Antiviral Research, 9 (1988) 233–247
Elsevier

AVR 00289

Modification of experimental rhinovirus colds by receptor blockade

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(Received 11 November 1987; accepted 18 February 1988)

Summary

Human rhinovirus (HRV) infection can be inhibited in vitro by antibody directed against the cellular receptor for the major HRV group representing 90% of serotypes. We assessed the prophylactic effectiveness and safety of intranasally administered rhinovirus receptor murine monoclonal antibody (RRMA) in two double-blind, place-controlled, randomized studies of volunteers experimentally inoculated with HRV-39. In the first study, RRMA administration (135 μg/subject in 9 applications, −17 to +48 h) did not reduce infection (RRMA 12/15 vs. placebo 13/15) or illness (8/12 vs. 7/13) rates or modify the clinical course of experimental HRV-39 colds. In the second trial, a higher RRMA dosage (1 mg/subject in 10 applications, −3 to + 36 h), similarly did not reduce overall infection (11/13 vs. 12/13) or illness (7/11 vs. 9/12) rates, but was associated with a 1–2 day delay in the onset of viral shedding and cold symptoms and with significant reductions in viral titers and nasal symptoms on the second day after challenge and in mucus weights on the third day after challenge. No toxicity related to RRMA was recognized. The results indicate that intranasal RRMA modified infection and illness after experimental HRV-39 challenge and suggest that blockade of host cell receptors offers a novel antiviral approach against HRV infections.

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Introduction

Human rhinoviruses (HRVs) are the most frequently recognized cause of the common cold and account for approximately 40% of such illnesses (Gwaltney, 1982). Previous studies have established that HRV infection of susceptible host cells depends on viral attachment to specific cellular receptors (Lonberg-Holm and Korant, 1972; Lonberg-Holm et al., 1976). Reciprocal competitive binding assays (Lonberg-Holm et al., 1976; Abraham and Colonno, 1984) with radiolabeled virus have demonstrated that approximately 90% of HRV serotypes (designated the major HRV group), as well as group A coxsackieviruses, utilize a single type of cellular receptor for attachment. The remaining HRV serotypes compose the minor group and attach to cells by utilizing a different cellular receptor. Thus, the finding that the 100 HRV serotypes interact with only two cellular receptors represents a novel target for the application of antiviral agents to prevent HRV infection.

Using HeLa cells and membranes as antigens, Colonno and his coworkers were able to isolate a murine hybridoma cell line which produced monoclonal antibody (IgG_1 isotype), that is capable of binding specifically to the major HRV group receptor on human and chimpanzee cells and protecting HeLa cells from HRV infection (Colonno et al., 1986). This antibody, called the rhinovirus receptor monoclonal antibody (RRMA), demonstrated the same receptor tropism as the major group of HRVs. Studies using HeLa cell membranes demonstrated that the RRMA had a strong affinity for the major HRV group receptor and was capable of displacing previously-bound virus (Colonno et al., 1986). In addition, no viral plaques could be detected in a standard plaque assay when RRMA-treated HeLa cell monolayers were exposed to over 3000 infectious virions per cell of three different serotypes (Colonno et al., 1986). These experiments strongly suggest that HRVs can not readily bypass the receptor blockade under in vitro conditions.

Cell binding studies using radiolabeled RRMA revealed that the major HRV group receptor is present on a wide range of human cells (Colonno et al., 1986), although certain human cell lines (e.g., HEp-2, RD, erythrocytes) appeared to lack this receptor. Although the function of this receptor in man is not known at the present time, binding of RRMA to the receptor in human cells in vitro has not resulted in detectable alterations in cell morphology, growth or macromolecular synthesis (Colonno et al., 1987). Using the RRMA and immunoaffinity chromatography, a 90 kDa membrane protein was isolated from detergent-solubilized HeLa cell membranes (Tomassini and Colonno, 1986). Polyclonal rabbit antisera raised against this purified protein selectively blocked the binding and infectivity of major HRV group viruses in HeLa cells, a finding which confirmed that the 90 kDa glycoprotein is involved in HRV attachment.

Placebo-controlled animal toxicology studies, including the administration of RRMA intranasally and intravenously to rats and cynomolgus monkeys for 14 day periods, have not found evidence of drug-related toxicity. However, cells from these animal species do not have detectable levels of the major HRV group receptor protein and thus might fail to manifest toxicity. Subsequent studies involving in-

tranasal administration of the RRMA (3.0 mg given twice at 5 day intervals) in chimpanzees, a species that does have the receptor, found no evidence of local or systemic intolerance. Extended administration for several months failed to produce detectable local or systemic toxicity (unpublished observations).

Preliminary challenge studies in a small number of chimpanzees found that a single intranasal instillation of RRMA (250 μg/nostril), prior to intranasal challenge with HRV-15, inhibited the serum antibody response to the virus (Colonno et al., 1987). The current studies were undertaken to assess the effectiveness and safety of intranasal RRMA in the prevention of experimental rhinovirus infection and illness in human volunteers and thus to ascertain whether the 90 kDa receptor protein which is crucial for HRV attachment in vitro is also involved in in vivo infections. These studies are the first human trials to determine the activity of a monoclonal antibody which acts by specifically blocking the cellular receptors required for initiating viral infection of susceptible cells.

Materials and Methods

Subjects

Healthy, young adult volunteers, who had screening serum neutralizing antibody titers to HRV-39 of ≤1:2, were randomly assigned to RRMA or placebo groups in two separate studies (30 in the first and 27 in the second). Individuals with an upper respiratory tract illness or fever within one week prior to the study, medical illness requiring concomitant medication, exposure to an investigational drug within the previous month, and those who were pregnant or breast-feeding were excluded from participation. Concurrent medications, except oral contraceptives and vitamin preparations, were discouraged. Volunteers were housed individually in separate motel rooms from the day of initiating drug administration for a total of five (study 1) or six (study 2) days.

Drug administration

RRMA was prepared from mouse ascites fluid and purified by chromatography on DEAE, protein (A), and mono Q (Pharmacia) columns to greater than 99% purity at the Merck Sharp and Dohme Research Laboratories. The antibody preparations were subjected to numerous safety and quality control tests and supplied in individual vials containing a phosphate buffered saline solution with 0.2% albumisol and 22.5 μg (30 μg/ml) or 140 μg (200 μg/ml) of RRMA. The placebo solution contained the same excipients without RRMA and was identical in appearance and consistency. Solutions were administered as nasal drops (0.25 ml/nostril/treatment) using a calibrated Eppendorf pipette by a member of the project staff. During administration and for 1 min afterward, subjects were supine with the neck extended, so that the chin and external ear were in the vertical plane (Aoki and Crawley, 1976). Thereafter, the subjects were asked to remain supine for 10 min and to avoid blowing their nose for at least 30 min.

Experimental plan

Both studies were randomized, double-blind, and placebo-controlled in design. In the first study, antibody or placebo solutions were administered every 8 h for three days (total of 9 applications) beginning 17 h prior to the first virus challenge and continued for 48 h afterward (−17, −9, −1, +8, +16, +24, +32, +40, +48). Each treatment consisted of 15 μg of RRMA, so that the total dosage administered was 135 μg per subject. The challenge virus (HRV-39) was administered by nasal drops (0.10 ml/nostril) in the same manner as the treatment drops at 1 h after the third (900 h) and fourth (1700 h) treatments on the second study day. The total inoculum was approximately 2000 $TCID_{50}$ per subject.

In the second study, the treatment schedule was altered so that more applications were given in the period immediately before and after virus challenge. Beginning three hours prior to virus challenge, a total of 10 applications containing 100 μg of RRMA were given (−3, −2, −1, +1, +4, +10, +18, +24, +30, and +36 h), so that the total dosage was 1 mg per subject. Virus challenge (0.05 ml/nostril) was administered 1 h after the third dose of study drug and again 15 min later. The total inoculum of HRV-39 administered was approximately 300 $TCID_{50}$ per subject.

Clinical monitoring

The frequency and severity of clinical illness were determined by monitoring clinical symptoms and weighing of expelled nasal secretions by previously described methods (Hayden and Gwaltney, 1983). To determine the onset of subjective colds, the subjects were asked, "do you feel that you had a common cold during the past day?" on each morning of the postchallenge days. Nose and throat examinations were performed before initiation of treatment, on the day following the last treatment, and three days later. Routine laboratory studies consisting of CBC with differential leukocyte and platelet counts, blood chemistries (blood urea nitrogen, creatinine, bilirubin, alkaline phosphatase, SGOT, SGPT), and urinalysis were performed before treatment and on the fourth and tenth days after initiating treatment.

Sera collected from RRMA recipients before treatment and approximately three weeks later were tested for the presence of anti-murine antibodies with a radioimmunoassay. Duplicate samples of 1:5 diluted serum were adsorbed to nitrocellulose paper overnight at 4°C. After addition of a blocking buffer (20% dialyzed fetal calf serum, 0.15 M NaCl, 0.05 M Tris-HCl, pH 7.6), the paper was immersed in a solution containing ^{125}I-labeled RRMA and 0.02% bovine serum albumin in PBS for 3.5 h at 4°C. Each nitrocellulose sample was washed and then assayed for radioactivity using a gamma counter. A positive control consisting of antisera (diluted 1:20) from a rabbit immunized with RRMA was included in each assay. Negative control sera from a placebo-treated subject gave values of 460–3606 counts per minute (cpm), while the rabbit anti-RRMA antisera generated values of 8260–39,222 cpm in four separate assays.

Virologic sampling

The rates of infection were determined by viral isolation and measurement of homotypic serum neutralizing antibody titers in paired serum specimens obtained prior to viral challenge and three weeks later. Nasal wash specimens were collected before viral challenge and for eight (first study) or nine (second study) consecutive days beginning on the day after challenge. The first isolate from each subject was confirmed to be HRV-39 by standard neutralization testing.

As described below, preliminary experiments determined that residual RRMA in nasal washings could potentially inhibit the in vitro recovery of rhinoviruses. Consequently, nasal wash samples collected during and for two days after RRMA administration were preincubated with Sepharose-4B anti-mouse IgG (Cooper Biomedical, West Chester, PA), to remove any remaining murine immunoglobulin. Briefly, 4 ml of nasal washings were added to 1 ml of 4-fold concentrated beef heart infusion broth containing 1.0% bovine albumin and antibiotics. After transfer to the laboratory on wet ice, 100 μl of rinsed Sepharose gel (diluted 1:5 in phosphate buffered saline) was added to 1 ml of the specimen, and the mixture incubated for 30 min on a rocker apparatus at room temperature. After centrifugation for 10 min at 2,000 RPM, the supernatant was mixed with a second 100 μl volume of the Sepharose gel. Following a 30-min incubation at 4°C on a rocker and centrifugation, the supernatant was used for inoculation of MRC-5 strain human embryonic lung fibroblast monolayers. After a 1 h absorption period at 34°C, the monolayers were washed three times with Hanks' buffered salt solution and refed with maintenance media consisting of Eagle's Minimal Essential Media containing 5% fetal bovine serum (HyClone Laboratories, Logan, UT), glutamine, and antimicrobics.

In the second study, samples were treated with Sepharose anti-mouse IgG as described above and rhinovirus titers in nasal wash samples were determined by culturing 10-fold dilutions (10^{-1}–10^{-3}) of initially positive, once frozen (−70°C) specimens on MRC-5 monolayers in quadruplicate. Specimens negative on initial culture were retested undiluted after one freeze-thaw cycle. Titers were calculated by the method of Kärber and expressed as $\log_{10}$ $TCID_{50}$/0.2 ml of nasal wash.

Assay of RRMA in nasal washings

Nasal washings collected from volunteers in the second study were assayed in a cell protection assay to determine whether any RRMA could be detected. Duplicate volumes (0.1 ml) of each nasal wash were incubated with 10 μl of a 1:25 dilution of anti-HRV-39 antiserum (American Type Culture Collection, original titer 1:1600/ml) for 1 h to neutralize any residual HRV-39. The treated nasal washings were then placed on HeLa cell monolayers in 48-well plates for 1 h at 34°C, removed, and fresh media put onto the cells. The cells were then challenged with 3×10^5 plaque forming units of HRV-14 and incubated overnight at 34°C. Based on previous assays in this system (Colonno et al., 1986), the development of cytopathic effect in monolayers was not altered and indicated that little, if any, RRMA (<0.05 μg/ml) was present in the sample. Assay controls included monolayers exposed to media only (cell control), nasal washings without RRMA followed by

HRV-14 challenge (virus control), and purified RRMA in medium followed by HRV-14 challenge (RRMA control). Nasal washes representing postchallenge days 1–5 were tested for both RRMA and placebo recipient samples in duplicate.

In vitro studies

To determine whether residual RRMA in nasal washings could inhibit the recovery of rhinoviruses, simulated nasal washings were used for inoculation of monolayers of MRC-5 fibroblasts in quadruplicate. Aliquots of lactated Ringer's solution containing HRV-39 at a titer of 50 $TCID_{50}$/0.2 ml and RRMA at a concentration of 10 μg/ml were used. Prior to inoculation, some of the samples were preincubated with Sepharose-4B anti-mouse IgG chromatography gel once or twice for varying times (15–180 min) and at two temperatures (room temperature or 4°C). According to the manufacturer, each ml of gel should bind 2.1–2.3 mg of purified IgG. During Sepharose treatment, the samples were held on a rocker apparatus to insure adequate mixing and then clarified by centrifugation at 2000 RPM for 10 min. Monolayers were inoculated with 0.2 ml of each sample in quadruplicate and observed daily for the development of rhinovirus cytopathic effect. The extent of monolayer involvement was scored on a daily basis.

Data analysis

All treatments, clinical evaluations, and virologic studies were conducted under double-blind conditions. The significance of differences in proportion was calculated by Fisher's exact test, of differences in symptom scores by Mann-Whitney U test, and of differences in other measures by Student's *t*-test. In each instance *P*-values were those for two-tailed testing.

Results

In vitro studies

After inoculation of MRC-5 monolayers with HRV-39 in lactated Ringer's solution without RRMA, cytopathic effect was readily detectable by 48 h (Table 1). In contrast, samples which contained 10 μg/ml of RRMA failed to develop any cytopathic effect after four days of incubation. By day six, only one of eight inoculated monolayers showed evidence of monolayer destruction. A single preinoculation treatment with Sepharose anti-mouse immunoglobulin gel once for 180 min at 4°C partially reversed this inhibitory effect. Two treatments at room temperature or 4°C almost completely reversed the inhibitory effect (Table 1). Subsequent studies using two gel treatments for 15, 30, or 45 min each, found that the duration of treatment within this time range did not appear to influence rhinovirus recovery (data not shown). Consequently, in processing nasal wash samples from infected volunteers, two 30-min treatments, one at room temperature followed by one at 4°C, were used.

TABLE 1

Removal of RRMA activity from simulated nasal wash samples by Sepharose anti-mouse IgG

RRMA 10 μg/ml	No. Sepharose treatments	Conditions of processing		Mean ± SD CPE rating* on post challenge day		
		Duration (min)	Temp.	2	3	4
0	0	Not applicable		75 ± 0	100 ± 0	–
+	0**	45	RT	0	0	0
		180	4°C	0	0	0
+	1	45	RT	0	0	0
		180	4°C	3 ± 5	53 ± 31	81 ± 38
+	2	45	RT	38 ± 14	100 ± 0	–
		180	4°C	10 ± 0	75 ± 0	–

Quadruplicate MRC-5 strain monolayers were inoculated with 0.2 ml of lactated Ringer's solution containing 50 $TCID_{50}$ HRV-39. Except for the virus control monolayers, all other inocula contained RRMA at a final concentration of 10 μg/ml. Prior to inoculation, some of the samples were preincubated with Sepharose anti-mouse IgG gel (100 μl of 1:5 dilution) once or twice under the conditions indicated. RT = room temperature.

* Rating was defined as the average proportion (0, 12.5, 25, 50, 75 or 100%) of each monolayer showing typical rhinovirus cytopathic effect.

** Samples were held without Sepharose treatment under the conditions indicated.

Participants

All subjects completed the protocol. The first study had 15 participants in each of the RRMA (9 females, 6 males; mean age, 21.1 years) and placebo (10 females, 5 males; 20.9 years) groups. Two RRMA recipients, whose acute neutralizing antibody titer to HRV-39 was 1:4, were included in the analysis of efficacy. Both shed virus and developed illness. The second study had 13 participants in the RRMA group (8 females, 5 males; 20.5 years) and 14 in the placebo group (7 females, 7 males; 20.6 years). One placebo recipient was culture-positive for an enterovirus before initiation of drug administration and was excluded from the efficacy analysis. One RRMA recipient, whose acute antibody titer was 1:4 and who did not develop infection or illness, was included in the analysis.

In the first study acetaminophen or salicylates were taken by 3 placebo recipients for relief of feverishness or headache on one or two days. One rhinovirus-infected RRMA recipient was given a 10-day course of erythromycin for presumed bronchitis beginning on the ninth study day. In the second trial, acetaminophen was used for relief of headache on one or two days by 1 RRMA and 2 placebo recipients.

Toxicity

Abnormal values were reported on routine laboratory studies in several subjects, but no clinically significant changes attributable to RRMA administration were detected. In the first trial, mild nasal burning or irritation associated with administration of the nose drops was reported by 5 placebo and 4 RRMA recipients following one or two applications. Excessive tearing or eye watering was reported by 2 placebo recipients. In general, the symptoms lasted only a few min-

utes, although 1 RRMA recipient reported marked nasal pressure and burning lasting 10 min on a single occasion. In the second trial, slight burning or irritation was noted after one or two doses by 3 RRMA but no placebo recipients. One placebo subject reported an association between the nasal drops and a chemical odor.

Nasal examinations detected mucosal abnormality (punctate bleeding sites and/or mucosal friability) that were not present on prestudy exams in 2 RRMA and 3 placebo recipients in the first study. One placebo recipient had a 10-min period of epistaxis on the third study day, and one RRMA recipient reported blood-tinged mucus on the fifth study day. In the second trial, new nasal exam abnormalities were found in 1 RRMA recipient and in 4 placebo recipients. In addition, 1 placebo recipient had a 1-min period of epistaxis on the sixth study day. Follow-up nasal examinations found that these abnormalities resolved within several weeks.

Antibodies directed toward the RRMA could not be detected in any of the acute and convalescent sera of RRMA recipients using an assay with sensitivity in the ng range.

Antiviral activity of intranasal RRMA

In the first study, the proportion of subjects who had virus recovered from nasal washings was similar in the RRMA (80%) and placebo-treated (87%) groups (Table 2). Likewise, the proportion of subjects who seroconverted (27% and 47%, respectively) was also similar. The fraction of the eight post-challenge days on which virus could be recovered from nasal washings was comparable in the RRMA (61% of days) and placebo (53%) groups, as was the proportion of subjects who shed virus on the first day after challenge (53% and 47%, respectively). No significant differences were found in the frequency of virus shedding on any of the post-challenge days (data not shown).

In the second study, no important differences were seen in the proportion of virus-positive subjects, in seroconversion rate, or in the fraction of virus-positive days after challenge between the groups (Table 2). However, the onset of virus shedding tended to be later in the RRMA group. On the first day after challenge, 46% of placebo compared to 23% of RRMA recipients shed virus (P=NS). This

TABLE 2

Infection rates and virus shedding in HRV-39 challenged subjects

Study	Treatment group	(N)	No. (%)			No. (%) virus-positive days**
			Virus positive	Sero-conversion	Infection*	
1	RRMA	(15)	12(80)	4(27)	12(80)	73/120(61)
	Placebo	(15)	13(87)	7(47)	13(87)	62/117(53)
2	RRMA	(13)	11(85)	7(54)	11(85)	69/117(59)
	Placebo	(13)	12(92)	4(31)	12(92)	70/117(60)

* Defined by occurrence of HRV-39 shedding and/or ≥4-fold rise in serum homotypic neutralizing antibody.

** Expressed as proportion of observation days after virus challenge.

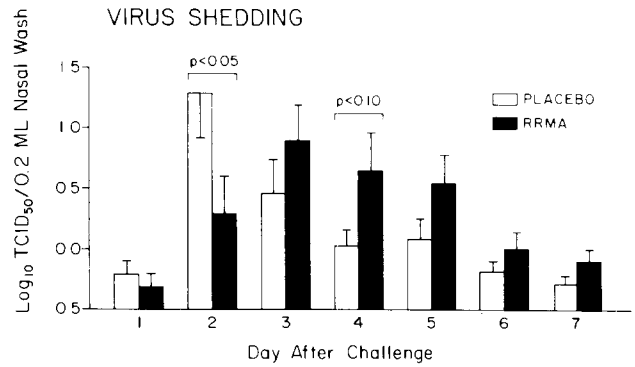

Fig. 1. Titers of HRV-39 in nasal washings from RRMA and placebo recipients of the second study. RRMA administration continued until the evening (2100 h) of the second post-challenge day. Infectious virus was recovered and titered as described in Materials and Methods. The values are expressed as mean ± SEM $\log_{10}$ $TCID_{50}$/0.2 ml of nasal wash for each group. Negative cultures were assigned a value of −0.50 for purposes of analysis. Statistical analysis was done by two-tailed *t*-test.

tendency continued on the second post-challenge day (72% versus 46%). For those who shed virus, the median time to onset of shedding tended to be later in the RRMA group (day 3 after challenge) than in the placebo group (day 1 after challenge) (P=NS). As shown in Fig. 1, the treatment groups differed in their patterns of quantitative virus shedding after challenge. Low titers of virus were present in nasal washings in both groups on the first day after challenge. The placebo group had peak titers on the second post-challenge day with a relatively rapid decline thereafter. In contrast, the RRMA group had significantly lower titers on the second post-challenge day (Fig. 1) followed by a gradual rise in titer, so that by the fourth day the RRMA group tended to have higher titers of virus in nasal washings.

Nasal washes were later assayed to determine if there was sufficient RRMA present to interfere with virus isolation. Using a cell protection assay, in which HRV-39 is neutralized by antiserum prior to challenging cells with HRV-14, found no detectable levels (<0.05 μg/ml) of RRMA present.

Efficacy against illness after rhinovirus challenge

In both studies, the proportions of all subjects or of those who had documented HRV infection who developed clinical colds did not differ between the groups (Table 3). Similarly, the mean scores on the five days after virus challenge for groups of related symptoms (nasal, non-nasal respiratory, or systemic) did not differ significantly between the groups in either study (Table 2). There were no differences between the groups in nasal mucus weights or in tissue counts on the four days after virus challenge in the first study. In the second study these measures were followed for six days after challenge; here nasal mucus weights (37% reduction) and tissue counts (36% reduction) tended to be lower in the RRMA than in the placebo-treated group (Table 3). However, the differences were not statistically significant.

TABLE 3

Illness occurrence in rhinovirus type 39-challenged volunteers

Study	Treatment group	(*N*)	No. (%) colds*	No. colds/ no. infected (%)	Symptom scores†			Nasal mucus** weight (g)	Nasal tissue** count (no.)
					Nasal	Respiratory	Systemic		
1	RRMA	(15)	9(60)	8/12(75)	5.5(5.3)	4.5(4.8)	0.3(0.7)	14.4(10.4)	46(38)
	Placebo	(15)	7(47)	7/13(54)	5.7(4.7)	4.3(7.4)	1.6(2.2)	14.1(16.9)	44(49)
2	RRMA	(13)	7(54)	7/11(64)	5.7(5.2)	4.4(4.2)	1.0(1.7)	17.5(21.3)	50(41)
	Placebo	(13)	9(69)	9/12(75)	7.8(5.2)	3.5(4.9)	2.8(3.4)	27.6(24.1)	78(58)

* Colds were determined according to modifications of the method of Jackson et al. (1958; Gwaltney et al., (1980).

† Days 1–5 after virus challenge. Nasal = sneezing, discharge, obstruction; Respiratory = sore throat, hoarseness, cough; Systemic = headache, fever, chill, malaise. The values are listed as mean (standard deviation) scores for all subjects.

** Days 1–4 (study 1) or 1–6 (study 2) after virus challenge. The values are listed as mean (standard deviation) for all subjects.

The clinical data were further analyzed to determine whether RRMA administration may have delayed the onset of subjective or objective measures of illness. In the first study no differences were noted in nasal symptom scores or nasal mucus weights when the results were analyzed on a day-by-day basis (data not shown). Similarly, when the subjects of the first study were asked each day whether they had a cold, the proportion of subjects in the RRMA and placebo groups who responded positively did not differ on the first (13% and 7%, respectively), second (47% in both), or third (53% in both) post-challenge days. In contrast, significant differences in these measures were found in the second study. The proportion of subjects indicating that they had a cold differed significantly ($P=0.01$) between the RRMA (15% of subjects) and placebo (77%) groups on the second day after challenge (Fig. 2), and this trend continued on the third post-challenge day (31% versus 62%; P=NS).

As shown in Fig. 3, there were corresponding differences in the time course of nasal mucus production and nasal symptom scores. For either one of the parameters, the placebo group had its peak on the third post-challenge day, whereas the maximum values in the RRMA groups were delayed by one day for each of these measures. Significant differences were present in nasal mucus weights in the second post-challenge day and in nasal symptom scores on the third post-challenge day between the groups.

Discussion

These studies are the first human trials to assess the safety and efficacy of receptor blockade with monoclonal antibodies for preventing HRV infection. The first trial, which used a total RRMA dosage of 135 μg given over 65 h, found no evidence of an effect on the virological or clinical course of experimental rhino-

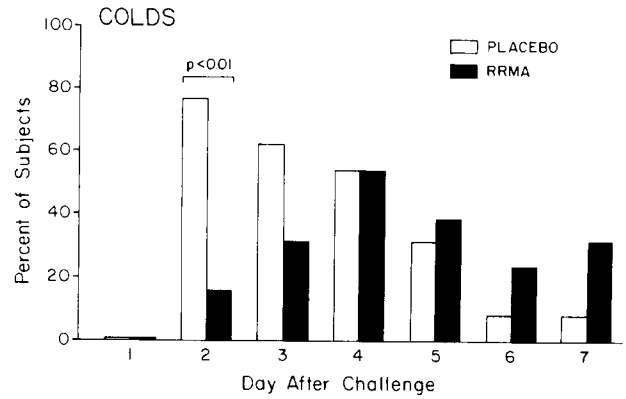

Fig. 2. Subjective assessment of whether RRMA- and placebo-treated volunteers developed a clinical cold. Proportion of subjects responding yes to the question "do you have a cold today?" is shown for each group. Statistical analysis by two-tailed Fisher's exact test.

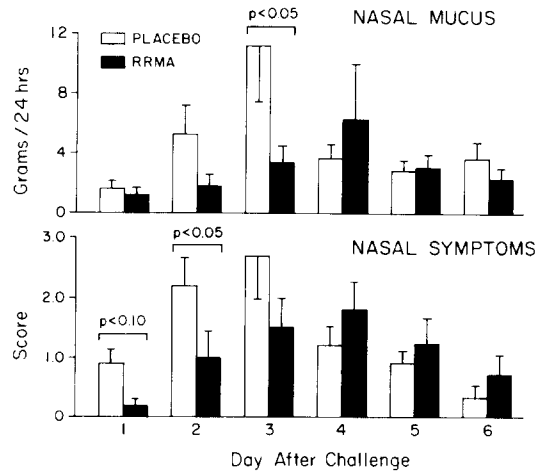

Fig. 3. Nasal mucus weights and symptom scores of RRMA and placebo recipients in the second study. RRMA administration continued until the evening (2100 h) of the second post-challenge day. The weight of mucus discharge was measured by previously described techniques (Hayden and Gwaltney, 1983). The mean ± SEM mucus weights (g/24 h) or scores are shown for each group. Nasal symptoms include obstruction, discharge, and sneezing which were rated from absent (0) to severe (3). Statistical analysis by Mann-Whitney U test (symptom scores) or *t*-test (mucus weights).

virus infection. In contrast, the second trial, which utilized an approximate 8-fold greater dosage (1000 μg) given over 36 h and administration of multiple doses just prior to virus challenge, found significant decreases in peak viral titers, and delays in the onset of colds, nasal symptoms, and nasal mucus production. Even in this trial, however, no overall reductions in the frequency of illness or infection were noted. The one to two day delay observed in viral shedding, nasal symptoms, and mucus production were followed by increases in these markers on the third and fourth days after viral challenge.

Several explanations could account for the subsequent rises in viral titers and clinical manifestations of infection in RRMA recipients in the second study. The most obvious is that the initial treatment period provided only partial protection of nasal mucosal cells. When the RRMA administration was stopped at 36 h after challenge, virus released from unprotected cells was able to further replicate and led to the development of clinical symptoms. The detection of virus in the nasal secretions of some RRMA recipients, even during the period of antibody administration, indicates that initial protection against rhinovirus infection was incomplete. It is possible that the initial dosage or concentration of RRMA was inadequate to block all available rhinovirus receptors. In the first study the unintentionally large viral challenge (2000 $TCID_{50}$/subject) may have overcome any blocking effect of the antibody. However, under in vitro conditions RRMA treatment protects HeLa cells against very large virus concentrations (over 3000 pfu per cell) (Colonno et al., 1986).

Because the dose schedules differed in RRMA amount, frequency of adminis-

tration, and temporal relationship to viral challenge, it is not possible to assess whether there was a dose-response effect. Perhaps higher dosages of antibody could provide greater levels of protection than observed in the second study. This is supported by chimpanzee studies in which a single large dose (0.5 mg) of RRMA showed efficacy in preventing HRV infection (Colonno et al., 1987). In this regard, it is also worth noting that the early interferon studies which utilized relatively low dosages (150–5000 U) had negative results, and it was not until much higher dosages (10–45 MU) were used that consistent evidence of protection was found in experimental challenge models (Hayden and Gwaltney, 1983; Scientific Committee on Interferon, 1965; Merigan et al., 1973; Scott et al., 1980, 1982; Samo et al., 1984). In addition, it is possible that the antibody did not distribute to regions in the nasal passages where the virus inoculum was able to initiate infection. Studies in this model, using conjunctival or nasopharyngeal inoculation of virus, have found that the nasopharynx appears to be the initial site of infection and that regional differences are present within the nasal passages in terms of timing and extent of involvement (Winther et al., 1986). We attempted to overcome this problem in the second study by using a larger volume for RRMA administration than for viral inoculation. It is possible that RRMA may not be able to readily penetrate mucus secretions to reach cellular receptors or that the antibody may interact with host cell receptors in vivo in a different manner than the avid binding observed in vitro in a variety of human cell lines.

Another possible explanation for the incomplete protection observed might be the rapid turnover of HRV receptors after exposure to RRMA. Under in vitro conditions, a single exposure of HeLa cells or MRC-5 cells to RRMA induces a state of receptor blockade that lasts for at least two days (R. Colonno, unpublished observations). No data are currently available regarding the number or distribution of rhinovirus receptors on human nasal epithelial cells, the presumed target cells of rhinovirus infection, or the turnover of such receptors after exposure to RRMA. Another possible explanation for incomplete protection is that RRMA may be inactivated or its effects inhibited by some substance in nasal secretions. In vitro studies, in which human nasal mucus samples were incubated with 2 μg of RRMA and titrated in a cell protection assay, found no evidence of significant inactivation (R. Colonno, unpublished observations).

We do not feel that the reductions in peak viral titers and the trend toward delayed onset of rhinovirus shedding observed in RRMA-treated subjects in the second study were the result of artifactual inhibition of rhinovirus recovery due to residual RRMA in nasal washings. Assays for RRMA in nasal wash samples indicated that no detectable antibody was present. Furthermore, the methods used to process the samples were shown to be effective for reserving inhibitory effect of RRMA concentrations as high as 10 μg/ml. Finally, there was a good temporal relationship between the delays in the virologic and clinical course of experimental colds in this study.

Tolerance to intranasal RRMA was good in these studies with no evidence of systemic or local toxicity observed during either study. However, repetitive use of RRMA does not appear to be feasible at this time due to potential development

of immune responses that could lead to both reduction in antibody activity (Houghton and Scheinberg, 1986), as well as possibly to allergic manifestations. Serum antibodies to murine monoclonal antibodies have been observed commonly after systemic IV administration of milligram quantities (1–1000 mg/day) of murine monoclonals in other clinical settings (Houghton and Scheinberg, 1986; Ortho Multicenter Transplant Study Group, 1985). Utilization of intranasal or topical administration should markedly decrease the chance of an immune response to the RRMA. Considering the limited exposure of volunteers to RRMA, it is not surprising that antibodies to RRMA could not be detected in convalescent sera. In addition, repeated intranasal administration of RRMA to chimps over a 1-year period failed to generate a detectable titer of anti-murine immunoglobulin antibodies (R. Colonno, unpublished observations).

In summary, intranasal administration of RRMA modified but did not prevent infection or illness after experimental HRV-39 challenge. RRMA administration delayed the onset of viral shedding and clinical colds by one to two days compared to placebo. Further studies are needed to determine whether higher dosages or more prolonged administration of RRMA can provide higher levels of protection. Given the relatively modest protective effects observed and remaining concerns about the cellular function of the HRV major group receptor and the immunogenicity of intranasal murine antibody, it is too early to speculate about the practical value of this approach. It may be possible that other peptides or small molecules could be developed that would be useful in establishing receptor blockade. Alternatively, it may be possible to inhibit viral infection through the use of exogenous molecules, which mimic portions of the 90 kDa receptor glycoprotein, and bind to the virus at the virion attachment site. Despite these uncertainties, the results of these studies document that blockade of host cell receptors can provide partial protection against rhinoviral infection in humans and warrant further studies.

Acknowledgements

The authors thank Dr. Kenneth Brown, Felicia Geist, Diane Woerner, R.N., Gloria Hipskind, R.N. and Martha Morris, R.N., for help in conducting these studies; Pia Callahan and Abner Schlabach for preparation of the RRMA; Dr. Hank Allen and Dr. Thomas Nolan for their help in conducting animal safety studies; and Margaret Belew for assistance in preparation of the manuscript.

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