Human Receptor for Measles Virus (CD46) Enhances Nitric Oxide Production and Restricts Virus Replication in Mouse Macrophages by Modulating Production of Alpha/Beta Interferon

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Complement regulatory protein CD46 is a human cell receptor for measles virus (MV). In this study, we investigated why mouse macrophages expressing human CD46 restricted MV replication and produced higher levels of nitric oxide (NO) in response to MV and gamma interferon (IFN-γ). Treatment of MV-infected CD46-expressing mouse macrophages with antibodies against IFN-α/β blocked NO production. Antibodies against IFN-α/β also inhibited the augmenting effect of MV on IFN-γ-induced NO production in CD46-expressing mouse macrophages. These antibodies did not affect NO production induced by IFN-γ alone. These data suggest that MV enhances NO production in CD46-expressing mouse macrophages through action of IFN-α/β. Mouse macrophages expressing a human CD46 mutant lacking the cytoplasmic domains were highly susceptible to MV. These cells produced much lower levels of NO and IFN-α/β upon infection by MV, suggesting the CD46 cytoplasmic domains enhanced IFN-α/β production. When mouse macrophages expressing tailless human CD46 were exposed to culture medium from MV-infected mouse macrophages expressing intact human CD46, viral protein synthesis and development of cytopathic effects were suppressed. Pretreating the added culture medium with antibodies against IFN-α/β abrogated these antiviral effects. Taken together, these findings suggest that expression of human CD46 in mouse macrophages enhances production of IFN-α/β in response to MV infection, and IFN-α/β synergizes with IFN-γ to enhance NO production and restrict viral protein synthesis and virus replication. This novel function of human CD46 in mouse macrophages requires the CD46 cytoplasmic domains.

Measles virus (MV) causes a common disease that accounts for about 10% of childhood mortality due to infectious diseases worldwide (5, 29). A major pathogenic factor of MV is its ability to suppress host cellular immune response, which can lead to severe secondary infections (6, 15). Monocytes and macrophages are major in vivo targets for MV in measles patients (10). These cells serve as a first line defense in the innate immune system against microbial pathogens (12, 26, 27). Interactions between MV and monocytes and macrophages therefore play a pivotal role in measles pathogenesis and host defense against MV. Immature human myelomonocytic cells support MV replication efficiently and produce infectious virus (16). By contrast, MV replication in monocytes and differentiated macrophages is highly restricted (16, 35, 37). The block in MV replication in those cells appears to be at both posttranscription and posttranslational levels (16). The mechanisms by which monocytes and macrophages suppress MV replication have not been characterized.

We recently established a system for studying the interactions between MV and mouse macrophages. Human complement regulatory protein CD46, a receptor for laboratory-adapted MV (9, 30), was expressed in RAW264.7 mouse macrophages. As expected, expression of human CD46 facilitated MV entry into mouse macrophages. Surprisingly, MV protein synthesis and virus production were more severely restricted in mouse macrophages expressing human CD46 than in CD46-negative mouse macrophages (20). Subsequently, we showed that mouse macrophages expressing human CD46 produced higher levels of nitric oxide (NO) than CD46-negative mouse macrophages when infected by MV in the presence of gamma interferon (IFN-γ) (17). Interestingly, deleting the CD46 cytoplasmic domains markedly attenuated NO production in mouse macrophages and rendered these cells highly susceptible to MV infection (17). NO has potent antimicrobial activities against a wide range of DNA and RNA viruses (32). These results raise the possibility that CD46 can augment antiviral functions in macrophages. To gain further insight into this phenomenon, we examined the IFN-α/β response in mouse macrophages expressing human CD46 upon MV infection, since IFN-α/β is important for antiviral defense against a wide range of viruses, including MV (22, 36).

In this study, we show that mouse macrophages expressing human CD46 produce IFN-α/β upon MV infection. Blocking IFN-α/β action by neutralizing antibodies against IFN-α/β reverses the inhibition on MV protein synthesis and intensifies viral cytopathic effects (CPE). These antibodies also abrogate the augmenting effect of MV on NO production in mouse macrophages expressing human CD46. Deleting the CD46 cytoplasmic domains greatly attenuates production of IFN-α/β from mouse macrophages upon MV infection but does not prevent these cells from acquiring an antiviral state when treated with culture fluid from MV-infected mouse macrophages bearing intact human CD46. These results provide evidence that human CD46 affects NO production and MV replication in mouse macrophages by modulating production of IFN-α/β.
MATERIALS AND METHODS

Cells. RAW264.7 mouse macrophages stably expressing human CD46 with the Cyt1 cytoplasmic domain or a tailless CD46 mutant were generated as described previously (17, 20). Cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) ( Gibco BRL, Grand Island, N.Y.) and 400 μg of the monoclonal analogue G18 (GIBCO BRL) per ml. Murine cell line L929 cells (gift from Masae Itoh, Osaka Public Health Institute) were maintained in Eagle’s minimum essential medium supplemented with 10% FBS.

Reagents. Recombinant murine IFN-γ was purchased from Pharmingen (San Diego, Calif.), and anti-mouse IFN-α/β antiserum and normal rabbit serum were purchased from Lee BioMolecular Research Laboratory (San Diego, Calif.) and Sigma Chemical Co. (St. Louis, Mo.), respectively.

Virus infection. Edmonston strain MV stocks were propagated in African green monkey kidney (CV-1) cells cultured in Eagle’s minimum essential medium supplemented with 10% newborn calf serum (GIBCO BRL). Mouse macrophages plated 6 h prior to infection were infected with MV at a multiplicity of infection (MOI) of 0.5, 5, and 50 for NO assay as described in Materials and Methods. Bars atop the columns show standard deviations from triplicate samples.

RESULTS

Neutralizing antibodies to IFN-α/β inhibit MV-induced NO production in mouse macrophages expressing human CD46. Mouse macrophages expressing human CD46 with the Cyt1 cytoplasmic domain (A24 line) produce high levels of NO in response to IFN-γ. This response is enhanced upon infection by MV (17). We investigated whether virus-induced IFN-α/β played a role in enhancement of NO production in mouse macrophages, using neutralizing antibodies against IFN-α/β.

A24 mouse macrophages were infected with MV Edmonston strain at an MOI of 0.5 and cultured in the presence or absence of antibodies against mouse IFN-α/β. The next day, the cells were replenished with fresh medium or without antibodies against IFN-α/β in the culture medium by a colorimetric method as described previously (17).

Bioassy of IFN-α/β. Production of IFN-α/β was measured by endpoint dilutions that suppress vesicular stomatitis virus (VSV) CPE on L929 cells using a colorimetric method as described previously (17). Neutralizing antibodies to IFN-α/β were added at 100 U/ml. Every day after infection, culture medium was collected for NO assay as described in Materials and Methods. Bars atop the columns show standard deviations from triplicate samples.

FIG. 1. Effects of antibodies against IFN-α/β on NO production in mouse macrophages. Mouse macrophages expressing human CD46 with the Cyt1 cytoplasmic domain (A24 line) were infected with the Edmonston strain of MV at an MOI of 0.5 and cultured in medium containing anti-IFN-α/β antibodies or normal rabbit serum. The next day, cells were replenished with fresh medium containing anti-IFN-α/β antibodies or normal rabbit serum in the absence or presence of IFN-γ. On day 2 or 3 after infection, culture medium was collected for NO assay as described in Materials and Methods. Bars atop the columns show standard deviations from triplicate samples.

Neutralizing antibodies to IFN-α/β enhance MV protein synthesis and CPE in mouse macrophages expressing human CD46. MV replication in mouse macrophages expressing human CD46 is restricted by inhibition of viral protein synthesis (20). To see whether this phenomenon is due to IFN-α/β, we examined viral protein synthesis in MV-infected A24 mouse macrophages in the presence or absence of antibodies against IFN-α/β.

Cellular proteins were labeled with [3S]methionine and immunoprecipitated with antibodies specific for the MV N protein. The N protein is translated from the most abundant MV mRNA species, and the N protein level is a good indicator of viral protein synthesis (17). Combining our previous report (20), MV N protein was maximally synthesized on the first day after infection (Fig. 2A, lane b), and viral protein synthesis declined on days 2 and 3 after infection (Fig. 2A, lanes c and h). The accumulation and decline of viral proteins were more gradual than we have previously observed, because the macrophages were infected at a lower MOI (0.5 instead of 2) in the present study. Most important, antibodies against IFN-α/β significantly enhanced the levels of MV N protein synthesis especially on days 2 and 3 after infection, when viral protein synthesis was typically restricted in the absence of antibodies against IFN-α/β (Fig. 2A, compare lanes c, f, and i to lanes b, e, and h).

Analysis of the gel bands in repeated experiments by the NIH Image program revealed that antibodies against IFN-α/β have restricted the levels of MV N protein synthesis especially on days 2 and 3 after infection. Most important, antibodies against IFN-α/β significantly enhanced the levels of MV N protein synthesis especially on days 2 and 3 after infection, when viral protein synthesis was typically restricted in the absence of antibodies against IFN-α/β (Fig. 2A, compare lanes c, f, and i to lanes b, e, and h).

MV caused a self-limiting infection in A24 mouse macrophages with localized multinucleated giant cells (syncytia) on days 2 and 3 after infection. Most of the syncytia contained fewer than 10 nuclei (Fig. 2B, panels b and e). Uninfected A24 macrophages exhibited no syncytia (Fig. 2B, panels a and d). Treatment with antibodies against IFN-α/β markedly intensified syncytium formation on days 2 and 3 after infection. Many of the syncytia developed in the presence of antibodies against IFN-α/β contained more than 15 to 20 nuclei (Fig. 2B, panels c and f).
These results, which have been observed in many experiments, indicate that MV-infected A24 mouse macrophages produce IFN-\(\alpha/\beta\), which inhibits viral protein synthesis and prevents further development of CPE beyond the second day of infection. Thus, type I IFN is largely responsible for the self-limiting characteristic of MV replication in A24 mouse macrophages.

Cytoplasmic domains of human CD46 modulate production of IFN-\(\alpha/\beta\) in response to MV infection in mouse macrophages. To see whether CD46 affected the IFN-\(\alpha/\beta\) response, we compared the relative levels of IFN-\(\alpha/\beta\) produced by mouse macrophages expressing human CD46 (A24 line), a CD46 mutant lacking the cytoplasmic domains (C11 line), or no human CD46 (F7 line). These cells were infected with MV, and the culture medium was collected after 2 days and assayed for IFN-\(\alpha/\beta\) antiviral activities against VSV on mouse L929 cells. Preliminary studies showed that A24 cells produced 10,000 to 30,000 U of IFN-\(\alpha/\beta\) after MV infection, whereas F7 mouse macrophages produced only background levels (less than 80 U). This difference may be due simply to differences in efficiency of virus entry into CD46-positive versus CD46-negative cells. Interestingly, C11 mouse macrophages expressing tailless human CD46 produced much lower levels of IFN-\(\alpha/\beta\) (less than 2,000 U) than A24 cells. Since C11 cells are fully susceptible to MV infection (17), these results implicate a role for CD46 in modulating IFN-\(\alpha/\beta\) production.

We further compared the time courses of IFN-\(\alpha/\beta\) production in A24 versus C11 mouse macrophages after MV infection. A24 mouse macrophages produced high levels of IFN-\(\alpha/\beta\), which peaked on day 2 and declined on day 3 after infection.

FIG. 2. Effects of antibodies against IFN-\(\alpha/\beta\) on MV replication. (A) Mouse macrophages expressing human CD46 with the Cyt1 cytoplasmic domain (A24 line) were infected with the Edmonston strain of MV at an MOI of 0.5 and cultured in medium containing anti-IFN-\(\alpha/\beta\) antibodies or normal rabbit serum. On day 1, 2, or 3 after infection, cells were labeled with \([^{35}S]\)methionine for 3 h. The cell lysates were immunoprecipitated with a monoclonal antibody against the N protein of MV and analyzed by SDS-PAGE. (B) MV-infected or uninfected A24 mouse macrophages cultured in the medium containing anti-IFN-\(\alpha/\beta\) antibodies or normal rabbit serum were examined by phase-contrast microscopy on day 2 or 3 after infection.
infection (Fig. 3, lanes c and e). By contrast, C11 mouse macrophages produced low levels of IFN-α/β on day 2 and only moderate levels on day 3 after infection (Fig. 3, lanes d and f). These cells were destroyed by MV after the third day of infection (17) and never produced high levels of IFN-α/β. These data show that the cytoplasmic domains of human CD46 affect production of IFN-α/β upon MV infection in mouse macrophages.

**CD46 cytoplasmic domains do not influence the antiviral response to exogenous IFN-α/β.** IFN-α/β induces an antiviral state by binding to IFN-α/β receptors. To test whether the cytoplasmic domains of human CD46 influence the mouse macrophage response to IFN-α/β, we treated C11 mouse macrophages with culture medium from MV-infected A24 mouse macrophages and examined MV replication in the C11 cells. MV infection of C11 mouse macrophages led to extensive CPE, with many syncytia containing over a dozen nuclei (Fig. 4A, panel a). Treating those cells with antibodies against IFN-α/β did not prevent development of CPE (Fig. 4A, panel b), consistent with the finding that C11 mouse macrophages failed to produce type I IFN effectively (Fig. 3). The culture medium from MV-infected A24 mouse macrophages was collected on day 2 after infection, when maximum levels of IFN-α/β were induced (Fig. 3). The medium was incubated with either normal rabbit serum or neutralizing antibodies against IFN-α/β for 30 min at 37°C. The treated medium was then added to the C11 mouse macrophages 1 day after MV infection, and the cultures were incubated for another day. Culture medium from uninfected A24 mouse macrophages failed to suppress development of CPE in MV-infected C11 cells, regardless of whether the medium was treated with normal serum or neutralizing antibodies against IFN-α/β (Fig. 4A, panels c and d).

In the presence of culture medium from uninfected A24 cells, MV efficiently synthesized viral proteins in these cells, as shown by immunoprecipitation of the MV N protein (Fig. 4B, lanes b and c). By contrast, syncytium formation in the MV-infected C11 culture was greatly suppressed by incubation with culture medium from MV-infected A24 cells which had been treated with normal rabbit serum (Fig. 4A, panel e). Viral protein synthesis in these cells was also drastically suppressed (Fig. 4B, lane d). Treatment with neutralizing antibodies against IFN-α/β reduced the effectiveness of the culture medium from MV-infected A24 cells to inhibit development of CPE (Fig. 4A, panel f) or viral protein synthesis in C11 mouse macrophages (Fig. 4B, lane e).

Analysis of the gel bands by the NIH Image program revealed that the culture medium from MV-infected A24 cells suppressed MV N protein synthesis in C11 cells about 15-fold (Fig. 4B, compare lanes b and d). Treatment with antibodies against IFN-α/β alleviated much of the suppression, so that the treated MV-infected A24 medium reduced N protein synthesis in C11 cells only 1.8-fold (Fig. 4B, compare lanes c and e). These results suggest that the cytoplasmic domains of human CD46 modulate production of IFN-α/β in mouse macrophages in response to MV infection. However, the CD46 cytoplasmic domains are not required for mouse macrophages to acquire an antiviral state in response to exogenous IFN-α/β.

**DISCUSSION**

This study links human CD46 expression to IFN-α/β production in mouse macrophages and establishes the role of IFN-α/β in NO response and restriction of MV replication in these cells. These data therefore suggest opposite roles of human CD46 in MV infection. On one hand, CD46 serves as a receptor to facilitate MV entry. On the other hand, expression of human CD46 appears to enhance antiviral responses, including production of IFN-α/β and NO. The extracellular domains of human CD46 are sufficient to facilitate MV entry, but the cytoplasmic domains of CD46 are important for augmenting IFN-α/β and NO production. These findings lend further credence to the hypothesis that human CD46 may serve a host defense function that augments antimicrobial responses in macrophages (17, 38).

MV, a single-stranded RNA virus, presumably produces double-stranded RNA as a by-product during RNA transcription and replication (19) to induce IFN-α/β (24, 33, 40). As shown in Fig. 3, in mouse macrophages expressing human CD46 lacking cytoplasmic domains, MV replicates efficiently yet provokes low levels of IFN-α/β production. This finding suggests that virus replication is insufficient to maximally induce IFN-α/β production, and the cytoplasmic domains of CD46 provide additional stimuli to augment IFN-α/β production. We speculate that interaction between MV and its receptor may trigger signaling events that affect IFN-α/β and NO production in mouse macrophages. Preliminary data from our recent CD46 cross-linking studies are consistent with such a function (A. Hirano, unpublished data). Removing the cytoplasmic domains of the receptor may prevent this function, reducing IFN-α/β and NO responses in macrophages against MV. In this context, it is noteworthy that glycoproteins of other viruses have been implicated in IFN-α/β induction. For example, recombinant gp120 glycoprotein of human immunodeficiency virus type 1 (HIV-1) induces IFN-α production (1, 7), and antibodies against gp120 of HIV-1 or the cognate cellular receptor CD4 block HIV-1-induced IFN-α/β production in human peripheral blood mononuclear cells (13). Similarly, glycoprotein gD of herpes simplex virus type 1 has been shown to induce IFN-α production (2).

The present study further shows that IFN-α/β is largely responsible for NO production and restriction of viral protein synthesis in mouse macrophages upon MV infection. A likely scenario is that IFN-α/β acts as an autocrine or paracrine to stimulate NO production, and antibodies against IFN-α/β prevent this action. There is precedence for various cytokines acting synergistically with microbial products to stimulate inducible NO synthase (iNOS) gene expression (26). IFN-γ alone can induce iNOS expression in some murine macrophage lines, and it greatly augments iNOS expression together with bacterial lipopolysaccharide (LPS) (25, 39). Several studies have shown that neutralizing antibodies against IFN-α/β attenuate NO production in primary mouse macrophages and RAW264.7 cells in response to IFN-γ and LPS (14, 41, 42), even though adding exogenous IFN-α/β alone is insufficient to...
induce NO production from these cells (41). However, other investigators have observed an inhibitory effect of exogenous IFN-α/β on IFN-γ-induced NO production (8, 11, 21). Experimental conditions and timing of IFN treatment can affect the outcome of these experiments. For instance, treating murine peritoneal macrophages with IFN-α/β blocked the cooperative action of IFN-γ and LPS in NO expression through rapid decrease in NF-κB activity, but this effect was absent when IFN-α/β was added 2 h after IFN-γ and LPS treatment or when the cells were stimulated exclusively by LPS (23). Interestingly, when mouse macrophages expressing human CD46 are infected by MV in the absence of IFN-γ, antibodies against IFN-α/β almost completely block NO production (93 to 98%). However, when these cells are infected in the presence of IFN-γ, anti-IFN-α/β antibodies do not reduce NO to the same levels (65 to 75%) as that induced by IFN-γ alone (Fig. 1). This indicates that in the absence of IFN-γ, NO response of mouse macrophages to MV infection is almost completely dependent on IFN-α/β. Stimulation by IFN-γ enables mouse macrophages to produce NO in response to MV by mechanisms independent of IFN-α/β.

The implications of the present findings for MV infection in vivo remain to be determined. Earlier studies showed that transgenic mice expressing a human CD46 cDNA were non-permissive for MV, and macrophages from those animals were especially resistant to MV infection (18). Removal of IFN-α/β receptors from CD46-expressing mice facilitated MV replication and spread in those animals (28). These observations are consistent with our findings that human CD46 augments IFN-α/β production and restricts MV replication in mouse macrophages (this study and reference 20). More recently, transgenic mice that express high levels of multiple CD46 isoforms from a genomic CD46 clone have been generated, and those mice are susceptible to MV infection (31). Perhaps high levels of CD46 allow efficient spread of MV to overwhelm the host defense in these animals. Interestingly, the mouse CD46 homologue is expressed mainly in testicular germ cells, and it lacks most of the cytoplasmic sequences found in human CD46 (34). It is possible that the human and mouse CD46 homologues have evolved to serve different functions, and the IFN-modulating effect of human CD46 may be exerted by other molecules in mouse macrophages. It will be informative to define the sequences within the human CD46 cytoplasmic domains responsible for modulating IFN-α/β production in this system.

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REFERENCES