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Intranasally administered alpha/beta interferon prevents extension of mouse hepatitis virus, strain JHM, into the brains of BALB/cByJ mice

Abigail L. Smith[1,2], Stephen W. Barthold[1], and Deborah S. Beck[1]

[1]*Section of Comparative Medicine and* [2]*Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut, U.S.A.*

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Summary

Intranasally administered alpha/beta interferon blocked extension of the coronavirus, mouse hepatitis virus, strain JHM (MHV-JHM), from the nose to the brain of BALB/cByJ mice following intranasal inoculation with the virus. Two hundred units of alpha/beta interferon were administered intranasally to BALB/cByJ mice daily over a five day period. The mice were exposed intranasally to 10^3 median tissue culture infectious doses of MHV-JHM on the third day of interferon treatment. Two days after virus exposure, the proportion of mice with MHV in nasal turbinates was reduced from 10 of 10 in the untreated group to 7 of 10 in the interferon-treated group, and mean titers in virus-containing noses were lower in the interferon-treated group. Five days after virus exposure, the proportion of mice with infectious virus in the brain was significantly lower in the interferon-treated group (1 of 10 mice) than in the untreated group (10 of 10 mice). Systemic infection, as measured by presence and concentration of virus in the spleen, was not affected by intranasal interferon treatment. These results suggest that intranasally administered interferon protects against local extension of MHV-JHM from nose to brain, but not against dissemination of virus to other organs, such as the spleen.

Mouse hepatitis virus; Coronavirus; Interferon

Correspondence to: A.L. Smith, Section of Comparative Medicine, 375 Congress Avenue, Yale University School of Medicine, New Haven, CT 06510, U.S.A.

Introduction

Intranasally administered recombinant alpha interferon (IFN) has been shown to reduce upper respiratory clinical signs, nasal secretion and virus excretion after challenge of human volunteers with coronavirus 229E (Higgins et al., 1983). In that study, intranasal recombinant IFN treatment shortened the duration and reduced the severity of cold symptoms following challenge with coronavirus 229E (Turner et al., 1986). In this case, the role that intranasal IFN therapy plays in prevention or treatment of coronavirus colds is unclear due to the difficulties in studying viral pathogenesis in the natural human host.

Respiratory coronavirus infections in the laboratory mouse may provide needed insight into respiratory coronavirus pathogenesis and mechanisms of IFN protection. Strains of the murine coronavirus, mouse hepatitis virus (MHV), have primary respiratory or enteric tropisms (Barthold and Smith, 1984, 1987; Barthold et al., 1985) as described for human coronaviruses. Respiratory MHV strains initially replicate in upper respiratory mucosa. In susceptible mice, virus can also disseminate to other internal organs (Barthold and Smith, 1984, 1987), and several MHV strains can extend directly from nose to brain along olfactory nerve pathways (Barthold et al., 1987; Barthold and Smith, 1983, 1987; Goto et al., 1977; Taguchi et al., 1977, 1979). Because of these systemic and central nervous system effects, MHV may not be a perfect model for human respiratory coronavirus infections. Nevertheless, the possible association of coronaviruses in human patients with multiple sclerosis (Burks et al., 1980; Gerdes et al., 1981) may make these MHV characteristics highly relevant.

Intranasal, as well as intravenous, administration of alpha/beta IFN significantly reduced mortality among susceptible BALB/cByJ mice that were intranasally exposed to respiratory MHV strain, JHM (MHV-JHM) (Garlinghouse and Smith, 1985), but the mechanism of IFN protection was not examined. Since mortality due to MHV-JHM is related to encephalitis (Barthold and Smith, 1987), it is likely that IFN treatment affects replication in nose or brain or prevents extension of virus into brain. The purpose of the current study was to determine the mechanism of protection afforded mice given intranasal IFN, using virus and antigen markers in key target tissues, rather than mortality.

Materials and Methods

Mice

Three- to four-week-old BALB/cByJ mice were obtained from The Jackson Laboratory, Bar Harbor, ME, and pregnant Swiss mice were obtained from Charles River Breeding Laboratories, Portage, MI. Based on indirect immunofluorescence serology, these mice were free of antibody to MHV on arrival. Mice were housed in micro-isolator cages (Lab Products, Maywood, NJ) which were opened only in a biological safety cabinet.

Inoculations

BALB/cByJ mice were given 200 U of alpha/beta IFN per inoculum (20 μl) intranasally on each of days −2, −1, 0, 1 and 2 relative to virus exposure. The IFN was produced in mouse L929 cells as described earlier (Barthold et al., 1985) with induction by Newcastle disease virus. Antiviral activity was acid-stable, protease-sensitive and virus-nonspecific and was thus designated alpha/beta interferon. International units were with reference to a WHO international standard obtained from the National Institute of Allergy and Infectious Diseases, NIH (G002-904-511). The intranasal virus challenge consisted of 10 μl containing 10^3 $TCID_{50}$ of MHV-JHM propagated in 17 Cl 1 cells (Sturman and Takemoto, 1972). MHV-JHM was obtained from the American Type Culture Collection (Rockville, MD) and passaged twice in NCTC 1469 cells and once in adult BALB/cByJ mouse brain prior to one passage in 17 Cl 1 cells. On the day of virus exposure, virus was inoculated approximately seven hours after interferon administration. Additional groups of mice were untreated or sham-treated with clarified L929 cell supernatant fluid and challenged with virus to rule out a non-specific protective effect of intranasally administered fluid.

Tissue processing

At necropsy, mice were killed with carbon dioxide gas and exsanguinated. Half of each organ of interest was fixed in 10% neutral buffered formalin for histological evaluation and half was frozen at −70°C for virus recovery attempts. Each organ was collected at the time when peak virus titers were expected (Barthold and Smith, 1987). Nasal turbinates were collected on day 2 post-inoculation, spleen on day 3 and brain on days 3 and 5 post-inoculation. Spleen was chosen as a marker of virus dissemination, and nose and brain were assayed for evidence of nasoencephalopathy.

Paraffin-embedded tissues were sectioned at 5 μm and stained with hematoxylin and eosin. Selected tissue sections were stained for MHV antigen using a streptavidin-biotin-horseradish peroxidase method (Bethesda Research Laboratories, Gaithersburg, MD) and tissue treatment as previously described (Barthold and Smith, 1987; Barthold et al., 1985). Primary antibody was in the form of hyperimmune mouse ascitic fluid prepared in multiparous Swiss mice by 3 weekly intraperitoneal injections of MHV-JHM-infected infant mouse brain emulsified in Freund's complete adjuvant. Virus isolation and quantification were accomplished by intracerebral injection of 2-day-old Swiss mice. Virus titers were calculated by the method of Reed and Muench (1938).

Results

On the second day of infection, virus was detected in the nasal turbinates of all 10 untreated mice and in the noses of 7 of 10 IFN-treated mice (Table 1). Comparison of mean virus titers for positive mice in the 2 groups revealed lower titers among IFN-treated mice than among untreated mice ($P < 0.05$). At this interval,

TABLE 1

Virus recovery from tissues of BALB/cByJ mice given 1000 U of alpha/beta interferon (IFN) and MHV-JHM[a].

Day post-inoculation	Tissue	Treatment	Positive / tested (mean titer ± S.E.)
2	Nose	None	10/10 (5.1 ± 0.9)[b]
		IFN	7/10 (3.9 ± 0.9)[b]
3	Spleen	None	4/4 (5.8 ± 1.1)
		IFN	5/5 (5.0 ± 0.6)
	Brain	None	2/4 (4.2 ± 0.6)
		IFN	0/5
5	Brain	Sham	10/10 (5.5 ± 1.4)
		None	10/10[c] (5.2 ± 1.2)
		IFN	1/10[c] (5.6)

[a] Titers are $\log_{10}$ $ICLD_{50}$ per gram of tissue (means for positive mice only).
[b] $t = 2.76$ (15 d.f., unpaired t test); $P < 0.05$.
[c] $X^2 = 16.29$; $P < 0.005$.

MHV antigen was found in nasal respiratory epithelium (Fig. 1) of 5 of 5 untreated mice, whereas none of 5 IFN-treated mice contained antigen in nasal epithelium. Two of the 5 treated mice did have antigen restricted to vomero-nasal organ epithelium.

Fig. 1. Nose of an untreated (no IFN) BALB/cByJ mouse 2 days after inoculation of MHV-JHM. MHV antigen is present in respiratory epithelial cells, particularly at the apical cell surfaces. Immunoperoxidase and hematoxylin. × 410.

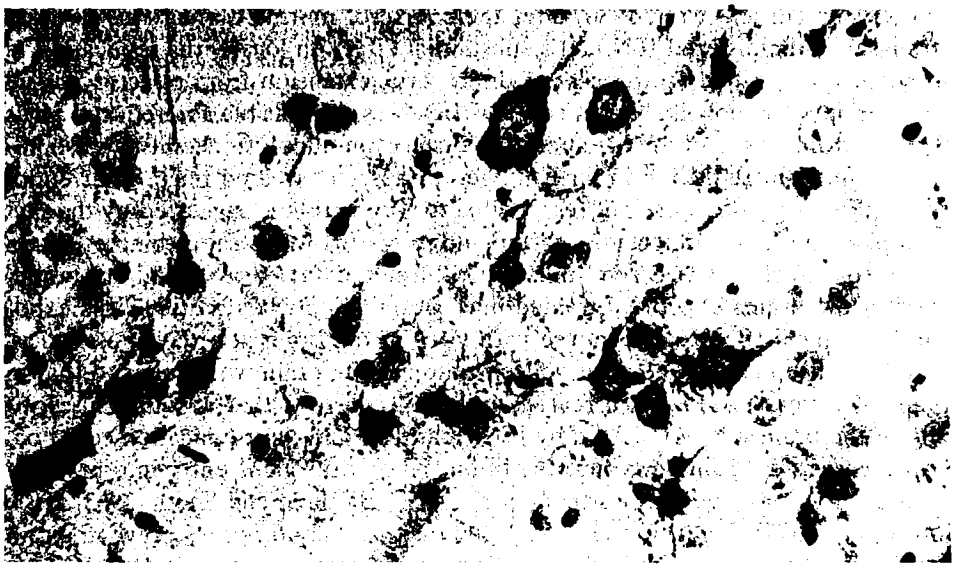

Fig. 2. Brain of an untreated (no IFN) BALB/cByJ mouse 5 days after intranasal inoculation of MHV-JHM. MHV antigen is present in cytoplasm of neuronal and glial cell bodies and processes. Immunoperoxidase and hematoxylin. × 410.

The spleens of all IFN-treated and untreated mice examined on day 3 contained MHV, and there was not a detectable difference in the mean virus titers in spleens of treated and untreated groups of mice (Table 1).

Interferon treatment substantially reduced the proportion of mice with virus in the brain (Table 1). The distinction between the treated and untreated mice was most marked on day 5 ($P < 0.005$), an interval when MHV titers and encephalitis peak (Barthold and Smith, 1987). Whereas all untreated or sham-treated mice contained virus in the brain, only one IFN-treated mouse harbored detectable infectious virus in the brain. The brain of this single mouse did, however, contain a concentration of virus similar to that in brains of mice in the untreated and sham-treated groups. Histologic examination confirmed that IFN treatment prevented morphologic changes associated with MHV replication in the brain. In brains of the untreated mice and the single interferon-treated mouse from which virus was recovered, necrotizing encephalitis was present in the olfactory bulb and anteroventral brain. Immunohistochemical staining revealed the presence of MHV antigen in the cytoplasm of neurons and glia in areas of encephalitis (Fig. 2). Neither antigen nor encephalitis was detected in virus-negative brains.

Discussion

Based on the data presented here, we conclude that IFN doses approximately 10-fold lower per unit weight than those reported in human clinical trials (Higgins

et al., 1983; Turner et al., 1986) alter the course of murine coronavirus disease. Intranasally administered IFN had a substantial effect on local virus replication in the nose and modified the process of direct virus extension from olfactory tissue to the brain. It is not likely that residual IFN was responsible for reduced virus recovery from noses of IFN-treated mice, since immunohistochemistry also revealed significant reduction in viral antigen in noses of treated mice. That endogenous IFN responses were not responsible for protection is suggested by the fact that alpha/beta IFN is detected in serum and spleen, not in nasal turbinates, brain or liver, of infected BALB/cByJ mice (unpublished data). Virus could not be detected in the brains of 9 of 10 treated mice examined at 5 days after virus exposure. The single IFN-treated mouse with virus in the brain at 5 days post-inoculation had encephalitis, and virus concentration in the brain was similar to that in brains of untreated and sham-treated mice. This suggests that absolute susceptibility of the brain was not altered by IFN treatment and that replication could occur if the virus did gain entry into the brain. Since virus gains entry into brain by extension from nasal tissues (Barthold et al., 1987; Barthold and Smith, 1983, 1987; Goto et al., 1977; Taguchi et al., 1977, 1979), it is likely that IFN prevents brain infection by interference with virus replication in nasal tissues. Since MHV-associated mortality is largely due to encephalitis (Barthold and Smith, 1987), previous observations of a protective effect of intranasally administered IFN (Garlinghouse and Smith, 1985) appear to be due to this mechanism. Despite the local effect of IFN in nose and brain, blood-borne transit to secondary organs such as the spleen was unaffected by the treatment regimen used in this study. Since MHV replicates in many different tissue types (Barthold and Smith, 1987), nasal infection is probably not a requisite for dissemination to other organs.

Acknowledgements

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