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Humoral Immune Response and Protection from Viral Infection in Mice Vaccinated with Inactivated MHV-68: Effects of Type I Interferon

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ABSTRACT

Infection of mice by murine gammaherpesvirus 68 (MHV-68) represents a suitable animal model in which to investigate the immune response against gammaherpesviruses and to test the efficacy of vaccination strategies. In this study, we evaluated the efficacy of heat-inactivated MHV-68 as a vaccine as well as the adjuvant activity of type I interferon (IFN-I) administered together with the vaccine. Mice vaccinated with inactivated MHV-68 and subsequently infected with the virus exhibited a significant augmentation of the virus-specific humoral immune response and a considerable inhibition of MHV-68 acute replication in the lungs compared with unvaccinated control mice. The coadministration of IFN-I with inactivated MHV-68 significantly enhanced the humoral immune response elicited by the vaccine by stimulating the production of virus-specific IgG2 antibodies but did not significantly enhance protection from viral challenge. We conclude that IFN-I, recently shown to exhibit a powerful adjuvant activity to a poorly immunogenic subunit vaccine in mice, can also enhance the humoral immune response when used as adjuvant of an inactivated viral vaccine, even though this effect is less marked as a result of the strong immune response elicited by the inactivated virus alone, which may also involve the contribution of endogenous IFN.

INTRODUCTION

THE INCIDENCE OF HUMAN DISEASES associated with gammaherpesviral infections has led to considerable interest in developing vaccines to control this class of viral infections.[1] However, the study of gammaherpesviruses suffered for many years the lack of a relevant small animal model system. Murine gammaherpesvirus (MHV-68) is a natural pathogen of free-living small rodents able to infect inbred strains of mice.[2] Studies on the genetic and pathogenic features of MHV-68 revealed a close relationship with human gammaherpesviruses, namely, the Epstein-Barr virus (EBV) and human herpesvirus-8 (HHV-8).[3–7] Inoculated intranasally (i.n.) into mice, MHV-68 establishes a productive infection in lung alveolar epithelial cells that lasts for 7–10 days and a subsequent latent infection in the spleen,[3–9] where an intense lymphoproliferation of B and T cells and an expansion of Vβ4$^+$ CD8 T lymphocytes occurs.[10] The role of the immune response in the control of MHV-68 infection has been almost completely elucidated.[3,11] T cells appear to be involved in the early immune-mediated control of both the acute and latent phases of MHV-68 infection,[11] whereas humoral immune responses intervene in antiviral surveillance at late times postinfection. Antibody production occurs between days 15 and 20 postinfection.[3] These pathogenic and immunologic features render the MHV-68 model an amenable small animal model for the study of gammaherpesvirus biology and a versatile, highly defined experimental setting in which to explore the immune response against gammaherpesvirusesand to define the requirements for effective control against their infection. Indeed, recent studies made use of the MHV-68 model of infection to verify the efficacy of various immunization strategies in protecting against MHV-68 infection.[12–14]

Vaccines based on whole inactivated viruses may represent an advantageous strategy with respect to the use of recombinant or purified viral proteins or subunits, as they contain a mixture of virtually all viral proteins and can prime the immune system against multiple viral antigens of immunologic relevance. Notably, the use of inactivated viral particle does not imply the risk of infection associated with living attenuated viral vaccines.

The efficacy of any vaccine against infectious diseases is strongly dependent on the identification of potent adjuvants. In

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recent years, the use of cytokines as natural adjuvants has received particular attention because of their important role in the development and regulation of the immune response.[15] Recently, renewed attention has been focused on type I interferons (IFN-I) as cytokines endowed with potent adjuvant activity.[16–18] IFN-I were originally described as antiviral substances, which are rapidly produced following viral infection and play a key role in inhibiting viral replication by inducing an antiviral state in target cells. IFN-I also exhibit antitumor, antiproliferative, and immunomodulatory properties.[19] Studies published in the past decade proved that IFN-I, in addition to being important modulators of the early innate response against infections and tumors,[19,20] play an important role in regulation of the adaptive immune response and in the generation and maintenance of specific immunologic memory against both viral antigens and tumor cells.[21] Le Bon et al.[16] showed that IFN-I coinjected as an adjuvant with a soluble protein potently enhances the primary antibody response to the antigen and also induces long-lived antibody production and immunologic memory. In a recent study, Proietti et al.[18] reported that IFN-I was highly effective as vaccine adjuvant in a model of influenza viral infection in mice. In light of this evidence, it was of interest to evaluate whether these cytokines could also exert some adjuvant activity using an inactivated virus as a vaccine. The study reported here represents the first example of the use of heat-inactivated MHV-68 as a vaccine. We show that mice vaccinated with inactivated MHV-68 and subsequently infected with the virus exhibited a marked augmentation of the virus-specific humoral immune response associated with considerable inhibition of MHV-68 acute replication in the lungs and that the use of IFN-I as adjuvant results in a significant enhancement of the antibody response in the infected animals.

MATERIALS AND METHODS

Mice

C57BL/6 mice (H-2^b) were purchased from Banton and Kingman Universal Limited (Grimston, Aldborough, Hull, U.K.).

Cell lines

BHK-21 cells were grown in Glasgow's minimum essential medium (GMEM) complemented with 10% newborn calf serum (NBCS), 10% tryptose phosphate broth (TPB), penicillin/streptomycin (70 μg/ml/10 μg/ml respective final concentrations), and L-glutamine (2 mM final concentration).

Interferons

High-titer IFN-α/β (2×10^7 U/mg protein) was obtained using a method adapted from Tovey et al.[22] Briefly, confluent C243-3 cells were primed by the addition of 10 U/ml IFN in MEM enriched with 10% fetal bovine serum (FBS) and 1 mM sodium butyrate and incubated at 37°C and 0.5% CO_2. Sixteen hours later, C243-3 cells were infected by Newcastle disease virus (NDV) (multiplicity of infection [moi] of 1) in MEM + 0.5% FBS + 5 mM theophylline. Culture supernatant was collected 18 h postinfection and centrifuged at 1500 rpm for 10 min. IFN was concentrated and partially purified by ammonium sulfate precipitation and dialysis against phosphate-buffered saline (PBS).

Viral stocks and viral infections

MHV-68 was originally obtained from Professor D. Blaskovic.[2] Working stocks of MHV-68 were prepared by infection of BHK-21 cells with MHV-68 clone g2.4[6] at low moi (0.01 plaque-forming units [pfu]/cell), as previously described.[7] Male and female mice were infected at 4–11 weeks of age i.n. with 4×10^5 pfu of MHV-68 under halothane anesthesia.

Vaccination

Inactive MHV-68 was obtained by incubating viral stock at 65°C for 30 min in a waterbath. Plaque assays were used to confirm that live virus was not detectable after heat treatment. Prior to vaccination, all mice were anesthetized using halothane. Mice were then administered a subdermal injection of 1×10^7/pfu inactivated MHV-68 in the nape of the neck alone or in association with 200,000 IU IFN-α/β per injection, administered on the day of vaccination and on the 2 subsequent days. Mice receiving two vaccinations were boosted 2 weeks later with an identical administration of inactivated virus, alone or in association with 200,000 IU IFN-α/β per injection, administered on the day of vaccination and on the 2 subsequent days. Four weeks after the second vaccination, all mice were challenged i.n. with 4×10^5 pfu MHV-68.

Viral assays

Infectious viral titers in the lungs were determined by plaque assays as previously described.[4] Briefly, lungs were frozen at −70°C, thawed, and homogenized and then refrozen at −70°C prior to assay. After thawing, the homogenates were centrifuged to remove particulate matter, and serial dilutions of homogenized lung tissues were added to BHK-21 cells and incubated for 4 days. Plaques were counted after formal 4% saline fixation and 0.1% toluidine blue staining of the monolayers.

ELISA for anti-MHV-68 antibody

Serum samples were prepared from blood obtained by cardiac puncture. Dynatech Immulon 4 Plates (McLean, VA) were coated with rabbit hyperimmune anti MHV-68 serum[4] in carbonate/bicarbonate buffer at pH 9.8 overnight at 4°C. Plates were washed six times in borate-buffered saline plus 0.001% Tween 20 (BBS-Tween) after incubation with each reagent. Blocking of nonspecific binding was accomplished by incubating with 2% normal rabbit serum at 37°C for 1 h. UV-irradiated MHV-68 was added to each well in BBS-Tween plus 1% normal rabbit serum at a concentration of approximately 10^7 pfu/ml. Twofold serial dilutions of mouse sera were prepared in the range 1:40–1:320, added to the plates, and incubated at 37°C for 90 min. Normal mouse serum and mouse hyperimmune anti-MHV-68 serum acted as negative and positive controls, respectively. Specific binding was detected with either horseradish peroxidase (HRP)-conjugated rabbit antimouse immunoglobulins or HRP-conjugated rabbit antimouse IgM, IgG1, IgG2a, or IgG2b-specific immunoglobulin (Dako, Carpinteria, CA). Plates were developed using Sigma Fast

tablets (St. Louis, MO), the reaction was stopped after 10–15 min using 12.5% H_2SO_4, and absorbance was quantitated at 490 nm using a Dynatech MR5000 ELISA plate reader. A standard curve was drawn for the positive control serum, and the equation of this curve was used to extract the arbitrary antibody titer from the OD value of each sample.

Statistical analysis

Data were analyzed by Mann-Witney test. p values are indicated in the Results section.

RESULTS

Total anti-MHV-68 antibody production in mice vaccinated with heat-inactivated MHV-68 alone or in association with IFN-I

In a first set of experiments, mice were subjected to a single vaccination with heat-inactivated MHV-68 alone or together with IFN-I, administered on the day of vaccination and on the 2 subsequent days. Four weeks later, vaccinated mice as well as control untreated mice were infected i.n. with MHV-68. Sera collected at different times after the infection were tested for the presence of total anti-MHV-68 antibodies. The results (Fig. 1A) indicated that no significant antibody response was detectable in any of the groups at early time points after MHV-68 infection (day 4 and day 6 postinfection). A similar increase in the titer of virus-specific antibodies occurred in all groups of mice from day 6 to day 11. On day 14 postinfection, the sera of mice vaccinated with inactivated virus together with IFN-I showed a statistically significant enhancement of virus-specific antibody titer compared with both the control mice ($p < 0.03$) and the mice vaccinated with inactivated virus alone ($p < 0.05$). Moreover, the peak level of serum antibody was found earlier (day 14 postinfection) in the sera of mice receiving IFN together with the vaccine compared with control mice or mice vaccinated with inactivated virus alone (day 26). These results indicated that coadministration of IFN-I with a single injection of heat-inactivated MHV-68 led to an increase in the virus-specific humoral response after infection.

We then evaluated the effects on the humoral immune response exerted by IFN-I when coadministered with each of two subsequent immunizations with inactivated-virus MHV-68 (Fig. 1B). Four days after infection with MHV-68, the sera obtained from mice vaccinated either in the absence or in the presence of IFN-I contained higher titers of virus-specific antibodies compared with sera from control mice ($p < 0.03$), suggesting that an antiviral humoral immune response was elicited by the vaccination prior to infection. Overall, the levels of anti-MHV-68-specific antibodies in the sera of both groups of vaccinated mice stayed higher than those of control mice during the whole course of the infection. Interestingly, in the sera of mice vaccinated in the presence of IFN-I, the virus-specific antibody titer peaked on day 11, 3 days earlier than in mice vaccinated with inactivated virus alone.

Taken together, these results show that IFN-I exerted a significant adjuvant effect when administered together with heat-inactivated MHV-68, increasing the level of virus-specific antibodies when administered together with a single injection of vaccine and anticipating the achievement of the antibody peak level when given in association with two injections of the vaccine.

Anti-MHV-68 antibody isotypes produced in mice vaccinated with heat-inactivated MHV-68 alone or in association with IFN-I

To characterize the antibody isotype, sera collected from mice on day 11 postinfection were analyzed by ELISA using antimouse IgM, IgG1, IgG2a, or IgG2b-specific immunoglobulins. As shown in Figure 2A, the sera of mice vaccinated with a single injection of inactivated MHV-68 contained significantly increased levels of IgG1 compared with unvaccinated mice ($p < 0.05$). Interestingly, in the sera of mice vaccinated with a single injection of heat-inactivated MHV-68 in association with IFN-I, a significant enhancement in the titer of IgG2a, persisting on day 14 postinfection (data not shown), was observed compared with untreated mice ($p < 0.03$) or mice injected with inactivated virus alone ($p < 0.05$). Moreover, a significantly higher titer of virus-specific IgG2b was found in the sera of mice receiving IFN-I in association with the vaccine compared with control mice ($p < 0.05$). A similar increase in MHV-68-specific IgG2b was induced by vaccination with inactivated virus alone, although it was not significantly different from the level found in control mice. Figure 2B shows the results of the isotype assays on the sera of mice vaccinated with two injections of inactivated virus with or without IFN-I. Vaccination of mice with inactivated virus alone induced a marked increase in the serum levels of IgG1 and IgG2a, and no significant difference in the levels of these antibody isotypes was observed in the sera of mice receiving IFN-I in association with the vaccine. In contrast, the titer of IgG2b antibodies appeared significantly higher in the group of mice that received IFN-I together with the two injections of vaccine compared with control mice ($p < 0.03$) and mice vaccinated with the inactivated virus alone ($p < 0.03$).

These observations indicated that IFN-I administered as an adjuvant together with heat-inactivated MHV-68 enhanced the magnitude of the humoral immune response elicited by the vaccine by preferentially increasing the production of IgG2a and IgG2b virus-specific antibodies.

MHV-68 replication in the lungs of mice vaccinated with heat-inactivated MHV-68 alone or in association with IFN-I

To verify whether the vaccination with heat-inactivated MHV-68 alone or together with IFN-I could confer some protection against the acute phase of the infection, we evaluated the extent of MHV-68 replication in the lungs of vaccinated and control mice. Lung viral titers were assayed 4 and 6 days postinfection, when lung viral titer reaches its peak,[3] and the results of the assays are shown in Figure 3. On day 4 postinfection, mice receiving two vaccinations with inactivated MHV-68 showed a significant reduction in lung viral titer compared with untreated mice ($p < 0.03$). In contrast, no significant difference in lung viral titer was found in mice receiving one injection of the vaccine with respect to control mice. Interestingly, on day 6 postinfection, both groups of mice vaccinated with either a single or two injections of inactivated MHV-68 exhibited similar and significantly lower titers of virus in the

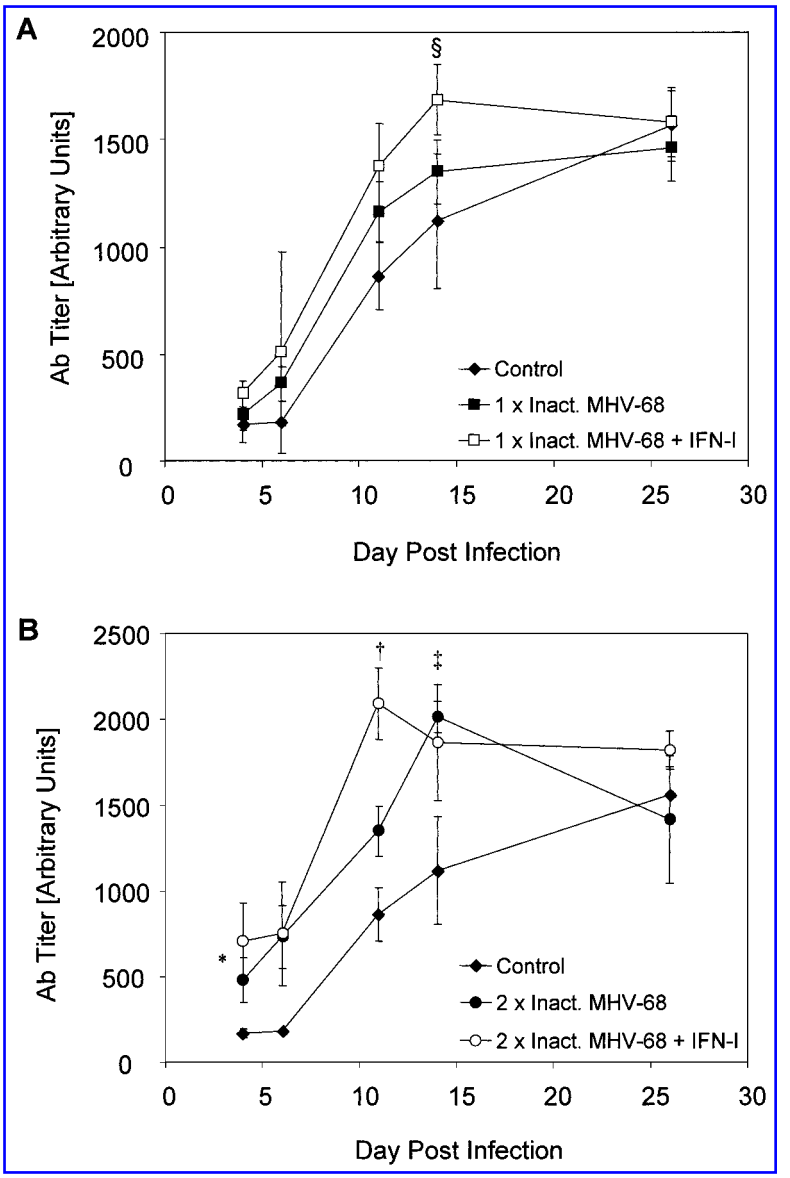

FIG. 1. Total anti-MHV-68 antibody production in control mice and mice vaccinated with one or two doses of inactivated MHV-68 in the presence or absence of IFN-I. Groups of four C57BL/6 mice were injected subcutaneously (s.c.) with 1×10^7 pfu heat-inactivated MHV-68, alone or in association with IFN-α/β (200,000 IU/injection), administered on the day of vaccination and on the 2 subsequent days (**A**). Groups of four C57BL/6 mice received two s.c. injections, 2 weeks apart, of heat-inactivated MHV-68, alone or in association with IFN-α/β (200,000 IU/injection), administered on the day of the first vaccination and on the 2 subsequent days (**B**). A group of untreated mice served as control group for MHV-68 infection. Four weeks after the second vaccination, all mice were challenged i.n. with 4×10^5 pfu MHV-68. At different times after the infection, sera were collected from vaccinated and control mice and tested for the presence of total anti-MHV-68 antibodies by ELISA as described in Materials and Methods. The mean antibody titer (expressed in arbitrary units) ± standard deviation (SD) for four mice per group is shown for each time point. $^{\S}p < 0.05$ compared with control and 1 × inactivated MHV-68 group; $^{*}p < 0.03$ compared with control group; $^{\dagger}p < 0.03$ compared with 2 × inactivated MHV-68 and control groups; $^{\ddagger}p < 0.03$ compared with control group.

lung than did the control group ($p < 0.03$). No difference in the viral load in the lung was observed between the groups of mice subjected to a single or two vaccinations with inactivated virus alone and mice receiving IFN-I in association with the vaccine at any of the times considered. The results of this assay indicated that vaccination with heat-inactivated MHV-68 significantly reduces the extent of viral replication in the lung. This inhibition occurred earlier in mice receiving two injections of the vaccine compared with mice receiving a single vaccination.

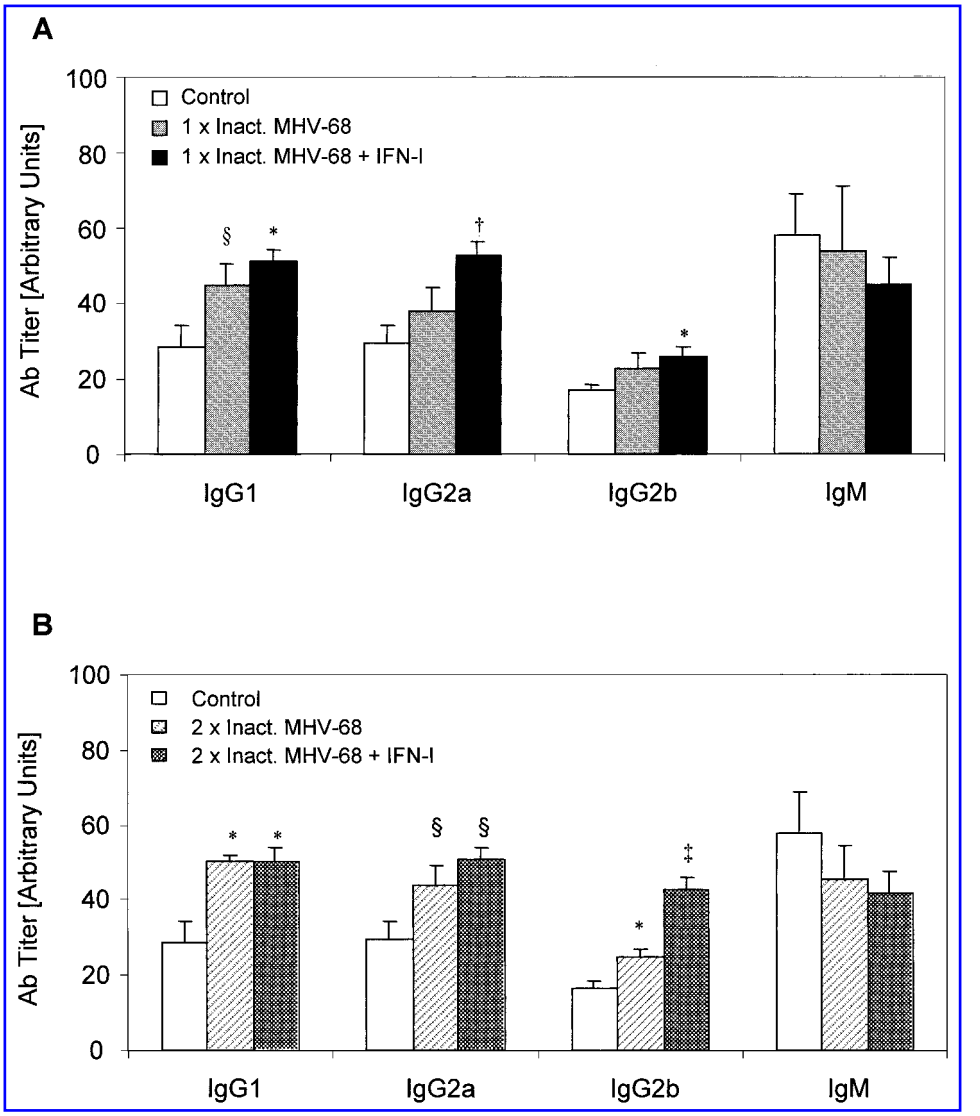

FIG. 2. Anti-MHV-68 antibody isotypes in the sera of mice infected with 4×10^5 pfu MHV-68 previously vaccinated as indicated in the legend to Figure 1. On day 11 after MHV-68 infection, sera were collected from vaccinated and control mice and tested for anti-MHV-68-specific isotypes as described in Materials and Methods. The mean antibody titer (expressed in arbitrary units) ± SD for four mice per group is shown for each group. $^{\S}p < 0.05$ compared to control group; $^{*}p < 0.03$ compared with control group; $^{\dagger}p < 0.05$ compared with control and 1 × inactivated MHV-68 group; $^{\ddagger}p < 0.03$ compared with control and 2 × inactivated MHV-68 groups.

DISCUSSION

The MHV-68 mouse model of infection has been used for evaluation of the efficacy of different immunization strategies potentially useful in protecting against infection with human gammaherpesviruses.[12–14] A significant inhibition of the peak levels of viral latency has been observed in mice immunized with recombinant vaccinia vectors encoding gp150,[12] the major viral membrane antigen, or with plasmid DNA encoding the MHV-68 latent antigen M2.[14] Concerning the acute phase of MHV-68 infection, a significant protection has been obtained in mice vaccinated with dendritic cells pulsed with peptide epitopes derived from MHV-68 antigens expressed during the lytic cycle.[13]

In the present study, we assessed the immunizing potential of inactivated MHV-68 as a vaccine and evaluated the adjuvant activity of IFN-I associated with this type of vaccine. Inactivation of viral particles is the basis for several vaccination regimens used successfully for many years.[23] Although inactivated viruses represent less defined immunogens compared with recombinant purified viral proteins or synthetic peptides, they offer the advantage of being a mixture of almost all viral proteins, with the potential of priming the immune system to a variety of antigens of immunologic significance. Our results show that a single immunization with heat-inactivated MHV-68 caused only a weak enhancement of the production of virus-specific antibodies compared with control mice at any time postinfection (Fig. 1A). This effect was probably related to a modest in-

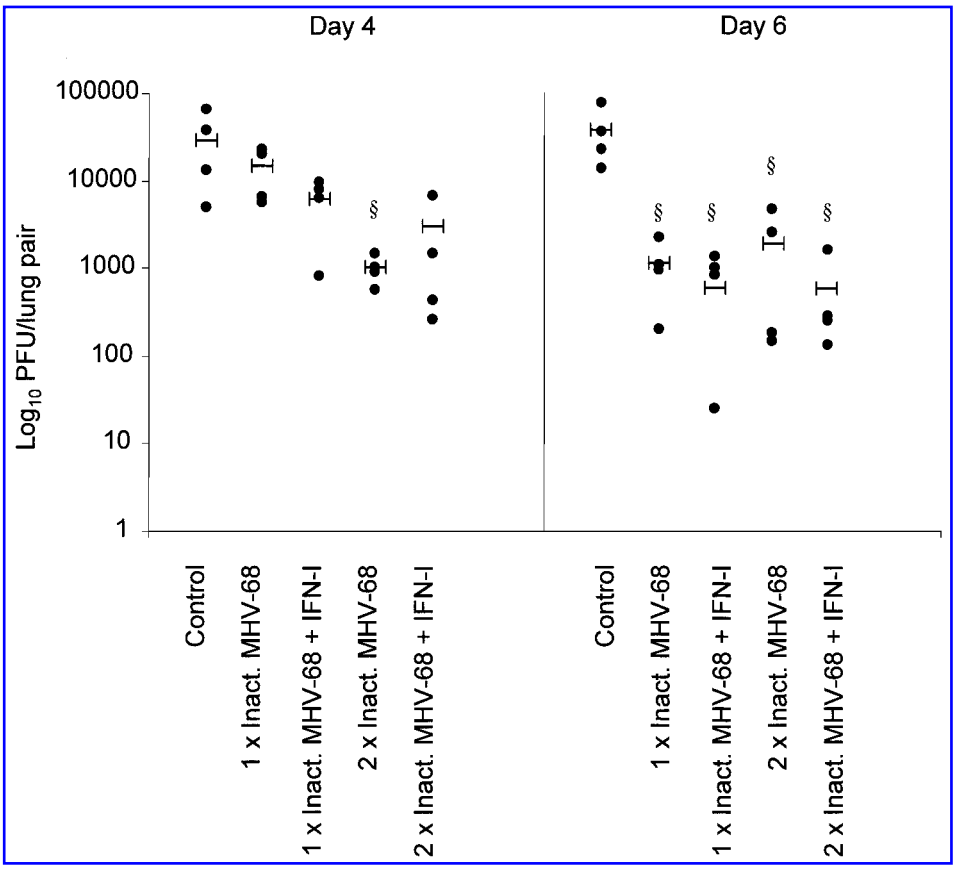

FIG. 3. Infective MHV-68 titers in the lungs of mice infected with 4×10^5 pfu MHV-68 previously vaccinated as indicated in the legend to Figure 1. On days 4 and 6 postinfection, lungs were collected from vaccinated and control mice, and the infectious viral titers were determined by plaque assays as described in Materials and Methods. The $\log_{10}$ of the mean viral titer per lung pair ± SD for four mice per group is shown for each time point. $^{\S}p < 0.03$ compared with control group.

crease in the levels of IgG1 and IgG2b (Fig. 2A). Two injections of inactivated MHV-68 vaccine caused the induction of a significant anti-MHV-68 humoral immune response prior to infection (Fig. 1B), followed by enhancement of the humoral immune response against MHV-68 during the whole time course of the infection compared with control mice (Fig. 1B).

It is well known that most live viruses elicit strong immune response, whereas nonliving vaccines are poorly immunogenic unless supplemented with exogenous adjuvants. In this study, we show that IFN-I exerts a significant adjuvant effect when administered together with a single injection of heat-inactivated MHV-68 by enhancing the virus-specific humoral response induced by the vaccine (Fig. 1A). This enhancement was associated with a more evident increase in the serum levels of IgG1 and IgG2b, with respect to the mice vaccinated with the inactivated virus alone, and with a significant induction of IgG2a, observed exclusively in mice receiving IFN-I together with the vaccine (Fig. 2A). Significant adjuvant effects were also observed when IFN-I was administered with each of two injections of the vaccine. In fact, this vaccination regimen resulted in a significant anticipation of the achievement of the antibody peak level (day 11 postinfection) with respect to two vaccinations with inactivated virus alone (day 14 postinfection) (Fig. 1B). Whereas administration of IFN-I together with two vaccinations did not cause a further enhancement of the virus-specific IgG2a antibodies with respect to two injections of the vaccine alone (Fig. 2B), a statistically significant increase in the level of IgG2b was specifically observed in mice receiving the cytokine as an adjuvant, suggesting that this antibody isotype might be responsible for the enhancement of the humoral response exerted by IFN-I (Fig. 2B).

The effect of IFN-I on the humoral response has been controversial for many years, with some authors showing an inhibitory effect on antibody production and others finding a modest stimulation exerted by this cytokine.[24–29] In two recent studies,[16,18] however, IFN-I was shown to potently enhance humoral immunity against a soluble antigen[16] or to the subunit influenza vaccine[18] when coinjected with the immunogen in mice and to markedly promote the switching toward the IgG2a.[16,18] In our study, we show that IFN-I used an adjuvant in a heat-inactivated virus-based vaccination regimen enhances the humoral immune response elicited by the vaccine by stimulating the production of virus-specific IgG2 antibodies. The ability of IFN-I to affect the isotype of the antibody production has been shown previously in mouse models.[30,31] Of interest, early studies had shown that the injection of IFN-I in tumor-bearing immunocompetent mice caused tumor rejection, which was mediated by host mechanisms and associated with the in-

duction of tumor-specific antibodies of the IgG2a isotype.[21,30] Finkelman et al.[31] found that administration of IFN-α during a large polyclonal Ig response was responsible for a switch toward the IgG2a isotype. Antibodies of the IgG2 isotype are known to be characteristic of the immune response to viral infection, as they effectively activate complement,[32] exhibit neutralizing activity,[33] and cooperate with antibody-dependent effector cells in inducing a protective immune response.[34] Therefore, the specific induction of IgG2 antibodies by IFN-I, reflecting a shift toward a Th1 type of immune response, supports the efficacy of this cytokine when administered as an adjuvant.

The heat-inactivated MHV-68 vaccination regimen used in our study effectively inhibited the acute replication of MHV-68 in the lungs of vaccinated mice (Fig. 3). In a recent report, significant inhibition of the acute phase of the infection, similar to that achieved in our study, was obtained in mice immunized with dendritic cells, pulsed with epitopes derived from MHV-68 lytic cycle antigens, and subsequently boosted with the peptides.[13] In our experimental setting, two injections of inactivated MHV-68 appeared to be more effective than a single injection in protecting against the acute phase of MHV-68 infection in the lung. In fact, early after the infection (day 4), a significant reduction of viral load in the lung was observed in mice subjected to two vaccinations compared with control mice, whereas in mice receiving a single injection of the vaccine, a similar inhibition of viral replication was found at a later time (day 6) (Fig. 3). On day 4 postinfection, significant levels of MHV-68-specific antibodies were present exclusively in the sera of mice vaccinated with two injections of inactivated virus (Fig. 1B). This observation suggests that the humoral response induced by this vaccination regimen can mediate the early protection from MHV-68 replication in the lung observed in these mice, even though we have not performed an antibody neutralization assay to support this suggestion. On day 6 postinfection, when similar titers of virus-specific antibodies were found in the sera of mice subjected to a single or two vaccinations (Fig. 1), no difference in the viral load in the lung was observed between the two groups of vaccinated mice (Fig. 3).

The adjuvant effect exerted by IFN-I on the immune response elicited by heat-inactivated MHV-68 was not associated with an improved inhibition effect on replication of the virus in the lungs (Fig. 3). In fact, mice receiving IFN-I at the time of vaccination showed MHV-68 viral loads in the lung similar to those of mice injected with the vaccine alone (Fig. 3). This result is not surprising for at least two reasons. First, no direct effect on viral replication in the lungs could be exerted by the cytokine under our experimental conditions, as it was administered 4 weeks prior to viral infection. Second, the IFN-induced increase in the virus-specific humoral response occurred after day 6 postinfection, when it is known that the MHV-68 acute infection in the lung rapidly declines and resolves.[3] Notably, it is possible to assume that the enhancement of the virus-specific humoral immune response observed in mice vaccinated with two injections of inactivated MHV-68 is due, at least partially, to the induction of endogenous IFN-I production. In fact, it might be possible to assume that inactivated MHV-68 can induce IFN. The importance of endogenous IFN-I in the control of MHV-68 acute replication in the lungs was reported by Dutia et al.,[35] who showed that mice unresponsive to IFN-I are highly susceptible to the virus and exhibit 100–1000-fold higher lung viral titers than wild-type mice. Moreover, we have recently found that mice knockout for type I IFN receptor exhibit a defective immune response when immunized with a reference antigen in the presence of adjuvants,[18] suggesting an important role for endogenous IFN-I in the generation of a protective immune response. In the light of this evidence, it is tempting to speculate that IFN-I endogenously produced in mice vaccinated with heat-inactivated MHV-68 may contribute to the effects ascribed to the vaccine *per se*, thus diminishing the importance of exogenously administered IFN-I. On the basis of these considerations and taking into account that IFN-I exhibits a powerful adjuvant activity when administered in combination with either proteins of low immunogenicity[16] or subunit vaccines,[18] we conclude that the most promising use of IFN-I as an adjuvant of human viral vaccines could be represented by the association of these cytokines with virus-derived proteins.

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