22, 24, 29). We investigated whether poly I:C enhances the NK cell activity of peritoneal cells in SCID mice. As shown in Figure 1, administration of poly I:C (0.01, 0.1, or 1 mg/kg) significantly increased the NK cell activity. Furthermore, when the NK cell pool was depleted by *in vivo* treatment with anti-asialo $GM_1$ antibody, poly I:C failed to restore NK activity (Fig. 2). However, when SCID mice were pretreated with anti-$Mac_1$ antibody (a specific monoclonal antibody to macrophages), poly I:C induced stimulation of NK cell activity to a similar extent as in the control.

**Table I.** Protective Activity of Poly I:C Against MCMV Infection in SCID Mice

| Agent[a] | Dose (mg/kg) | Mean day of death[b] (MDD ± SD) |
|---|---|---|
| Experiment 1 | | |
| Virus control ($1 \times 10^4$ PFU) | | 9.4 ± 1.8 |
| PolyI:C | 3.7 | 17.6 ± 3.4[c] |
| | 15 | 20.4 ± 0.5[d] |
| Experiment 2 | | |
| Virus control ($1 \times 10^3$ PFU) | | 16.0 ± 0.6 |
| PolyI:C | 15 | 21.8 ± 0.4[d] |

[a] Poly I:C was administered ip at the indicated doses 18 hr before ip infection with $1 \times 10^3$ or $1 \times 10^4$ PFU of MCMV.
[b] The results are expressed as the mean day of death (MDD) ± standard deviation (SD) for five mice per group.
[c] $P < 0.01$, as compared with virus control group (two-tailed Student's *t* test).
[d] $P < 0.001$.

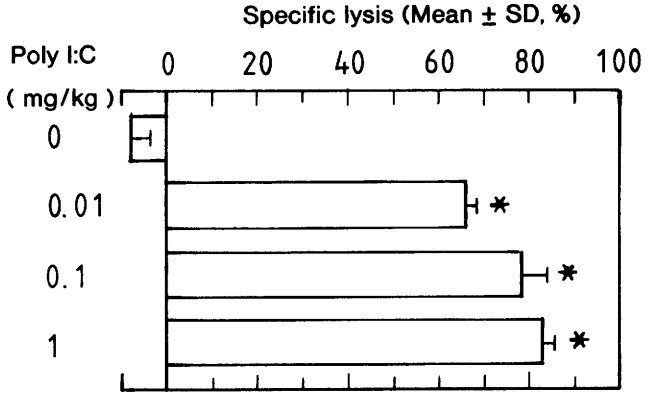


**Figure 1.** Activation of NK-cell activity of SCID mice by poly I:C. Peritoneal NK cells were obtained from SCID mice which had been treated ip with the indicated doses of poly I:C 18 hr before the assay (three mice/group). Cytolytic activity of the NK cells was assessed based on the radioactivity released from target [$^{51}$Cr]-labeled YAC-1 cells. Results are expressed as mean lysis (%) ± SD of triplicate cultures. *$P < 0.001$, as compared with the peritoneal NK cells from untreated mice (two-tailed Student's *t* test).

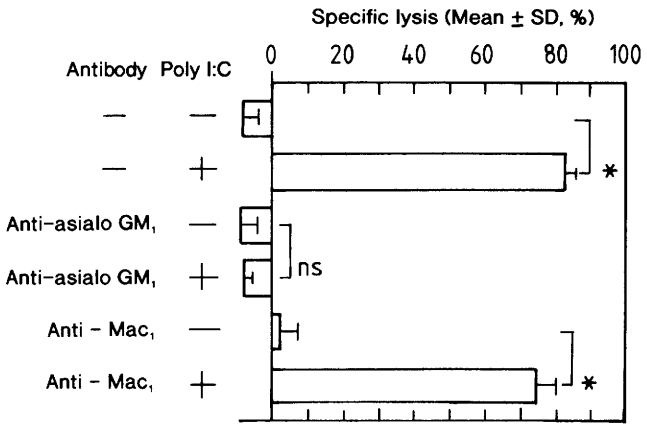


**Figure 2.** Effect of monoclonal antibodies on activation of NK cells by poly I:C in SCID mice. SCID mice were treated ip with 0.2 ml of anti-asialo $GM_1$ (1:30) or anti-$Mac_1$ (1:30) antibody 1 day before ip administration of poly I:C (1 mg/kg) (three mice/group). Eighteen hours after administration of poly I:C, peritoneal NK cell activity was determined. *$P < 0.001$; ns, not significant, as compared with poly I:C-untreated groups (two-tailed Student's *t*-test).

**Activation of Macrophages by Poly I:C in SCID Mice.** In addition to NK cells, macrophages play an important role in the early defense mechanisms against viral infections (26, 30, 31). Therefore, we investigated the activation of macrophages by poly I:C in SCID mice. Macrophage activity was assessed by determining phagocytic activity of peritoneal macrophages obtained from SCID mice that had received poly I:C 18 hr earlier. As shown in Figure 3, polyI:C markedly enhanced the phagocytic activity of macrophages in a dose-dependent manner (0.01–1 mg/kg). The phagocytic activity of resident macrophages was reduced by *in vivo* treatment with anti-$Mac_1$ antibody (Fig. 4).

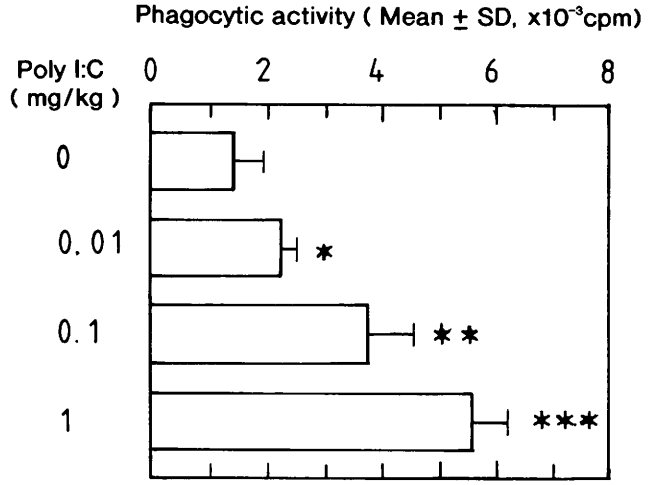


**Figure 3.** Activation of macrophages by poly I:C in SCID mice. Peritoneal macrophages were obtained from SCID mice which had been treated ip with the indicated doses of poly I:C 18 hr before the assay (three mice/group). The macrophages were cultured for 1 hr with [$^{51}$Cr]labeled antibody-sensitized erythrocytes (EA). Phagocytic activity is expressed as mean counts/min (cpm) ± SD of triplicate cultures. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, as compared with the macrophages from untreated mice (two-tailed Student's *t* test).

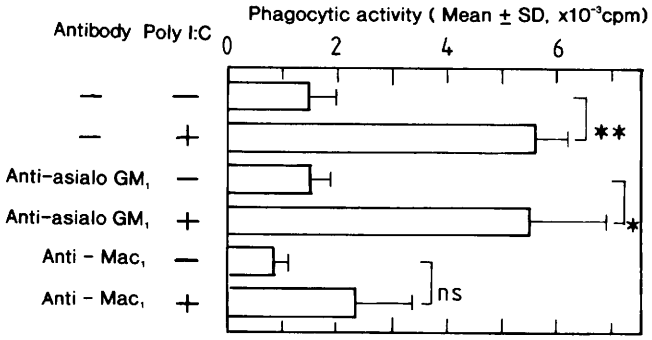


**Figure 4.** Effect of monoclonal antibodies on activation of macrophages by poly I:C. Peritoneal macrophages were obtained from mice that had been treated with monoclonal antibodies 24 hr before administration of poly I:C (1 mg/kg), and their phagocytic activity was determined 18 hr after administration of poly I:C (three mice/group). $^{*}P < 0.01$; $^{**}P < 0.001$; ns, not significant, as compared with poly I:C-untreated groups (two-tailed Student's *t* test).

Furthermore, treatment of SCID mice with anti-$Mac_1$ antibody abrogated the stimulatory effect of poly I:C on the phagocytic activity of macrophages, but did not do so after treatment with anti-asialo $GM_1$ antibody.

**IFN-Inducing Activity of Poly I:C in SCID Mice.** IFN plays a role in activating NK cells and macrophages (32, 33). We investigated whether poly I:C induces IFN in the peritoneal cavity or blood of SCID mice. Poly I:C, when administered ip, induced in a dose-dependent manner "early-type" IFN (2 hr after induction) in peritoneal fluid and serum (Table II). Moreover, poly I:C administered at doses of 1 or 10 mg/kg induced "late-type" IFN (at 18 hr after administration). IFN titers induced by poly I:C were higher in peritoneal fluids than in sera. Since both NK cells and macrophages can produce IFN, we next investigated whether poly I:C is able to induce IFN in SCID mice in which either NK cells or macrophages had been depleted by antibody treatment. Poly I:C was found to induce both peritoneal and serum IFN, at 2 hr after administration, in either NK cell- or macrophage-depleted SCID mice (Table III).

**Table II.** IFN Induction by Poly I:C in SCID Mice

| Dose[a] (mg/kg) | IFN titer (IU/ml)[b] | | | |
|---|---|---|---|---|
| | Peritoneal fluid | | Serum | |
| | 2 hr | 18 hr | 2 hr | 18 hr |
| 0 | <43 | <25 | <43 | <25 |
| 0.01 | 300 | — | <43 | — |
| 0.1 | 330 | <25 | 160 | 38 |
| 1 | 660 | 300 | 190 | 70 |
| 10 | 800 | 300 | 800 | 70 |

[a] Poly I:C was injected ip and peritoneal fluid and serum were taken at 2 hr and 18 hr after induction.
[b] IFN titers of samples were assayed in L-929 cells with vesicular stomatitis virus as the challenge virus. The results represent the mean for two independent experiments (three mice per group).

**Protective Activity of Poly I:C Against MCMV-Induced Mortality in NK Cell- and Macrophage-Depleted SCID Mice.** We next investigated whether administration of poly I:C could increase the life span of MCMV-infected SCID mice in which NK cells or macrophages had been depleted by treatment with antibodies to these cells (Table IV). Poly I:C significantly delayed MCMV-induced mortality in either NK cell- or macrophage-depleted SCID mice. However, protective effect was less pronounced in anti-asialo $GM_1$ antibody-treated (NK-defective) SCID mice (34% increase in life span) or anti-$Mac_1$ antibody-treated (macrophage-defective) SCID mice (73% increase in life span), as compared with mice that had not been pretreated with antibodies (92%), suggesting that NK cells may be somewhat more important than macrophages in the early defense mechanisms induced by poly I:C against MCMV infection in SCID mice.

**Table III.** IFN Induction by Poly I:C in SCID Mice Treated with Anti-Asialo $GM_1$ or Anti-$MAC_1$ Antibody

| Antibody[a] | Poly I:C[b] (1 mg/kg) | IFN titer (IU/ml)[c] | |
|---|---|---|---|
| | | Peritoneal fluid | Serum |
| — | + | 800 | 400 |
| Anti-asialo $GM_1$ | + | 800 | 800 |
| Anti-$Mac_1$ | + | 800 | 800 |

[a] Anti-asialo $GM_1$ (1:30) or anti-$Mac_1$ (1:30) antibody (0.2 ml) were injected ip.
[b] Poly I:C (1 mg/kg) was injected ip 1 day after administration of the antibody.
[c] IFN titers in peritoneal fluid and serum were determined at 2 hr after injection of poly I:C.

**Table IV.** Protective Activity of Poly I:C Against MCMV in SCID Mice Treated with Anti-Asialo $GM_1$ or Anti-$Mac_1$ Antibody

| Antibody[a] | Poly I:C[b] (10 mg/kg) | Mean day of death[c] (MDD ± SD) | Increase in life span[d] (%) |
|---|---|---|---|
| — | — | 10.0 ± 1.8 | — |
| — | + | 19.2 ± 1.5[e] | 92 |
| Anti-asialo $GM_1$ | — | 9.4 ± 0.5 | — |
| Anti-asialo $GM_1$ | + | 12.6 ± 1.2[f] | 34 |
| Anti-$Mac_1$ | — | 9.0 ± 0.9 | — |
| Anti-$Mac_1$ | + | 15.6 ± 1.9[g] | 73 |

[a] SCID mice were treated ip with 0.2 ml of anti-asialo $GM_1$ (1:30) or anti-$Mac_1$ (1:30) antibody at 48 and 2 hr before ip challenge with $10^{3.5}$ PFU of MCMV.
[b] Poly I:C (10 mg/kg) was administered ip at 18 hr before infection.
[c] See footnotes to Table I.
[d] The percent increase of life span was determined for each poly I:C-treated group in comparison with the corresponding untreated group (no antibody, anti-asialo $GM_1$, or anti-$Mac_1$, respectively).
[e] $P < 0.001$ (two-tailed Student's $t$ test).
[f] $P < 0.05$.
[g] $P < 0.01$.

## Discussion

A variety of cell types and mechanisms (i.e., NK cells, macrophages, antibody-dependent cell-mediated cytotoxicity (ADCC), cytotoxic T cells and antibodies) probably cooperate in the host defense to acute MCMV infections (22). NK cells are considered to play a key role in the early defense mechanisms against acute MCMV infection, and cytotoxic T cells in the late defense mechanisms (22). Our aim was to elucidate the early defense mechanism against MCMV infection in mice that do not have B- and T-cell responses (i.e., SCID mice). We assumed that through the specific suppression of NK cell or macrophage activity in these T and B cell-deficient mice, together with the enhancement of the immune response by means of poly I:C, we would be able to further dissect the early host-cell response against MCMV infection. We demonstrated that poly I:C enhances both NK cell activity and macrophage activity in SCID mice. Monoclonal antibodies against NK cells or macrophages abolished this stimulatory effect, since poly I:C was unable to boost NK cell or macrophage activity in SCID mice in which these cells had been depleted by antibody treatment.

IFN is recognized as an important factor in the host defense mechanisms, and is also well-known to play a role in the activation of NK cells and macrophages (33). Poly I:C induces both "early-type" and "late-type" IFN in the peritoneal cavity and blood of SCID mice (Table II). "Early-type" IFN is usually produced by macrophages and fibroblasts (some 2 hr after treatment with the inducer), whereas B cells and other leukocytes release a "late-type" IFN (some 16 and 18 hr later) (27, 36). In NK cells of immunocompetent mice poly I:C induces IFN-γ, and in macrophages it induces IFN-α and IFN-β (37–39). We found that poly I:C is still able to induce IFN in NK cell- or macrophage-depleted SCID mice.

Since the stimulation of "early-type" IFN by poly I:C is much more prominent than that of the "late-type", it is assumed that the "early-type" IFN efficiently activates NK cells and macrophages to become functional in the early defense of the viral infection. Kern *et al.* (19) reported that poly I:C and poly I:C/LC protect 6-week-old Swiss Webster mice against a lethal MCMV infection when administered 6 to 18 hr before virus challenge. We demonstrate here that polyI:C at doses of 3.7 or 15 mg/kg causes a significant increase in the life span of MCMV-infected SCID mice when administered ip at 18 hr before virus challenge. If, however, the administration of poly I:C was delayed until 5 days after infection, it was no longer able to increase the animals' life span (data not shown).

We recently reported that a synthetic lipid A analogue GLA-60 completely protects NK cell-defective beige mice against MCMV-induced mortality, whereas it increases the life span of nude and SCID mice without protecting them from MCMV-induced lethality (31). We suggested that macrophages, together with functional B and T cells, are essential in the host defense induced by GLA-60 against MCMV infection. Lipid A analogues differ from poly I:C in that (i) lipid A analogues have stronger macrophage-stimulating effect than poly I:C, and (ii) poly I:C is superior to lipid A analogues as IFN inducer and NK-cell stimulator (26, 27, 30, 31).

In conclusion, poly I:C was found to induce IFN in the peritoneal cavity and blood of SCID mice, and poly I:C-induced IFN enhanced both NK-cell and macrophage activities, thus resulting in a significant increase in the life span of MCMV-infected SCID mice. Both NK cells and macrophages appear to contribute to the nonspecific defense of SCID mice against MCMV infection.

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1. Rubin RH. Impact of cytomegalovirus infection on organ transplant recipients. Rev Infect Dis **12**(Suppl 7):754–765, 1990.
2. Tyms AS, Taylor DL, Parkin JM. Cytomegalovirus and the acquired immunodeficiency syndrome. J Antimicrob Chemother **23**(Suppl A):89–105, 1989.

3. Winston DJ, Ho WG, Champlin RE. Cytomegalovirus infections after allogeneic bone marrow transplantation. Rev Infect Dis **12**(Suppl 7):776–791, 1990.
4. Emanuel D. Treatment of cytomegalovirus disease. Semin Hematol **27**(Suppl 1):22–27, 1990.
5. Stagno S, Pass RF, Dworsky ME, Henderson RE, Moore EG, Walton PD, Alford CD. Congenital cytomegalovirus infections: The relative importance of primary and recurrent maternal infections. N Engl J Med **306**:945–949, 1982.
6. Bale JF Jr., O'Neil ME, Folberg R. Murine cytomegalovirus ocular infection in immunocompetent and cyclophosphamide-treated mice. Invest Ophthalmol Vis Sci **32**:1749–1756, 1991.
7. Shellam GR, Allan JE, Papadimitrious JM, Bancroft GJ. Increased susceptibility to cytomegalovirus infection in beige mutant mice. Proc Natl Acad Sci USA **78**:5104–5108, 1981.
8. Shellam GR, Flexman JP, Farrell HE, Papadimitrious JM: The genetic background modulates the effect of the beige gene on susceptibility to cytomegalovirus infection in mice. Scand J Immunol **22**:147–155, 1985.
9. Starr SE, Allison AC. Role of T lymphocytes in recovery from murine cytomegalovirus infection. Infect Immun **17**:458–462, 1977.
10. Neyts J, Balzarini J, Naesens L, De Clercq E. Efficacy of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine and 9-(1,3-dihydroxy-2-propoxymethyl)guanine for the treatment of murine cytomegalovirus infection in severe combined immunodeficiency mice. J Med Virol **37**:67–71, 1992.
11. De Clercq E, De Somer P. Effect of interferon, polyacrylic acid, and polymethacrylic acid on tail lesions in mice infected with vaccinia virus. Appl Microb **16**:1314–1319, 1968.
12. Homma JY, Mastuura M, Kumazawa Y. Studies on lipid A, the active center of endotoxin—Structure-activity relationship. Drugs of the Future **14**:645–665, 1989.
13. Ikeda S, Negishi T, Nishimura C. Enhancement of non-specific resistance to viral infection by muramyldipeptides and its analogs. Antiviral Res **5**:207–215, 1985.
14. Ikeda S, Tominaga T, Nishimura C, Homma JY, Kiso M, Hasegawa A. Antiherpes activity of chemically synthesized lipid A-subunit analogue GLA-60 in immunosuppressed mice. Antiviral Res **11**:173–180, 1989.
15. Kotani S, Takada H, Tsujimoto M, Ogawa T, Mori Y, Koga T, Iribe H, Tanaka A, Nagao S, McGhee JR, Michalek SM, Morisaki I, Nishimura C, Ikeda S, Kohashi O, Ogawa H, Ogawa S, Hammada S, Kawata S, Shiba T, Kusumoto S. Lipophilic muramyl peptides and synthetic lipid A analogs as immunomodulators. Progress Immunol **5**:1359–1377, 1983.
16. De Clercq E. Interferon inducers. Antibio Chemother **27**:251–287, 1980.
17. Field AK, Tytell AA, Lampson GP, Hilleman MR. Inducers of interferon and host resistance. II. Multistranded synthetic polynucleotide complexes. Proc Natl Acad Sci USA **58**:1004–1010, 1967.
18. Johnston AG. Modulation of the immune system by synthetic polynucleotides. Springer Semin Immunol **2**:149–168, 1979.
19. Kern ER, Olsen GA, Overall JC Jr., Glasgow LA. Treatment of a murine cytomegalovirus infection with exogenous interferon, polyinosinic-polycytidylic acid, and polyinosinic:polycytidylic acid-poly-L-lysine complex. Antimicrob Agents Chemother **13**:344–346, 1978.
20. Adams A, Mohrman M, Johnson AG, Morin A, De Paillette ED. Polyadenylic:polyuridylic acid-induced protection of BALB/c mice against acute murine cytomegalovirus infection. J Gen Virol **73**:2409–2413, 1992.
21. Janeway CA. A primitive immune system. Nature **341**:108, 1989.
22. Staczek J. Animal cytomegalovirus. Microbiol Rev **54**:247–265, 1990.
23. Koszinowski UH, Del Val M, Reddehase MJ. Cellular and molecular basis of the protective immune response to cytomegalovirus infections. Curr Top Microbiol Immunol **154**:189–220, 1990.
24. Welsh RM, Brubaker JO, Vargas-Cortes M, O'Donnell CL. Natural killer (NK) cell response to virus infections in mice with severe combined immunodeficiency: the stimulation of NK cells and the NK cell-dependent control of virus infections occur independently of T and B cell function. J Exp Med **173**:1053–1063, 1991.
25. De Clercq E, Wells RD, Grant RC. Merigan TC. Thermal activation of the antiviral activity of synthetic double-stranded polyribonucleotides. J Mol Biol **56**:83–100, 1971.
26. Ikeda S, Nishimura C, Natkatsuka M, Homma JY, Kiso M, Hasegawa A. Antiviral and immunomodulating activities of chemically synthesized lipid A-subunit analogues GLA-27 and GLA-60. Antiviral Res **9**:37–46, 1988.
27. Ikeda S, Matsuda Y, Nishimura C. Enhancement by muramyldipeptides of the activities of early-type inducers of interferon. Antiviral Res **5**:345–353, 1985.
28. Ikeda S, Tominaga T, Nishimura C. $Thy_1^+$ asialo$GM_1^+$ dendritic epidermal cells in skin defense mechanisms of vaccinia virus-infected mice. Arch Virol **117**:207–218, 1991.
29. Welsh RM. Do natural killer cells play a role in virus infections? Antiviral Res **1**:5–12, 1981.
30. Ikeda S, Neyts J, Matsuura M, Kiso M, Hasegawa A, Nishimura C, De Clercq E. Protective activity of lipid A analogue GLA-60 against murine cytomegalovirus infection in mice. J Med Virol **40**:222–227, 1993.
31. Ikeda S, Neyts J, Matsuura M, Kiso M, Hasegawa A, Nishimura C, De Clercq E. Protective activity of the lipid A analogue GLA-60 against murine cytomegalovirus infection in immunodeficient mice. J Gen Virol **74**:1399–1403, 1993.
32. Morahan PS. Interactions of herpesviruses with mononuclear phagocytes. In: Rouse BT, Lopez C, Eds. Immunology of Herpes Simplex Virus Infection. Boca Raton, FL: CRC Press, pp 71–89, 1984.
33. Kirchner H, Engler H, Schroder CH, Zawatzky R, Storch E. Herpes simplex virus type 1-induced infection and activation of natural killer cells in mice. J Gen Virol **64**:437–441, 1983.
34. Bosma GC, Custer RP, Bosma MJ. A severe combined immunodeficiency mutation in the mouse. Nature **301**:527–530, 1983.
35. Smee DF, Morris JLB, Leonhardt JA, Mead JR, Holy A, Sidwell RB. Treatment of murine cytomegalovirus infections in severe combined immunodeficient mice with ganciclovir, (S)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cyto-sine, interferon, and bropirimine. Antimicrob Agents Chemother **36**:1837–1842, 1992.
36. Maehara N, Ho M. Cellular origin of interferon induced by bacterial lipopolysaccharide. Infect Immun **15**:78–83, 1977.
37. Djeu JY, Heinbaugh JA, Holden H, Herberman RB. Augmentation of mouse natural killer cell activity by interferon and interferon inducers. J Immunol **122**:175–181, 1979.
38. Fileit HB, Rabinovitch M. Production of interferon by *in vitro* derived bone marrow macrophages. Cell Immunol **57**:495–504, 1981.
39. Martinez D, Lynch RJ, Meeker JB, Field AK. Macrophage dependence of polyriboinosinic acid-polyribocytidylic acid-induced resistance to herpes simplex virus infection in mice. Infect Immun **28**:147–153, 1980.