A 2',5'-oligoadenylate analogue inhibits murine hepatitis virus strain 3 (MHV-3) replication in vitro but does not reduce MHV-3-related mortality or induction of procoagulant activity in susceptible mice

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Exposure of inbred mice to murine hepatitis virus strain 3 (MHV-3) causes a strain dependent spectrum of disease symptoms which correlates with induction of procoagulant activity (PCA) by macrophages. Previous studies have demonstrated a role for interferons in resistance to MHV-3 infection. These cytokines have both antiviral and immunoregulatory effects which may be crucial for MHV-3 resistance. One of their antiviral effects is the ability to induce 2',5'-oligoadenylate (2-5A) synthetase leading to activation of the latent endoribonuclease RNase L. Once activated, RNase L degrades ssRNA thereby inhibiting viral-induced protein synthesis. These studies were undertaken to determine the effects of Oragen 0004 (Oragen), an RNase L activating 2-5A analogue, on MHV-3 replication and induction of PCA in vitro and on the course of MHV-3 infection in susceptible BALB/cJ mice in vivo. Oragen inhibited MHV-3 replication in peritoneal macrophages derived from resistant A/J and susceptible BALB/cJ mice in a dose-dependent fashion. Concentrations of Oragen greater than 110 μg/2 x 10⁶ macrophages decreased viral replication by greater than 89% in peritoneal macrophages in vitro obtained from both BALB/cJ and A/J mice and by 86% in livers from MHV-3-infected mice in vivo. However, Oragen failed to inhibit induction of PCA following in vitro exposure of BALB/cJ mice-derived peritoneal macrophages to MHV-3 and failed to prevent the development of fulminant hepatitis in BALB/cJ mice in vivo. Thus, these studies demonstrate clearly that induction of 2-5A synthase and inhibition of viral replication is not sufficient to prevent MHV-3-related hepatocellular injury, and these data further support the role of PCA in the pathogenesis of MHV-3 infection.

Introduction

Infection of inbred mice by the RNA coronavirus murine hepatitis virus strain 3 (MHV-3) produces a strain- and age-dependent spectrum of disease symptoms (Virelizier & Allison, 1976; MacNaughton & Patterson, 1980; Le Prevost et al., 1975; Levy et al., 1981). BALB/cJ mice are fully susceptible and die within 7 days of MHV-3 exposure from fulminant hepatitis whereas A/J mice are fully resistant and develop no clinical symptoms (Levy et al., 1981, 1982). The mechanisms underlying this variation in host response are not clear. We (Levy et al., 1982, 1983; Dindzans et al., 1985), as well as others (Lucchiari & Pereira, 1989; Le Prevost et al., 1975; Schindler et al., 1982), have demonstrated that viral replication occurs in resistant and susceptible strains both in vitro and in vivo and, thus, resistance to MHV-3 cannot be explained on the basis of a failure of viral replication in resistant strains. We have shown that susceptibility to MHV-3 correlates with induction of procoagulant activity (PCA) following viral exposure. High levels of PCA are produced in vivo and in vitro by macrophages from susceptible BALB/cJ mice following MHV-3 exposure whereas macrophages from resistant A/J mice fail to produce increased PCA either in vivo or in vitro after challenge with MHV-3. MHV induction of PCA is rapid, with increases being observed within 1 to 1.5 h and maximal activity occurring at 12 to 18 h. Viral growth in the susceptible cell line 17CL1 is not detectable until 6 h after infection and is not detected until 18 h after infection in monocytes/macrophages from susceptible BALB/cJ mice. Therefore, the induction of PCA precedes the replication of infectious virus (Levy et al., 1981, 1982; Dindzans et al., 1986). Furthermore, treatment of susceptible mice with antibody raised against PCA prevents the lethal effects associated with
MHV-3 infection thus indicating that there is a role for PCA in the pathogenesis of MHV-induced disease (Li et al., 1992).

Previous studies have demonstrated a crucial role for interferons (IFNs) in resistance to MHV-3 infection (Lucchiari & Pereira, 1989, 1990; Lucchiari et al., 1991, 1992; Virelizier & Gressor, 1978). These cytokines produce direct antiviral effects as well as effects on the immune response of the host which result in decreased viral infectivity and cellular injury (Whitaker-Dowling & Youngner, 1987; Borden, 1992; Grossberg, 1987; Staehehi, 1990; Samuel, 1987; Pestka et al., 1987).

An important antiviral effect of IFNs is the ability to stimulate synthesis of 2',5'-oligoadenylate 5'-triphosphates (2-5A) (Chebath et al., 1987; Kumar et al., 1988). In the presence of dsRNA, 2-5A molecules are synthesized from ATP by 2-5A synthetase, which is induced by IFNs. These molecules subsequently bind to and activate latent endoribonuclease L (RNase L) (Lengel, 1982; Pestka & Langer, 1987; Samuel, 1987). This enzyme degrades single-stranded mRNA and rRNA resulting in inhibition of cellular protein synthesis and viral replication.

Activation of RNase L is crucial for inducing host resistance following infection with certain viruses (Chebath et al., 1987; Kumar et al., 1988). Recently, bioactive analogues of the naturally occurring 2-5A have been synthesized (Charachon et al., 1990; Kariko et al., 1987; Kanou et al., 1990, 1991; Nagai et al., 1993). In vitro, these agents have been demonstrated to be capable of inhibiting replication of hepatitis B virus (Carter et al., 1993) and human immunodeficiency virus type 1 (Muller et al., 1991; Montefiori et al., 1989). There are, however, no reports of their efficacy in vivo.

The availability of these 2-5A analogues provides an opportunity to distinguish the antiviral from the immunomodulatory effects in IFN following MHV-3 infection both in vitro and in vivo. In this study, we examined the ability of Oragen 0004 (Oragen), a 2-5A analogue, to inhibit MHV-3 replication in vitro in peritoneal macrophages derived from both resistant and susceptible strains of mice and in vivo in susceptible mice. We subsequently determined the effects of this agent on PCA induction in macrophages derived from susceptible strains of mice in vitro and determined the effects of Oragen on the survival of susceptible mice following exposure to MHV-3.

**Methods**

**Mice.** Female BALB/cJ and A/J mice aged 6–8 weeks were purchased from Jackson Laboratories (Bar Harbor, Me., USA); they were maintained in the animal colony facility at the University of Toronto and fed a standard chow diet and water ad libitum prior to and during studies.

**Virus.** The origin and growth of 17CL1, DBT and L2 cells have been described previously (Sturman & Takemoto, 1972; Hirano et al., 1977; Rothfels et al., 1979). MHV-3 was plaque-purified on monolayers of DBT cells and grown to a titre of 4×10^7 p.f.u./ml in 17CL1 cells. Viruses were harvested by one cycle of freezing and thawing and then were clarified by centrifugation at 4500 g for 1 h at 4 °C. Viral titres were determined on monolayers of L2 cells in a standard plaque assay (Sturman & Takemoto, 1972; Lucas et al., 1977).

**Chemicals.** Oragen 0004 (Oragen) (HEM Pharmaceuticals, Rockville, Md., USA) was dissolved in distilled deionized water, filter-sterilized using Millex-GS 0.22 μm filter units (Millipore) and stored at 4 °C until use.

**Peritoneal macrophages.** Peritoneal macrophages were harvested from A/J and BALB/cJ mice 4 days after intraperitoneal (i.p.) administration of 1.5 ml 3% Brewer's thioglycollate, pH 6.9 (Difco) as previously described (Sinclair et al., 1990) and suspended in RPMI 1640 medium ( Gibco) supplemented with 2% heat-inactivated fetal calf serum (ICN Biomedicals) and 2 mM-glutamine (RPMI 2). Cell suspensions contained more than 95% macrophages as demonstrated by morphology and non-specific esterase staining. Viability exceeded 98% when assessed by Trypan blue exclusion and was not altered by incubation of macrophages with Oragen at concentrations of up to 150 μg/2×10^6 macrophages.

**Effects of Oragen on MHV-3 replication in murine peritoneal macrophages in vitro.** Oragen (0–110 μg) dissolved in 100 μl aliquots was added to 2×10^6 BALB/cJ mouse-derived peritoneal macrophages suspended in a final volume of 1 ml of RPMI 2, 1 h prior to and 24 h following infection with MHV-3 at an m.o.i. of 0.001. Similar studies in mice were carried out in macrophages derived from resistant A/J mice. Following incubations of 0 to 48 h, macrophages were harvested and then sonicated and viral titres were assayed on monolayers of L2 cells in a standard plaque assay as previously described (Sturman & Takemoto, 1972; Lucas et al., 1977). The percentage of viral growth inhibition occurring at each time point was calculated by the following equation: (viral titre in control sample – viral titre in study sample) / (viral titre in control sample) × 100%.

**PCA assay.** Peritoneal macrophages from susceptible BALB/cJ mice were incubated with MHV-3 in the presence or absence of Oragen. Samples were assayed for PCA by their ability to inhibit the spontaneous clotting time of recalculated platelet-reduced normal human plasma with results quantified by comparison with values obtained using serial dilutions of rabbit brain thromboplastin as a standard (Sigma) as previously described (Abecassis et al., 1987).

**Western blot analysis with anti-PCA monoclonal antibodies.** BALB/cJ mouse peritoneal macrophages, in 1 ml samples of 2×10^6 cells suspended in RPMI 2, were incubated for up to 8 h with 37.5 μg Oragen and stimulated with MHV-3 at an m.o.i. of 10. Samples of peritoneal macrophages were also incubated with Oragen in the absence of MHV-3 or in the presence of MHV-3 without Oragen. Following incubation, samples were dissolved in Tris-HCl sample buffer containing 2% SDS and 10% glycerol and heated for 10 min at 70 °C. Samples were subjected to 4–20% (w/v, acrylamide) SDS–PAGE according to Laemmli (1970) with prestained molecular mass markers (Amersham). After electrophoresis and electroblotting onto a nitrocellulose membrane using Tri-glycine, 20% methanol buffer markers (Amersham), the blot was blocked with a solution of 5% horse serum (Jackson Immunoresearch Laboratory) for 1 h at room temperature.
Autoradiography was performed after exhaustive washing and films were examined for evidence of proteins that were reactive with monoclonal antibody 3D4-3.

**Effects of Oragen on BALB/cJ mice in vivo**

*Toxicity studies.* BALB/cJ mice were treated with or without and 100 μg Oragen IP once daily and three mice per group were sacrificed 1, 3, 5, 7 or 10 days following initiation of therapy. Animals were also treated with 1000 and 10000 μg Oragen, but all showed marked clinical signs of toxicity with those animals treated with 10000 μg all dying within 6 h of Oragen injection. Thus these two groups were not further studied. Animals given 0 and 100 μg Oragen were bled and samples were analysed for L-alanine aminotransferase (ALT), creatinine and haematocrit content and white blood cell count. Livers and kidneys were excised and fixed by immersion in 10% formalin (Fischer Scientific) in 0.1 M-phosphate buffer, pH 7.4.

Effects of Oragen on intrahepatic MHV-3 replication and MHV-3-related tissue injury. BALB/cJ mice were pretreated with 100 μg Oragen administered once daily beginning 24 h prior to infection by injection of 1000 p.f.u. of MHV-3. An equal number of control mice, exposed to MHV-3 but not receiving Oragen, were also studied. Oragen treatment was continued at a dose of 100 μg i.p. once daily following administration of MHV-3 and mice were sacrificed either 24 or 48 h post infection. Following sacrifice, livers were excised and fixed in 10% formalin, 0.1 M-phosphate buffer, pH 7.4, prior to histological examination. The remaining liver tissue was homogenized at 4 °C as previously described (Li et al., 1992; Abecassis et al., 1987) and viral titres were determined on monolayers of L2 cells in a standard plaque assay. Viral titres, expressed as p.f.u./g liver tissue, in the Oragen-treated group was calculated as described above.

Histology. Samples of kidney and liver procured in the previously described studies which had been fixed by immersion in 10% formalin in 0.1 M-phosphate buffer, pH 7.4, to 10% formalin (Fischer Scientific) in 0.1 M-phosphate buffer, pH 7.4, prior to histological examination. The remaining liver tissue was homogenized at 4 °C as previously described (Li et al., 1992; Abecassis et al., 1987) and viral titres were determined on monolayers of L2 cells in a standard plaque assay. Viral titres, expressed as p.f.u./g liver tissue, in the Oragen-treated mice were compared with those of mice that were infected with 1000 p.f.u. of MHV-3 but did not receive Oragen. The percentage of inhibition of intrahepatic viral replication in the Oragen-treated group was calculated as described above.

Biochemistry. Blood, obtained by axillary bleeding into non-heparinized capillary tubes, was centrifuged at 8800 g (Eppendorf centrifuge 5402) for 10 min at 4 °C. Serum was subsequently collected and a determination of serum alanine aminotransferase (ALT) and creatinine levels was carried out as described previously (Abecassis et al., 1987).

Survival studies. The effects of Oragen on the survival of susceptible BALB/cJ mice following exposure to 1000 p.f.u. of MHV-3 were assessed. A group of five mice, each pretreated for 72 h prior to infection with MHV-3, had 100 μg of Oragen administrated i.p. once daily and this treatment was continued for 14 days or until the death of the animal. Control infected mice were given saline treatment in a similar protocol to the mice treated with Oragen. To determine whether the route of administration affected the outcome, mice (n = 5/group) were also pretreated with 100 μg Oragen intravenously (i.v.) 72 h prior to i.p. infection with 1000 p.f.u. of MHV-3 and this i.v. treatment was continued for 14 days or until the death of the animal.

Statistical analysis. Data are expressed as the mean ± 1 SD. Statistical analysis was carried out using analysis of variance (ANOVA) and the Wilcoxon Ranked Sum test. A P value of 0.05 or less was considered statistically significant.

**Results**

In vitro studies

**Viral replication.** The pattern of MHV-3 replication in vitro in macrophages from resistant A/J mice and susceptible BALB/cJ mice was similar (Fig. 1). The viral titres in macrophages from both A/J and BALB/cJ mice were not statistically different from each other for all time-points studied. Peak viral titres occurred at 24 h decreasing in value thereafter; however, virus could still be recovered even at 48 h post-infection in macrophages from both the BALB/cJ and A/J mice.

Oragen (Fig. 2) inhibited MHV-3 replication in peritoneal macrophages from BALB/cJ mice in a dose and time dependent manner with the maximum inhibition – of greater than 89%– occurring when a concentration of 110 μg Oragen was used. In parallel studies, Oragen also inhibited viral replication to a similar extent in peritoneal macrophages derived from A/J mice (data not shown). Inhibition of MHV-3 replication by Oragen in macrophages from A/J mice was greater than that seen in BALB/cJ mice at 12 and 24 h post infection (P < 0.02). At 36 h, inhibition of viral replication was greater in BALB/cJ mouse macrophages (P < 0.01), but by 48 h inhibition was similar in macrophages from both groups of mice (P > 0.24).

**Functional PCA.** Peritoneal macrophages from BALB/cJ mice expressed a marked increase in total PCA, from 87 ± 16 mU/10^6 macrophages to 923 ± 532 mU/10^6 macrophages (P < 0.05), when infected with
Table 1. Effect of Oragen on induction of macrophage PCA by MHV-3

<table>
<thead>
<tr>
<th>MHV-3 (p.f.u.)</th>
<th>Oragen (µg/ml)</th>
<th>PCA (mU/10⁶ macrophages)*</th>
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<td>87 ± 16</td>
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<tr>
<td>1 x 10⁶</td>
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<td>923 ± 532†</td>
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<td>1 x 10⁶</td>
<td>50</td>
<td>788 ± 144†</td>
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<td>1 x 10⁶</td>
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<td>790 ± 154†</td>
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* Macrophages were suspended at a concentration of 1 x 10⁶ cells/ml in RPMI 0. Oragen 0004 was added to macrophages 60 min prior to addition of MHV-3. Total content PCA was determined in a one-stage clotting assay. One way analysis of variance (ANOVA) demonstrated no significant difference between PCA results of study groups incubated in the presence of MHV-3 in the absence or presence of Oragen.

† Macrophages stimulated with MHV-3 had significantly higher PCA values than samples not stimulated with MHV-3. Oragen did not induce PCA when added to macrophages in the absence of MHV-3. Data represent the mean ± SD of three experiments done in triplicate.

MHV-3 in vitro for 8 h. Oragen, at concentrations of up to 100 µg when added 60 min prior to addition of MHV-3, did not inhibit induction of PCA (Table 1).

Antigenic PCA. Western blot analysis revealed that a PCA protein (70 kDa) was present in macrophages from BALB/cJ mice which had been stimulated with MHV-3 for 8 h (Fig. 3). PCA protein was not detected in either non-infected and non-treated BALB/cJ mouse-derived macrophages or BALB/cJ mouse macrophages incubated in the presence of Oragen which had not been stimulated with MHV-3. BALB/cJ mouse macrophages which had been pretreated with 37.5 µg Oragen 60 min prior to exposure to MHV-3 expressed PCA protein in an amount equivalent to that seen in Oragen-untreated MHV-3-stimulated macrophages (Fig. 3).

Thus, although Oragen inhibited viral replication in
BALB/cJ mouse peritoneal macrophages, it did not inhibit either the function or induction of PCA protein following MHV-3 stimulation.

**Effects of the Oragen compounds on BALB/cJ mice and the course of MHV-3 infection in vivo: toxicity study**

BALB/cJ mice receiving 100 µg Oragen by i.p. administration once daily for 10 days demonstrated no changes in physical appearance or activity levels for up to 10 days of treatment. Livers and kidneys excised from these animals showed no histological abnormalities. Furthermore, there was no biochemical evidence of renal, liver or haematologic toxicity. Serum ALT, a marker of liver necrosis, remained within normal limits [40 to 60 international units (IU)/l]; serum creatinine, a marker of renal function, also did not deviate from normal (40 ± 10 µmol/l); and no haematologic disturbances were noted as indicated by normal hematocrit (45 ± 5%) and white blood cell count (2.85 ± 0.5 x 10⁶ cells/ml).

**Effects of Oragen compounds on MHV-3 replication and MHV-3 related liver injury in BALB/cJ mice in vivo**

MHV-3 replication in vivo. There was a statistically significant reduction in growth of MHV-3 in vivo in livers recovered from mice at 24 and 48 h following infection with MHV-3 that had been treated with Oragen as compared with livers recovered from MHV-3-infected but untreated control mice (P < 0.05 at 24 and 48 h). Hepatic viral replication was inhibited by 82.8% ± 5.7% and 69.4% ± 10.7% at 24 and 48 h post-infection respectively, in the group of mice treated with Oragen as compared with MHV-3-infected but untreated mice.

Liver histology. Liver tissue was obtained from Oragen-treated (100 µg administered i.p. once daily) and untreated mice at 24 and 48 h following infection with MHV-3. Mice infected with MHV-3 and not treated with Oragen demonstrated, at 24 h post-infection, multiple small, discrete foci or liver necrosis associated with polymorphonuclear cell infiltration. At 48 h post-infection, these necrotic areas became more numerous and areas of confluent necrosis were seen. Morphometric analysis of liver specimens from both Oragen-treated and untreated mice demonstrated a similar pattern of necrosis with less than 5% necrosis per section in tissue obtained from mice exposed to MHV-3 at 24 h and 30–40% necrosis at 48 h and greater than 82% necrosis at 72 h.

Histological examination was similarly carried out on liver tissue obtained from BALB/cJ mice pretreated with 100 µg Oragen administered i.v. twice daily for 24 h prior to the i.p. administration of MHV-3 and continued thereafter. No significant differences in the extent or pattern of liver necrosis or polymorphonuclear cell infiltration were seen in specimens obtained at 24, 48 and 72 h post MHV-3 exposure as compared with results seen in control mice receiving MHV-3 but not treated with Oragen.

**Biochemistry.** MHV-3-infected, but Oragen-untreated mice developed a marked elevation in ALT from 40 to 23200 IU/l by 48 h which continued to increase until their demise at 72 to 96 h. In mice treated with 100 µg Oragen administered i.p. once daily beginning 24 h prior to MHV-3 infection, a similar marked rise in ALT was seen by 48 h (40 to 10600 IU/l) which also persisted to death of the animals although it was significantly less than that seen in untreated and MHV-3-infected mice at all time-points (P < 0.007).

**Survival studies.** Oragen treatment had no effect on the lethality of MHV-3 infection regardless whether it was administered i.v. or i.p. Mice infected with 1000 p.f.u. of MHV-3 whether treated or untreated with Oragen died within 7 days. In additional studies, mice which were pretreated with Oragen and infected with 10 or 100 p.f.u. of MHV-3 and had Oragen therapy continued daily, showed no reduction in mortality and all animals died within 7 days.

**Discussion**

A number of investigators have demonstrated that IFNs are necessary for host resistance to MHV-3 infection (Lucchiari et al., 1991). Unlike adult A/J mice, A/J mice less than 4 weeks old are fully susceptible to MHV-3 with susceptibility correlating with the inability of these mice to produce IFN-γ in vivo in response to MHV-3 (Lucchiari & Pereira, 1990). In mice resistant and semi-resistant to MHV-3, treatment with MAbs raised against IFN results in conversion from resistance to susceptibility (Lucchiari et al., 1992; Virelizier & Gresser, 1978). Treatment with IFN type 1 has been shown to prolong survival following MHV exposure, particularly when treatment is started prior to viral infection (Minagawa et al., 1987; Kato et al., 1986). Although the mechanisms for the protective effect of IFNs were not examined in these studies, the beneficial effects of IFN may have been due to either their antiviral or immune effects (Whitaker-Dowling & Younger, 1987; Borden, 1991; Grossberg, 1987; Staeheli, 1990; Samuel, 1987; Pestka & Langer, 1987).

IFNs induce the synthesis of two groups of proteins with established antiviral effects (Pestka & Langer, 1987; Staeheli, 1990): (i) protein p1/eIF-2 alpha protein kinase which causes inhibition of intracellular protein synthesis through inactivation of the eukaryotic translation initiation factor eIF-2 and (ii) 2-5A synthetase which catalyses the synthesis from ATP of oligonucleotides of
the general structure ppp(A2'p)A (2-5A) and causes activation of the 2-5A-dependent latent endoribonuclease RNase L. The role of these antiviral proteins in the host response to MHV-3 infection has not previously been examined.

The availability of the 2-5A analogue Oragen 0004 permitted us to examine whether activation of RNase L alone confers resistance to MHV-3. Potentially, susceptibility to MHV-3 might be a secondary effect of a lack of activation of RNase L, due either to failure of activation of 2-5A synthetase by IFN or the lack of an inducible RNase L in susceptible mice.

In vitro, Oragen inhibited MHV-3 replication in peritoneal macrophages derived from susceptible BALB/cJ mice. Inhibition of viral replication was dose dependent and increased with time over the interval 0 to 48 h following macrophage exposure to MHV-3. Furthermore, the amount of inhibition seen in BALB/cJ mice macrophages was similar to that seen in macrophages from A/J mice at equivalent dosages and time intervals. Oragen also significantly decreased intrahepatic MHV-3 replication in susceptible BALB/cJ mice in vivo. Thus, these results together suggest that an inducible RNase L is present in susceptible BALB/cJ mice which is capable of inhibiting viral replication to the same extent as occurs in resistant A/J mice.

Recent studies from our laboratory have demonstrated the crucial role of induction of a unique macrophage procoagulant (PCA) in susceptibility to MHV-3 infection (Li et al., 1992). The induction of PCA following exposure to MHV-3 correlates with susceptibility to MHV-3 with high levels of PCA expressed by monocytes/macrophages derived from susceptible strains of mice following exposure to MHV-3 both in vitro and in vivo (Levy et al., 1982; Dindzans et al., 1986; Levy et al., 1981). The importance of PCA in the pathogenesis of MHV-3-induced disease has been demonstrated by the recent observation that mortality following MHV-3 infection in susceptible BALB/cJ mice can be prevented by treatment with a MAb raised against PCA (Li et al., 1992).

Despite the potent antiviral properties of Oragen, it failed to inhibit functional or antigenic expression of PCA response to MHV-3 stimulation in macrophages from susceptible BALB/cJ mice or to prolong the survival of MHV-3-infected susceptible mice, even in studies when the infectious dose of MHV-3 was reduced to 10 p.f.u. (LD50, 100). Although there was a statistically significant reduction in serum ALT (a marker of hepatic necrosis) levels in treated versus untreated animals at 48 h, the level of ALT in the Oragen-treated mice was consistent with severe hepatic necrosis as revealed by liver histology and the differences in serum ALT between the two groups were insignificant. Thus, the results of this study suggest that, although the antiviral effects of IFNs may have an important influence on resistance to MHV-3, this property by itself is insufficient to prevent MHV-3-related mortality, and therefore the immune effects of IFNs must be crucial to the development of resistance of MHV-3.

T lymphocytes have been established as being important in the development of resistance to MHV infection (Le Prevost et al., 1975; Woodward et al., 1984; Lucchiari et al., 1992). In vivo studies of mice infected with MHV-JHM, a strain of virus closely related to MHV-3, have demonstrated that resistance could be conferred by adoptive transfer of virus-specific Lyt-1+2−, L3T4+ T cell clones (Stohlman et al., 1986). Subsequent studies (Korner et al., 1991) demonstrated that T cell clones which were able to confer resistance to MHV-JHM expressed IFN-γ and interleukin 2 (IL-2), consistent with a TH1 T lymphocyte profile (Mosmann & Coffman, 1989). We have demonstrated that immunization of susceptible mice with MHV-3 specific TH1 T lymphocyte clones derived from resistant A/J mice which produced IFN-γ and IL-2 and inhibited macrophage expression of PCA in vitro resulted in protection from the lethal effects of MHV-3 infection (Chung et al., 1994). In that study, although IFN-γ production by the T cell clones was necessary for resistance, the cytokine did not directly inhibit macrophage production of PCA, consistent with previous reports that have shown that IFN-γ has no direct effect on PCA (Moon & Geczy, 1988) and thus suggesting that the protective effects of IFNs in MHV-3 infection may relate primarily to their enhancement of a TH1 T helper lymphocyte response. This is similar to what has been reported in murine infections with cytomegalovirus (Lucin et al., 1992), influenza virus (Palladino et al., 1991) and measles virus (De Vries et al., 1989) infection in which resistance is associated with the activity of competent TH1 and CD8 cells and a loss of TH1 cells results in conversion of resistance to susceptibility.

In conclusion, despite having antiviral effects both in vitro and in vivo, Oragen failed to inhibit induction of MHV-3-induced PCA or prolong survival of susceptible BALB/cJ mice. The finding that administration of IFNs prolongs survival suggests that their protective effect is affected through their ability to induce a TH1 T helper lymphocyte response which has previously been shown to prevent both induction of PCA and mortality associated with MHV-3 infection. This present study, therefore, helps improve our understanding of the mechanisms of IFNs' beneficial effects in ameliorating the effects of MHV-3 infection.

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