Antiviral Research, 12 (1989) 231–238
Elsevier

AVR 00384

Protective effect of rhinovirus receptor blocking antibody in human fibroblast cells

Steven J. Sperber[1,*] and Frederick G. Hayden[1,2]

Departments of [1]Internal Medicine and [2]Pathology, University of Virginia School of Medicine, Charlottesville, Virginia, U.S.A.

(Received 15 May 1989; accepted 28 August 1989)

Summary

Infection of HeLa cells by human rhinoviruses (RV) of the major receptor group is inhibited by a HeLa-derived rhinovirus receptor murine monoclonal antibody (RRMA). In yield reduction studies in human embryonic lung fibroblast cells, pretreatment with 1.0 or 10 μg/ml of RRMA partially protected (>90% titer reduction) against infection by RV 39 or coxsackie A21 (members of the major receptor family), but not by RV 1A (member of the minor receptor family). The protection afforded by RRMA persisted at least 72 h after a 2-h exposure. These results suggest that RV receptors can be effectively blocked for prolonged periods in cultured fibroblast cells.

Rhinovirus; Antiviral; Receptor; Monoclonal antibody

Introduction

Human rhinoviruses (RV), which currently number 100 distinct antigenic serotypes (Gwaltney, 1989), have been divided into two groups based on competitive cell binding assays (Abraham and Colonno, 1984; Colonno et al., 1986). Using HeLa R-19 cells and membranes as antigens, Colonno and coworkers were able to isolate a murine monoclonal antibody, designated rhinovirus receptor monoclonal antibody (RRMA), that protected HeLa cells from infection by nearly 90%

Correspondence to: Steven J. Sperber, M.D., Division of Allergy, Immunology and Infectious Diseases, UMDNJ- Robert Wood Johnson Medical School, One Robert Wood Johnson Place, New Brunswick, New Jersey 08903-0019, U.S.A.

**Present address*: Division of Allergy, Immunology and Infectious Diseases, Department of Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, New Jersey, U.S.A.

of RV serotypes (Colonno et al., 1986). RRMA protected HeLa cells against infection by members of the major RV receptor group and 3 coxsackie A (Cox A) viruses, but had no effect on RV of the minor group or other viruses. A membrane protein of approximately 90 kDa was subsequently isolated from HeLa cells and shown to be involved in attachment of RV of the major receptor group (Tomassini and Colonno, 1986). This receptor protein has recently been reported to be intercellular adhesion molecule 1 (ICAM-1), a member of the immunoglobulin superfamily (Greve et al., 1989; Staunton et al., 1989). In human trials, Hayden, Gwaltney and Colonno demonstrated modification of experimental rhinovirus type 39 colds in subjects given RRMA intranasally beginning prior to virus challenge (Hayden et al., 1988). We conducted in vitro experiments to evaluate the protective effect of RRMA in human embryonic lung fibroblast cells, a cell type different from which the antibody was initially produced, and to better define the concentration-related inhibition of virus replication.

RRMA, viruses and cells

RRMA, prepared from mouse ascites fluid at Merck, Sharp and Dohme Research Laboratories, was kindly supplied by R.J. Colonno. Ten-fold dilutions to final concentrations of 10, 1.0 and 0.1 μg of antibody/ml were prepared using MEM with 5% fetal bovine serum and antimicrobics as a diluent. The viruses were originally obtained from clinical specimens and included representatives of the major receptor group, RV 39 (passage history WI-38/2, MRC-5/4) and Cox A21 (WI-38/2); and the minor receptor group, RV 1A (MK-5, WI-38/1, human fetal tonsil/1). Assays for RRMA activity were performed on monolayers of human embryonic lung fibroblast (WI-38) cells (purchased from Whittaker Bioproducts, Walkersville, MD) plated in 24-well tissue culture plates (Costar, Cambridge, MA). Titrations of the harvests from the yield reduction assays were performed in monolayers of HeLa-M cells (supplied by R.J. Colonno) grown in 24-well tissue cultures plates.

Effects of RRMA concentration and timing of addition on replication of RV 39, RV 1A and Cox A21

The concentration-related activity of RRMA against RV 39, RV 1A, and Cox A21 in human fibroblast cells and the effect of treating the monolayers before and/or after virus adsorption were determined. For each of the three viruses, quadruplicate monolayers of WI-38 cells were exposed for 2 h at 33°C to 0.5 ml of MEM containing 10, 1.0, 0.1 or 0 μg/ml of RRMA, and then rinsed twice with Hank's balanced salt solution (HBSS). Approximately 100 $TCID_{50}$ (0.2 ml) of virus was adsorbed to the monolayer at 33°C for 1 h, which was subsequently rinsed twice with HBSS. The monolayers were then replenished with 0.5 ml of MEM containing 10, 1.0, 0.1, or 0 μg/ml of RRMA, such that quadruplicate monolayers

TABLE 1

Reduction in 24-h virus titer by 2-h treatment of human fibroblast (WI-38) cell monolayers with antibody to rhinovirus receptor (RRMA) before, after, or before and after infection with RV 39 or 1A or Cox A21

Virus/ RRMA (μg/ml)	Reduction in titer (log_{10} $TCID_{50}$/0.2 ml) compared to untreated control								
	Pre treatment			Post treatment			Pre/Post treatment		
	10	1.0	0.1	10	1.0	0.1	10	1.0	0.1
RV 39	1.75	1.25	0.875	<0.5	<0.5	<0.5	≥2.375	1.75	1.0
RV 1A	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Cox A21	1.0	0.5	<0.5	0.5	<0.5	<0.5	1.25	0.875	0.5

RV 39, rhinovirus type 39; RV 1A, rhinovirus 1A, Cox A21, coxsackie A21. The 24-h yields of virus in untreated controls were 3.875 log_{10} for RV 39, 2.875 log_{10} for RV 1A, and 3.125 log_{10} for Cox A21.

for each virus and RRMA concentration were treated with RRMA before, after, or before and after inoculation with virus. After a 24-h incubation, the monolayers and supernatants were harvested and frozen at −70°C. Ten-fold dilutions of the harvests were subsequently prepared for determination of virus titer in HeLa-M cells (Sperber and Hayden, 1989). Reductions in virus titer by ≥90% (≥1.0 log_{10}) were considered to represent a definite protective effect.

Pretreatment with RRMA resulted in concentration-dependent reductions in virus titer for RV 39 and Cox A21 (Table 1). No definite effect of pre-treatment was observed for RV 1A. In contrast, addition of RRMA after adsorption of virus did not appreciably reduce the 24h yields of any of the viruses. For RV 39 and Cox A21, the combination of treatment before and after virus adsorption reduced virus yields to a slightly greater degree at each RRMA concentration than pretreatment alone.

Concentration-dependent effects on visualization of cytopathic effect

The degree of inhibition of cytopathic effect (CPE) by RRMA was determined in triplicate monolayers of WI-38 cells pre-treated with 0.5 ml of MEM containing 10, 1.0, or 0 μg/ml of RRMA. After 2 h, approximately 1000 $TCID_{50}$ of RV 39 in 0.2 ml was added to the supernatant, and the monolayers were observed daily for 7 days. Neither the RRMA nor virus was rinsed from the monolayers. All infected nontreated control monolayers showed complete CPE on day 2 (Table 2). In contrast, monolayers treated with 10 μg/ml of RRMA were completely protected from CPE through day 7. The monolayers treated with 1.0 μg/ml of RRMA showed minimal CPE beginning on day 4, which did not visibly progress any further through day 7.

TABLE 2

Cytopathic effect score of human fibroblast (WI-38) cell monolayers treated with antibody to rhinovirus receptor (RRMA) before infection with RV 39

RRMA (μg/ml)	Percent viral cytopathic effect on			
	Day 2	Day 3	Day 4	Day 7
10	0	0	0	0
1.0	0	0	17	17
0	100	100	100	100

Values represent mean of observations of triplicate monolayers for each concentration. Cytopathic effect of each monolayer was scored on a scale of 0%, 25%, 50%, 75% or 100%. RV 39, rhinovirus type 39.

Effect of increasing the interval between virus infection and harvest

To determine the duration of the protective effect of a single RRMA exposure, monolayers were pretreated with 10, 1.0 or 0 μg/ml of RRMA for 2 h, rinsed twice, and then infected with RV 39 (approx. 100 $TCID_{50}$). Quadruplicate sets of monolayers and supernatants were harvested at 24, 48, and 72h for determination of viral titers. No decrease in protective activity was observed to 72 h (Fig. 1). Relative to infected nontreated controls, the decrease in titer at a RRMA concentration of 1.0 μg/ml was the same at 48 h and 72 h as at 24 h (1.0 $\log_{10}$ $TCID_{50}$/0.2 ml). For monolayers pretreated with 10 μg/ml of RRMA, there was an apparent increase in protective effect relative to nontreated controls at 48 h and 72 h compared with

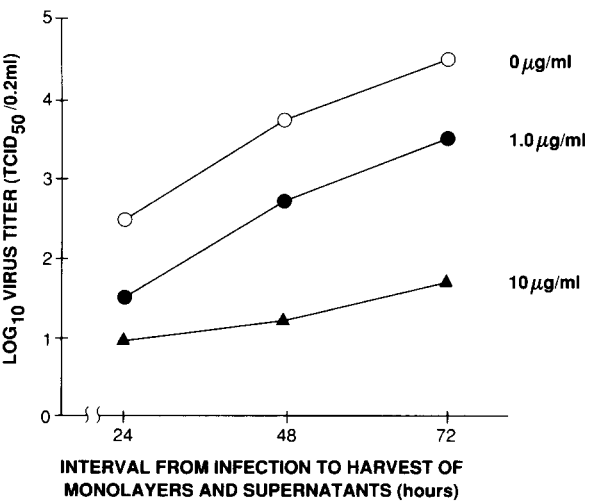

Fig. 1 Virus titer in human fibroblast (WI-38) cell monolayers pretreated with 10, 1.0, and 0 μg/ml of antibody to rhinovirus receptor (RRMA) and harvested at various intervals after inoculation with RV 39. The monolayers were pre-treated with RRMA for 2 h, rinsed twice, and infected with RV 39. Monolayers and supernatants were harvested at 24, 48, or 72 h, and virus titers were determined in HeLa-M cells.

TABLE 3

Reduction in 24-h virus titer by 2-h treatment of human fibroblast (WI-38) cell monolayers with antibody to rhinovirus receptor (RRMA) at 24, 48, or 72 h before infection with RV 39

RRMA (μg/ml)	Reduction in titer (log_{10} $TCID_{50}$/0.2 ml) compared to untreated control		
	24 h	48 h	72 h
10	1.125	1.125	1.375
1.0	1.125	1.125	1.0

RV 39, rhinovirus type 39.

24 h. This was due to a greater rise in titer of the infected untreated control monolayers with time.

Effect of increasing the interval between RRMA pre-treatment and infection

To determine the duration of the protective effect of a single RRMA exposure in relation to the time of harvest, monolayers were pretreated for 2 h with MEM containing 10, 1.0, or 0 μg/ml of RRMA, rinsed three times, and then fed with plain MEM. After 24, 48 or 72 h, RV 39 (approx. 100 $TCID_{50}$) was adsorbed to the monolayers for 1 h, followed by rinsing and replenishing with RRMA-free medium. The monolayers and supernatants were harvested at 24 h after virus adsorption and the virus yields determined in HeLa-M cells. Reductions in virus titer of $\geq$1.0 log_{10} compared to infected nontreated controls were observed at each time interval through 72 h between RRMA exposure and subsequent virus infection (Table 3). No differences were apparent in the magnitude of reductions observed at 24 h relative to those at 72 h.

Comment

We found that pretreatment of human fibroblast monolayers with RRMA resulted in dose-dependent protection against infection by RV 39 and Cox A21, but not RV 1A. These studies confirm that replication of a representative strain of the major group of RV and a Cox A virus can be inhibited in vitro by RRMA, whereas a member of the minor group of RV is not, and extend these observations to a cell line other than that from which the antibody was derived (Colonno et al., 1986). A concentration of 1.0 μg/ml, but not 0.1 μg/ml, provided consistent protective effects. Unfortunately, little is known about the concentration-related activity of RRMA in man. In one study, the concentrations of RRMA detected in nasal washings collected at various intervals after multiple 100 μg intranasal doses were less than 0.05 μg/ml (Hayden et al., 1988).

We were unable to demonstrate a definite decrease in virus titer compared with nontreated controls when RRMA (up to 10 μg/ml) was added immediately after

infection of human fibroblast cells with RV 39 or Cox A21. Colonno and co-workers previously reported that addition of 30 μg of RRMA up to 6 h after adsorption with RV 15 resulted in complete inhibition of plaque formation in HeLa cells (Colonno et al., 1986). In addition to differences in cell type, this difference may result from the fact that the plaque inhibition assay used by Colonno involves preventing virus spread to other cells from a single infectious focus over a period of days as opposed to infection of many cells of a monolayer during a 24-h experiment comprising few cycles of viral replication.

Studies to define the duration of RRMA effect found persisting protective activity with increased intervals between RRMA exposure and virus harvest (up to 72 h) relative to nontreated controls. A prolonged protective interaction between RRMA and fibroblast cells was also evident from visual observation of virus exposed monolayers, which showed complete protection from virus CPE through seven days at RRMA concentrations of 10 μg/ml. Partial protection from RV infection ($\geqslant$1.0 $\log_{10}$ reduction in virus titer) was found even when RRMA was rinsed off the monolayers 72 h prior to virus inoculation. It has recently been reported that HeLa cells pretreated with RRMA and then rinsed remained resistant to binding by RV 14 for 45 h (Colonno et al., 1989).

The mechanism by which RRMA can bind to the cellular receptor and retain a prolonged protective effect is not clear. In the human trials, in which RRMA was given intranasally beginning prior to rhinovirus infection, an increase in virus replication was observed after cessation of RRMA administration (Hayden et al., 1988). This result could have been due to incomplete initial protection, upregulation or turnover of receptors, or degradation of RRMA by nasal secretions. It has recently been proposed that the host cell response to rhinovirus infection, through stimulation of local cytokine production, should result in induction of receptor expression (Staunton et al., 1989). In vitro studies, conducted before the functional role of the receptor was identified, suggested that attachment of RRMA to the receptor does not cause receptor turnover or affect cell growth (Colonno, 1986; Tomassini and Colonno, 1986). It remains to be determined whether binding to the receptor by blocking substances, such as RRMA, will affect receptor expression in vivo. Additionally undefined is whether manipulations that block the rhinovirus receptor will impact on the interaction between ICAM-1 and lymphocyte function associated 1 (LFA-1) antigen. From the perspective of cytokine therapy for the control of rhinovirus colds, it is of interest that IFN-γ, which upregulates ICAM-1 expression (Dustin et al., 1986, 1988; Pober et al., 1986), appears to have less potent antirhinoviral activity in vitro (Ahmad and Tyrrell, 1986; Sperber and Hayden, 1989) than IFN-α and IFN-β, which have not been demonstrated to upregulate receptor expression (Dustin et al., 1986, 1988; Pober et al., 1986). Furthermore, intranasal administration of rIFN-α is effective in the prophylaxis of rhinovirus colds (Sperber and Hayden, 1988) whereas in a recent study of experimental rhinovirus colds IFN-γ was not (Higgins et al., 1988). With increasing understanding of the functional role of the receptor (Greve et al., 1989; Staunton et al.,1989) and of the physical properties of its interaction with virus (Abraham and Colonno, 1988; Colonno et al., 1988), studies in respiratory epithelial organ cul-

ture systems may be helpful to evaluate further the virus-receptor-RRMA interactions occurring in vivo.

Acknowledgements

The authors thank Richard J. Colonno for supplying the RRMA. This work was supported in part by grant T32AI-07046 from the National Institute of Allergy and Infectious Diseases.

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