Nasal Cytokine and Chemokine Responses in Experimental Influenza A Virus Infection: Results of a Placebo-Controlled Trial of Intravenous Zanamivir Treatment

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The local immune response to influenza virus infection was characterized by determining cytokine and chemokine levels in serial nasal lavage fluid samples from 15 volunteers experimentally infected with influenza A/Texas/36/91 (H1N1). The study was part of a randomized double-blind placebo-controlled trial to determine the prophylactic effect of intravenous zanamivir (600 mg 2×/day for 5 days), a highly selective inhibitor of influenza A and B virus neuraminidases, on the clinical symptoms of influenza infection. Nasal lavage fluid levels of interleukin (IL)-6, tumor necrosis factor-α, interferon-γ, IL-10, monocyte chemotactic protein–1, and macrophage inflammatory protein–1α and –1β increased in response to influenza virus infection and correlated statistically with the magnitude and time course of the symptoms. Treatment with zanamivir prevented the infection and abrogated the local cytokine and chemokine responses. These results reveal a complex interplay of cytokines and chemokines in the development of symptoms and resolution of influenza.

Influenza is an acute respiratory infection with high morbidity and significant mortality, primarily because of its complications in very young or old persons. The symptoms of influenza include the sudden onset of malaise and fever, followed by upper and lower respiratory symptoms, myalgia, and headache. In adults, the fever and other systemic features usually last 3–5 days, whereas respiratory symptoms, particularly a cough, may persist 1–2 weeks. In addition to inducing constitutional symptoms, influenza infection causes desquamation of the respiratory epithelium and provokes an acute inflammatory response, in which mononuclear cells and then neutrophils migrate into the mucosa.

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In a recent study of nasal lavage fluid collected from volunteers experimentally infected with influenza A, we documented a cascade of cytokine responses that implicate their involvement not only in disease resolution but also in symptom formation. Specifically, interleukin (IL)-6, interferon (IFN)-α, and tumor necrosis factor (TNF)-α levels increased and peaked by days 2 or 3, while IL-8 levels peaked later. These responses correlated significantly with the magnitude and kinetics of viral replication, mucus formation, and disease symptoms [1].

Here we extend these prior observations in 3 regards. First, in a placebo-controlled trial, we explored the ability of a potent new inhibitor of influenza virus replication, zanamivir, to prevent the development of disease symptoms and to abrogate local responses. Second, we quantitated IFN-γ and IL-10 levels and those of other cytokines to examine the role of regulatory and inflammatory cytokines in influenza. Finally, we quantitated levels of a series of chemokines, members of a family of >30 chemotactic factors that are believed to be important in recruiting leukocytes to sites of inflammation.

The two major subfamilies of chemokines are designated CC and CXC, based on the arrangement of conserved cysteines in the *N*-terminal domains of the proteins. The best characterized neutrophil-active chemokines are in the CXC subfamily, whereas members of the CC and CXC subfamilies target lymphocytes, monocytes, and/or other granulocytes. As indicated, we had already documented increased levels of the CXC chemokine IL-8 on day 4 after infection [1]. Here, we quantitated nasal lavage fluid concentrations of the 2 CC chemokines mac-

rophage inflammatory protein (MIP)–1α and –1β, monocyte chemotactic protein (MCP)-1, and of the regulated-on-activation normal T cell–expressed and –secreted cytokine (RANTES).

Methods

Subjects. We recruited 16 healthy susceptible male volunteers (hemagglutination-inhibition [HI] antibody titers $\leq$1:8 to influenza A/Texas/36/91 [H1N1] virus) for study participation. The volunteers were studied in 2 successive cohorts of 7 and 9 subjects, respectively. Chronic use of any medication, including over-the-counter medications, that could interfere with the evaluation was prohibited. Other concurrents were permitted if agreed upon by the investigator and the study monitor. Subjects were compensated for their participation.

Study outline. Screening assessments were begun within 60 days of the scheduled virus inoculation to determine HI antibody status. Volunteers were confined to the isolation unit in the Clinical Research Center, University of Virginia Medical Center, 1 day before and for $\geq$7 days after inoculation. The participants were inoculated intranasally (0.25 mL/nostril) with $\approx 10^5$ $TCID_{50}$ of influenza A/Texas/36/91 (H1N1) virus on day 0. This virus strain and challenge paradigm have been used in many prior studies [1–4]. The subjects were randomized to receive either intravenous zanamivir (600 mg 2×/day) or placebo for 5 days in a blind fashion, beginning 4 h before virus inoculation. Although developed primarily for topical treatment of influenza, zanamivir was used in this study as a test of concept to determine the effect of systemic administration. Nasal lavage fluids (6–8 mL/wash) were collected (days 0–7) for virus isolation and titration by standard methods and for cytokine and chemokine determinations. Acute and convalescent sera (3–4 weeks after virus inoculation) were assayed for the presence of HI antibody titers to the challenge virus.

Oral temperatures were measured 4×/day. Fever was defined as an oral temperature >37.7°C. Symptom assessments were done by the volunteers 2×/day on a 4-point scale (0–3 corresponding from absent to severe). The symptoms assessed were nasal stuffiness, runny nose, sore throat, cough, sneezing, earache/pressure, breathing difficulty, muscle aches, fatigue, headache, feverish feeling, hoarseness, chest discomfort, and overall discomfort. The total symptom score for each time point was obtained by adding the individual symptom scores for that particular time point. The individual symptom scores contributing to the total symptom score were divided into three subgroups: systemic symptoms (muscle aches, fatigue, headache, and fever), upper respiratory symptoms (nasal stuffiness, earache/pressure, runny nose, sore throat, and sneezing), and lower respiratory symptoms (cough, breathing difficulty, hoarseness, and chest discomfort). Nasal discharge weights were determined throughout the isolation period by methods described elsewhere [5].

Sample preparation. Nasal lavage fluids were collected and mixed thoroughly with a syringe and were placed on wet ice. The nasal lavage fluids were clarified by centrifugation (1000 *g* for 10 min at room temperature), and the supernatant fluids were aliquoted and frozen at −70°C.

Cytokine determinations. Daily cytokine and chemokine levels in nasal lavage fluids were determined by commercial ELISA kits following the manufacturers' protocols. The kits were obtained from the following sources: IL-6, IL-10, and IFN-γ (Endogen, Cambridge, MA); TNF-α, MIP-1α, and MIP-1β (R&D Systems, Minneapolis); and MCP-1 and RANTES (BioSource International, Camarillo, CA). The limits of sensitivity of these assays (in picograms per milliliter), as supplied by the manufacturers, were as follows: IL-6 (<1), IL-10 (<3), IFN-γ (<2), TNF-α (<0.18), MIP-1α (<6), MIP-1β (<4), MCP-1 (<20), and RANTES (<3).

Data analysis. Analyses were based on data collected from all infected subjects (culture positive and/or a $\geq$4-fold rise in HI antibody titer) in the placebo group and from all subjects in the treatment group, except for 1 in the zanamivir group who was proven in retrospect to have been infected with a nonchallenge virus. Changes in measures of infection and illness (virus titer, symptom assessments, temperature, and nasal discharge weight) and cytokine and chemokine levels from baseline were analyzed by the Wilcoxon signed rank test (StatView version 4.5; Abacus Concepts, Berkeley, CA). Comparisons between the measures of illness and the levels of each cytokine and chemokine were made using Spearman's rank correlation test (StatView). The effect of zanamivir on the cytokine and chemokine responses was ascertained by determining the area under the curve (Prism version 2.0; GraphPad, San Diego) for each group and comparing the responses by Mann-Whitney *U* test (StatView).

Results

Influenza Infection

Sixteen healthy male volunteers were inoculated intranasally with influenza virus (A/Texas/36/91 [H1N1]) and followed serially to determine the relationship among symptoms, the development of cytokine and chemokine responses, and the effect of zanamivir treatment. Eight subjects were randomly allocated to the zanamivir group and 8 to the placebo group. Demographic characteristics were comparable in the zanamivir and placebo groups. The median age of the placebo and zanamivir groups was 23 years (range, 19–33 years) and 20.5 years (range, 19–35 years), respectively. One subject in the zanamivir group was infected with a rhinovirus and was excluded from the analysis. The presence of influenza infection was determined by seroconversion and by recovery of the virus from the nasal lavage fluid. Influenza virus was recovered from 8 of 8 volunteers in the placebo group. As shown in figure 1, the mean virus titer in the nasal lavage fluids of patients in the placebo group peaked on day 2, rising significantly above baseline ($P < .05$) and gradually declined; in these subjects, virus shedding persisted for 4.6 ± 1.5 days. In contrast, persons in the zanamivir treatment group did not shed detectable virus on any day (F.G.H., unpublished data).

Symptom Patterns

All of the infected volunteers (i.e., the 8 subjects in the placebo group) developed $\geq$1 symptoms; these symptons peaked

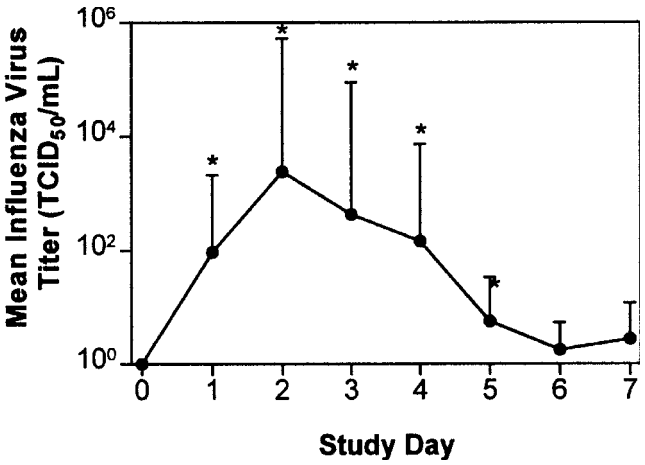

Figure 1. Mean nasal lavage fluid virus titers (±SD) after experimental influenza A/Texas/36/91 (H1N1) infection. Mean $TCID_{50}$/mL is shown for 8 volunteers in placebo group for each study day. $*P \leq .05$, Wilcoxon signed rank test, for differences from day 0.

on day 2 and returned to baseline by day 7. As shown in figure 2, 7 (88%) of the 8 subjects developed fever (oral temperature >37.7°C), which peaked on day 2 and was significantly higher than at study entry ($P < .05$).

As shown in figure 3, upper respiratory illness in infected (placebo) subjects, defined by the presence of symptoms such as nasal stuffiness, earache, runny nose, sore throat, and sneezing, occurred in 6 of 8 subjects by day 1 and was maximal 2–3 days after virus inoculation. Systemic symptoms scores (myalgia, fatigue, headache, and fever) also peaked and were significantly increased on day 2. Lower respiratory symptoms (cough, breathing difficulty, hoarseness, and chest discomfort) were milder and peaked on day 3 when they were significantly increased relative to day 0 ($P < .05$). As shown in figure 4, nasal discharge weights were significantly elevated on both days 2 and 3 ($P < .05$), peaking on day 3 and returning to baseline on day 5.

Treatment with zanamivir significantly reduced the systemic ($P = .01$) and upper respiratory symptoms ($P = .001$) reported by the volunteers, the temperature response ($P = .003$), and the amount of nasal discharge ($P = .004$). Details of these and other effects of zanamivir will be presented in a separate report.

Cytokine and Chemokine Responses in the Infected Placebo Group

Cytokine levels in nasal lavage fluid. Nasal lavage fluids collected daily on days 0–7 were assayed for IL-6, TNF-α, IFN-γ, and IL-10. As shown in figure 5, peak responses in nasal lavage fluid levels of all 4 cytokines occurred on day 3. The magnitude of the mean IL-6 and TNF-α responses were comparable, exhibiting 87- and 81-fold increases above baseline, respectively, on day 3. Six of 8 infected volunteers exhibited increases in IL-6, while 7 of 8 had increases in TNF-α. Both increases were statistically significant ($P < .05$). The IFN-γ and IL-10 responses were of a lower magnitude (4- and 18-fold, respectively) and did not achieve statistical significance.

Chemokine levels in nasal lavage fluid. The levels of MIP-1α, MIP-1β, MCP-1, and RANTES were determined by using a different aliquot of the same serial nasal lavage fluid samples, as used for the cytokine determinations. As shown in figure 6, the levels of MIP-1α, MIP-1β, and MCP-1 in infected volunteers increased in roughly the same pattern as the cytokines, with responses peaking on days 2–4 and returning to baseline on day 6. The magnitude of these responses were similar to that of IL-6 and TNF-α and were statistically significant compared with the baseline ($P < .05$), at least once in each case. Of interest, MCP-1 displayed a broad peak that only reached statistical significance on day 5, suggesting a prolonged response. As also shown in figure 6, the RANTES response did not show a significant elevation in the nasal lavage fluid over the course of the infection.

Correlation of Cytokine and Chemokine Levels with Measures of Infection and Symptoms in Influenza Infection

The relationship between the clinical features of influenza A virus infection and the levels of cytokines and chemokines observed in the nasal lavage fluid was investigated by subjecting the data from the placebo recipients to Spearman's rank correlation analyses.

Nasal lavage fluid cytokine responses. As shown in table 1, the rises in nasal lavage fluid IL-6 and IFN-γ content among the placebo recipients correlated significantly ($P < .05$) with virus titer, total symptoms, oral temperature, and nasal discharge weight. Levels of these 2 cytokines also correlated with the other

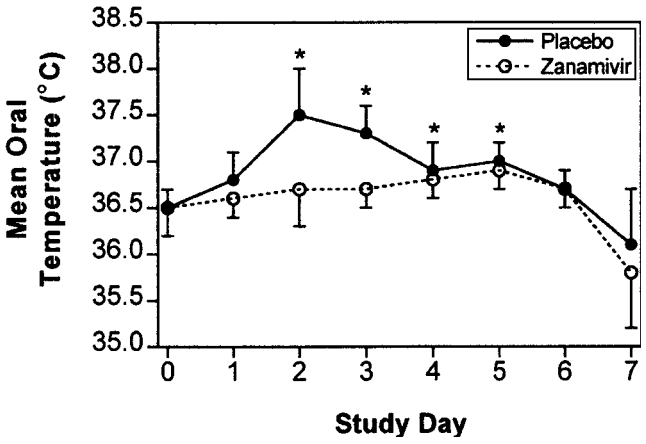

Figure 2. Mean oral temperature (±SD) of subjects experimentally infected with influenza A/Texas/36/91 (H1N1). Oral temperatures were determined 4×/day for duration of study. Values represent mean for 8 subjects in placebo group and 7 zanamivir recipients. $*P \leq .05$, Wilcoxon signed rank test, for differences from day 0.

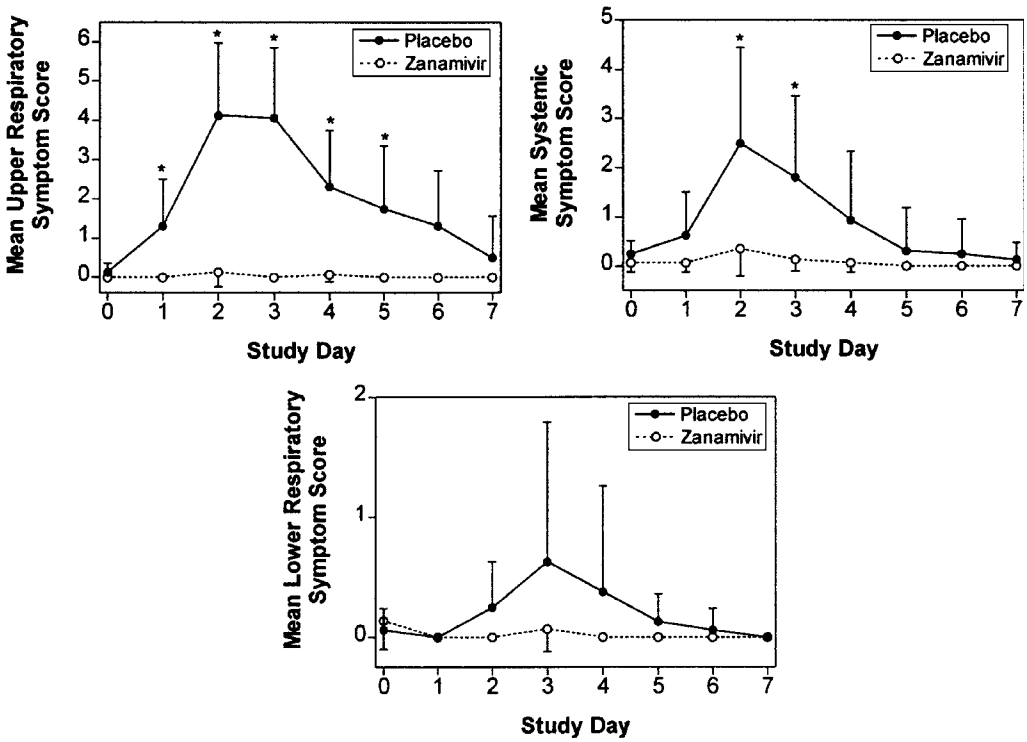

Figure 3. Mean symptom scores (±SD) of volunteers experimentally infected with influenza A/Texas/36/91 (H1N1). Subjects assessed symptoms twice daily on 4-point scale (absent = 0 to severe = 3). Score for each symptom group was obtained by adding individual scores for that particular group. Daily score was obtained taking average of 2 symptom scores for that particular day. Symptom subgroup scores for upper respiratory, systemic, and lower respiratory symptoms of placebo and zanamivir recipients are shown. *$P \leq .05$, Wilcoxon signed rank test, for changes in scores from study initiation (day 0).

categories of symptoms, namely, upper respiratory symptoms, lower respiratory symptoms, and systemic symptoms (IFN-γ only). TNF-α and IL-10 levels correlated with nasal discharge weight and variably with the symptom subgroup scores.

Nasal lavage fluid chemokine responses. As also shown in table 1, the MIP-1α response correlated with all the features of influenza infection studied; however, the MIP-1β response correlated with all features except temperature elevation and systemic symptoms. In contrast, the MCP-1 response correlated significantly only with the lower respiratory symptom score. RANTES levels, which were not elevated at any point, did not correlate with any of the clinical features measured.

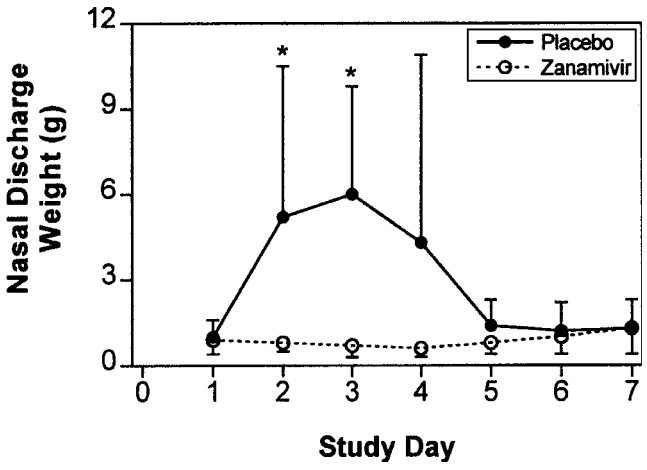

Figure 4. Mean nasal discharge weights (±SD) of volunteers experimentally infected with influenza A/Texas/36/91 (H1N1). Nasal discharge weights were determined daily using preweighed tissues. Mean nasal discharge weight for placebo and zanamivir groups are shown for each study day. *$P \leq .05$, Wilcoxon signed rank test, for differences from day 0.

Effect of Zanamivir Treatment

As expected from its effect on viral replication and clinical symptoms, prophylactic administration of zanamivir essentially abrogated all of the observed cytokine and chemokine responses that occurred during influenza A infection. Thus, comparison of integrated (area under the curve) responses showed zanamivir treatment to be associated with markedly smaller

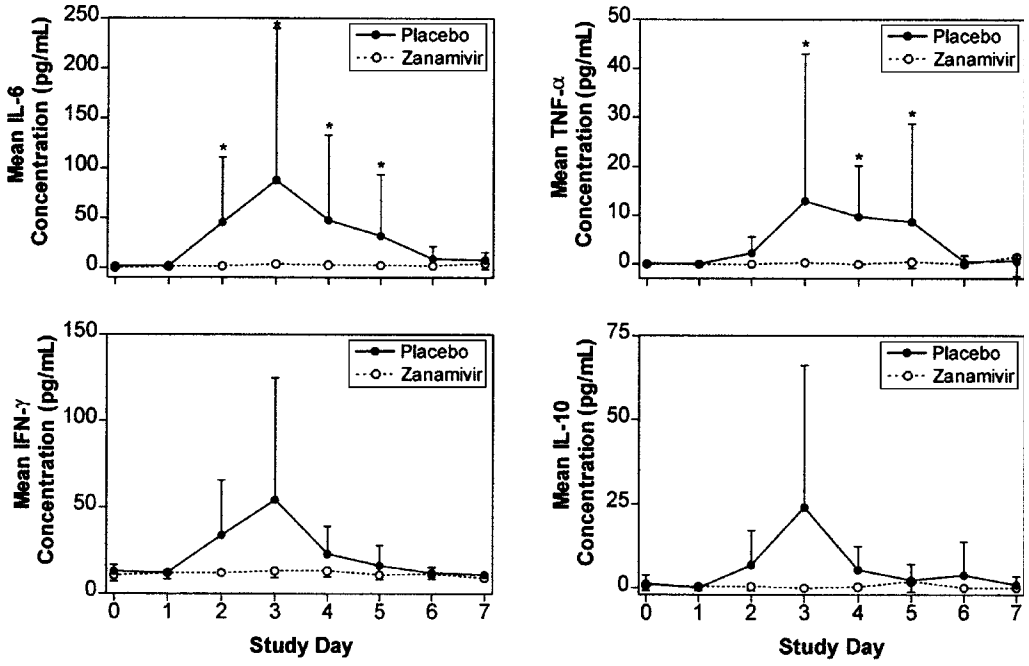

Figure 5. Mean nasal lavage fluid cytokine levels (±SD) in 15 volunteers experimentally infected with influenza A/Texas/36/91 (H1N1) and effect of treatment with intravenous zanamivir in 7 volunteers. Cytokine levels for each day were determined by commercial ELISA kits. $*P \leq .05$, Wilcoxon signed rank test, for differences from baseline for placebo recipients. IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

responses of IL-6, TNF-α, IFN-γ, IL-10, and MCP-1, relative to the placebo group (table 2).

Discussion

We recently reported [1] that nasal lavage fluid levels of IL-6, TNF-α, and IL-8 in humans acutely infected with influenza A virus increase and correlate with the severity of local and systemic illness symptoms. Here we confirm and extend these earlier observations. In addition to rises in IL-6 and TNF-α, there are concurrent rises in IL-10 and IFN-γ levels and in the chemokines MIP-1α, MIP-1β, and MCP-1. Moreover, prophylactic treatment with zanamivir, an agent that effectively prevents demonstrable infection, abrogates these cytokine and chemokine responses. Thus, measurement of nasal cytokine and chemokine levels reveals the fundamental immune responses in human influenza, while providing objective and convenient markers of illness severity and of the efficacy of novel therapeutic interventions.

In the study reported here, we confirm the significant elevations in the nasal lavage fluid levels of IL-6 and TNF-α. As compared with our prior results [1], the kinetics of the responses in this study were slightly different—the IL-6 peak occurred 1 day later (day 3), and the TNF-α peak occurred 1 day earlier (day 3). However, in parallel with our previous study, significant elevations of IL-6 were noted earlier than elevations in TNF-α, and IL-6 elevations correlated better with upper respiratory symptoms than did TNF-α. IL-6 was the only cytokine whose levels were significantly elevated by study day 2. Thus, the conclusions reached earlier, that IL-6 is the first cytokine to appear in influenza infection and the one likely responsible for much of the early symptom formation, still holds.

In the present study, we extended our characterization of the local immune response exhibited by influenza infection to include IL-10 and IFN-γ, 2 cytokines that regulate T cell differentiation during inflammation. IL-10 is a pleiotropic cytokine with the ability to block cytokine synthesis and several accessory functions of macrophages. Perhaps its most important effect in this regard is its potent capacity to turn off dendritic cell and macrophage production of IL-12, thereby shutting off the Th1 T cell response and ultimately the production of IFN-γ, the linchpin of Th1-mediated inflammation. This, plus the fact that IL-10 also inhibits macrophage production of TNF-α and IL-6, establishes IL-10 as a major anti-inflammatory cytokine and suggests that its production early in influenza virus infection is an important mechanism by which the body controls and limits inflammation induced by such infection. In contrast to these negative aspects of IL-10 are its positive effects on Th2 T cell differentiation (mainly through the above-mentioned effect on IL-12/IFN-γ production) and its ability to act as a terminal differentiation factor for B cells. This leads to the suggestion that IL-10 plays a role in the B cell response to influenza antigen and thus helps drive the protective antibodies that evolve during and after infection.

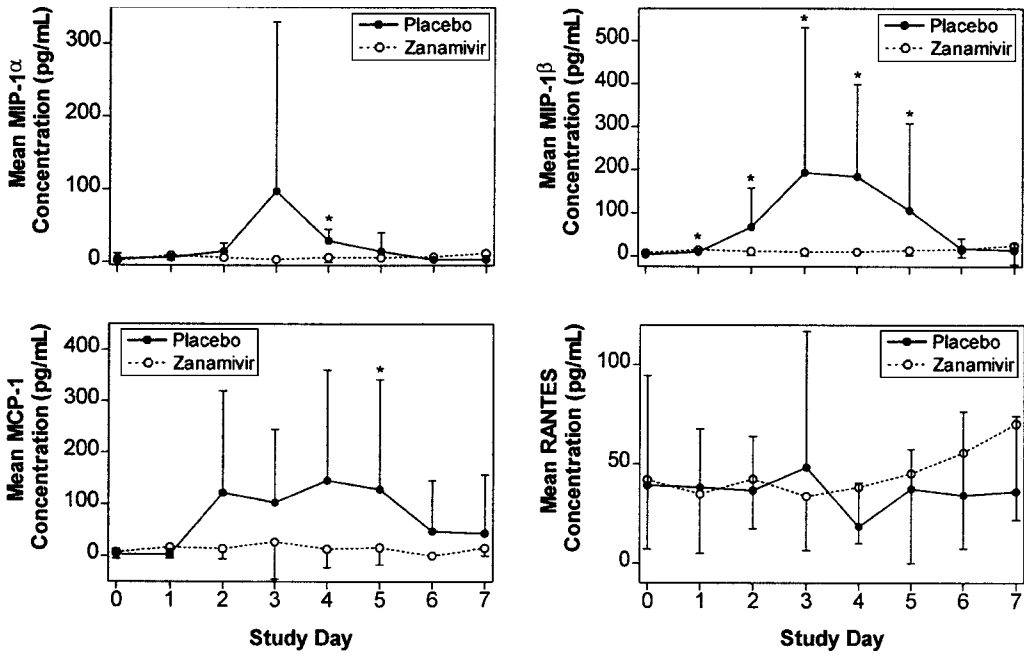

Figure 6. Mean nasal lavage fluid chemokine levels (±SD) in 15 volunteers experimentally infected with influenza A/Texas/36/91 (H1N1) and effect of treatment with intravenous zanamivir in 7 volunteers. Chemokine levels for each day were determined by commercial ELISA kits. *$P \leq .05$, Wilcoxon signed rank test, for changes in levels from study initiation (day 0) for placebo recipients. MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein.

The rise and fall of the nasal IFN-γ levels paralleled that of the other cytokines studied. However, the magnitude of the increase above baseline failed to reach statistical significance, suggesting that the response was not a major element of host defense in influenza infection in the relatively mild and short-lived experimentally induced upper respiratory infection studied here. These data therefore corroborate findings in mice with targeted disruption of the IFN-γ gene, which showed that mice lacking the capacity to release IFN-γ remain able to mount an effective cytotoxic T cell response to influenza infection [6]. A different situation may arise, however, if influenza infection establishes itself in the lower respiratory tract and becomes more severe. The IFN-γ appearing early in the infection probably arises from natural killer (NK) cells, whereas that occurring later in a more sustained lower respiratory infection would more likely arise from T cells mediating the Th1 response necessary for effective resolution of the infection.

The influx of inflammatory cells, particularly lymphocytes and monocytes, has long been recognized as the hallmark of host defense against viral infection [7]. Although we did not specifically enumerate or characterize these cells, the present findings, with respect to chemokine responses, suggest a mechanism by which these cells emigrate into the nasal mucosa. Chemokines, including MIP-1α, MIP-1β, and MCP-1, are induced in many types of cells, primarily in response to inflammatory cytokines such as TNF-α, IL-1, and IFN-γ [8]. The MIPs are produced primarily by monocytes/macrophages, lymphocytes, and fibroblasts, while MCP-1 is produced by monocyte/macrophages and a variety of tissue cells, including endothelial cells, epithelial cells, keratinocytes, and tumor cells. MIP-1α can act through the receptors CCR1, expressed on monocytes, T cells, dendritic cells, and NK cells [9]. MIP-1β also acts through CCR5, and MCP-1 acts through CCR2, which is expressed on monocytes, T cells, B cells, dendritic cells, and NK cells.

Several studies have reported the expression of CC chemokines in response to viral infections. These studies include lymphocytic choriomeningitis in mice [10], Epstein-Barr virus [11] and respiratory syncytial virus [12] infections in human cells, and cytomegalovirus encephalitis in human immunodeficiency virus–infected persons [13]. MIP-1α, in particular, has been implicated in the inflammatory reaction to viral infection. By use of antibodies against MIP-1α as well as MIP-1α knockout mice, this chemokine was shown to play a role in autoimmune myocarditis in mice infected with coxsackievirus [14], NK cell responses to murine cytomegalovirus [14], and experimental herpes stromal keratitis in mice [15]. Thus, as with the cytokine response, the pattern of chemokine release cannot be viewed as specific or unique to influenza. CC chemokines have been investigated in influenza virus infection of mice [14] and of human monocytes in vitro [16]. In MIP-1α knockout mice, there was reduced pulmonary inflammation and delayed clearance of virus from the lungs. Infected human monocytes secreted MIP-1α, MCP-1, and RANTES, not neutrophil che-

Table 1. Correlations between nasal lavage fluid cytokine and chemokine responses and clinical features of experimental influenza A virus infection in 8 placebo recipients.

Feature	IL-6	TNF-α	IFN-γ	IL-10	MIP-1α	MIP-1β	MCP-1	RANTES
Viral titer	.76[a] (.04)	—	.76 (.04)	—[b]	.83 (.03)	—[b]	—[b]	—[b]
Symptoms								
Total	.83 (.03)	—[b]	.81 (.03)	.73 (.05)	.83 (.03)	.74 (.05)	—[b]	—[b]
Upper	.89 (.02)	—[b]	.83 (.03)	.81 (.03)	.80 (.03)	.81 (.03)	—[b]	—[b]
Lower	.89 (.02)	.87 (.02)	.92 (.02)	.93 (.01)	.84 (.03)	.87 (.02)	.77 (.04)	—[b]
Systemic	—[b]	—[b]	.89 (.02)	—[b]	.86 (.02)	—[b]	—[b]	—[b]
Temperature	.76 (.04)	—[b]	.88 (.02)	.74 (.05)	.81 (.03)	—[b]	—[b]	—[b]
Nasal discharge	.93 (.02)	.89 (.03)	.86 (.04)	.89 (.03)	.86 (.04)	.86 (.04)	—[b]	—[b]

NOTE. IFN, interferon; IL, interleukin; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; TNF, tumor necrosis factor.
[a] Spearman's rank correlation coefficient (*P*).
[b] Not significant, $P > .05$.

motactic factors, and this was proposed as the basis for the mononuclear character of the infiltrate in influenza. The importance of chemokines in the host response to viral infection is emphasized by the discoveries that a number of viruses secrete potent chemokine inhibitors [16–20].

In view of the predominately mononuclear nature of the cells migrating into the upper respiratory mucosa in influenza, we investigated the role of the CC chemokines in the inflammatory process. The levels of MIP-1α, MIP-1β, and MCP-1 increased in response to infection and correlated with the kinetics of the symptom response. In addition, the MIP-1α response correlated with virus titer, temperature, and nasal discharge. The effect on these clinical features may be directly mediated by MIP-1α or indirectly by the cells recruited to the site of the infection and the soluble mediators they release.

It is noteworthy that among all of the chemokines studied, the most sustained response was by MCP-1, which has a potent capacity to recruit monocytes to sites of infection. MCP-1 favors Th2 responses [21–24] and therefore may also function to augment antibody responses to influenza virus infection.

Infection of human bronchial epithelial cells with influenza A virus (H3N2) induced the expression of RANTES [25]. The specificity of the induction was demonstrated by using antiserum against influenza A virus which inhibited the induction. We detected RANTES in the nasal lavage fluid from volunteers in this study, but the levels did not change over the course of the infection. Furthermore, treatment with zanamivir had no effect on the levels of this chemokine as it did with the other 3 chemokines we measured. The strain of virus used in this study (H1N1) produces a relatively mild disease and may not effectively induce RANTES expression, or RANTES might have little physiologic role in influenza infection.

Table 2. Effect of zanamivir on cytokine and chemokine levels (area under the curve [AUC]) in experimental influenza A virus infection.

	Mean AUC (SD)		
Cytokine/chemokine	Placebo (n = 8)	Zanamivir (n = 7)	P[a]
IL-6	229.6 (287.39)	16.1 (12.8)	.004
TNF-α	33.5 (43.7)	1.6 (3.9)	.004
IFN-γ	161.2 (104.0)	82.8 (19.2)	.04
IL-10	42.7 (57.7)	3.6 (4.1)	.01
MIP-1α	169.2 (236.4)	45.1 (26.3)	.08
MIP-1β	586.4 (632.6)	87.6 (67.5)	.06
MCP-1	576.3 (650.5)	99.9 (139.8)	.02
RANTES	250.8 (147.3)	305.7 (204.2)	—[b]

NOTE. IFN, interferon; IL, interleukin; MCP, monocyte chemoactic protein; MIP, macrophage inflammatory protein; TNF, tumor necrosis factor.
[a] Mann-Whitney *U* test on individual patient AUC values.
[b] Not significant, $P > .05$.

Zanamivir, a sialic acid analogue, is a potent inhibitor of influenza A and B virus neuraminidases [26, 27]. In this study, pretreatment with zanamivir prevented the infection and effectively abrogated the integrated responses of all of the cytokines and chemokines examined. These results confirm that active influenza virus replication is required for the induction of potent proinflammatory, regulatory, and chemotactic factors in the nose.

In summary, these studies verified our initial findings of the role of IL-6 and TNF-α in the pathogenesis of symptom production in influenza. In addition, we extended those findings to include 2 cytokines involved in the counterregulation of the immune response, IL-10 and IFN-γ, and 3 chemokines that mediate the influx of cells into the respiratory epithelium. The prevention of the infection and the abrogation of local responses with zanamivir treatment confirm the role of these cytokines and chemokines in the pathogenesis of influenza.

Acknowledgment

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