

Effect of rhinovirus 39 infection on cellular immune parameters in allergic and nonallergic subjects

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Patients with allergic rhinitis (AR), compared with nonallergic persons, have been reported to respond differently to a variety of stimuli, some of which are immunologic in nature. This study compared the systemic cellular immune responses to experimental rhinovirus (RV) 39 challenge in RV-39-seronegative AR (n = 20) and nonallergic (n = 18) subjects. Peripheral blood was obtained before, 4 or 7 days after, and 23 days after RV-39 intranasal challenge and assayed for the number and function of various white blood cells. All subjects were infected, as manifested by viral shedding in nasal secretions or seroconversion. RV-39 induced marked changes from baseline values in both immune cell number and functions. Compared with nonallergic subjects, AR subjects manifested different responses for the following parameters: (1) numbers of total white blood cells and lymphocytes (smaller increases on day 4), (2) helper/suppressor T cell ratio (absence of an increase on day 7 and presence of an increase on day 23), (3) number of IL-2 receptor-positive suppressor T cells (presence of a decrease on day 7), (4) natural killer (NK) cell numbers (absence of an increase on day 4 and presence of increases on days 7 and 23), (5) NK/T cell ratio (absence of an increase on day 4 and a decrease on day 7), (6) NK cell activity (a blunted decrease on day 7 and absence of a decrease on day 23), and (7) RV-39-induced lymphocyte proliferation (exaggerated increase on day 4). The results show that intranasal challenge with RV-39 induced RV-39-specific and nonspecific systemic cellular immune responses and a unique immunologic response pattern in AR subjects. (J ALLERGY CLIN IMMUNOL 1993;92:732-43.)

Key words: Rhinovirus, common cold, cellular immunity, allergy, lymphocytes, natural killer cells

One of the most common reasons for physician office visits and school or work absenteeism is the common cold and its sequelae. Although clinicians have long been aware of its symptomatic manifestations, researchers have only recently begun to identify the physiologic, inflammatory, and immune responses to common cold viruses.¹⁻⁷ Sev-

Abbreviations used

AR:	Allergic rhinitis
cpm:	Counts per minute
IL:	Interleukin
LU:	Lytic unit
NK:	Natural killer
PBMNC:	Peripheral blood mononuclear cells
PHA:	Phytohemagglutinin
RV:	Rhinovirus
WBC:	White blood cell

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eral variables confound the interpretation of the results of such studies. For example, certain effects associated with the common cold may be virus specific, and attempts to generalize findings obtained from one cold virus to those of all common cold viruses may not be appropriate. Moreover, failure to define vigorously host characteristics that might influence sensitivity to cold

viruses further confounds the interpretation of results. For example, the presence of upper airway allergy and inflammation could influence the local response to cold viruses.⁷ Additionally, alterations in function of different types of immune and inflammatory cells are strongly associated with allergy.⁸⁻¹²

Experimental rhinovirus (RV) infections have been reported to induce alterations in peripheral blood lymphocyte numbers and function,³⁻⁶ indicating systemic effects for a virus whose associated pathology is generally assumed to remain localized to the upper respiratory tract. In some studies these immune changes were related to clinical manifestations of the respiratory infection.^{3, 5}

Experimental trials conducted by our investigative group have shown that patients with asymptomatic allergic rhinitis (AR), when studied outside their relevant allergen seasons, do not hyperrespond physiologically to RV-39.¹ Yet these same AR subjects had an increased vascular permeability response to RV-39 challenge as measured by the relative protein content of nasal secretions collected during the experimental infection.²

The purpose of the current study, conducted in conjunction with a protocol to measure nasal physiology and biochemistry, was threefold: (1) to monitor a variety of immune parameters during infection with the common cold virus RV-39, (2) to compare these responses in allergic and non-allergic persons, and (3) to determine whether the development or resolution of cold-associated symptoms is related to any of these immune parameters.

METHODS

Study population and study protocol

Our research was approved by the Human Rights Committee, Children's Hospital of Pittsburgh, and informed consent was provided by each of the study subjects. The study population and methods employed for RV-39 inoculation and collection of physiologic and symptomatic data have been described previously.¹ Briefly, the study population included 20 adult AR patients, defined by the presence of a positive history, positive skin test, and elevated specific serum IgE antibodies to inhalant allergens, and 18 control adult patients who were negative for AR by history, skin tests, and serum IgE antibodies. Subjects with a history of asthma, perennial rhinitis, or a respiratory infection in the preceding 3 weeks were excluded from the study. Subjects were prescreened for neutralizing antibodies to RV-39 immediately before study entry, and all were found to be seronegative, as indicated by a titer of less than 2.

The study was conducted during March, when relevant allergens were not present in the environment. Subjects were randomly assigned to one of two cohorts, which were challenged with the virus and monitored 2 weeks apart in an identical fashion, except for the timing of blood sampling, as noted below. Cohort I included 10 AR and eight nonallergic subjects (4 male, 14 female), and cohort II consisted of 10 AR and 10 nonallergic subjects (13 male, seven female). During the study subjects were asked to refrain from taking medications, with the exception of birth control pills.

The challenge virus pool was a safety-tested clinical isolate, RV-39, passaged twice in WI-38 human embryonic lung fibroblasts. Subjects were infected with RV-39 on day 0 and cloistered in a local hotel from days 2 through 7 after infection. The following physiologic parameters were monitored throughout the study: secretion production, as assessed by weighed tissues; nasal patency, by active posterior rhinomanometry; nasal clearance, by the dyed saccharin technique; pulmonary function, by spirometry; eustachian tube function, by sonotubometry; and middle ear status, by tympanometry. None of these physiologic tests is invasive.¹ Additionally, daily nasal lavages were performed for viral culture and biochemical parameters.² Symptoms were measured by interview at baseline and daily after the RV-39 challenge. Subjects rated eight symptoms, including sneezing, nasal discharge, nasal congestion, malaise, headache, chills, sore throat, and cough, on a scale of 0 to 3, corresponding to none, mild, moderate, or severe. On the day of release from cloistering (day 7) the subjects were asked whether they believed that they had had a cold.

The presence of infection was defined by active shedding of virus on any day of cloister (as determined by the presence of virus in nasal lavage culture) or seroconversion to a neutralizing antibody titer of at least 8 when serum was assayed 23 days after the virus challenge. The presence of a cold was defined by the following modified Jackson criteria: a total interview symptom score for the period of cloister of more than 5 and either symptoms of nasal discharge for more than 2 days, or the subject's impression of having had a cold.¹

Blood samples for cellular immune tests were obtained at baseline and 23 days after RV-39 challenge in all subjects. In addition, samples were obtained on day 7 for cohort I or on day 4 for cohort II. All blood samples were obtained at approximately the same time each morning (6:30 AM to 8:30 AM) and delivered immediately to the laboratory for processing.

Assessment of viral infection

The methods for performing nasal lavages, culturing RV-39 for nasal lavage samples, and assaying serum neutralizing antibodies to RV-39 were described previously.¹

Assay of immune cell number and function

Overview. Lymphocyte subsets were enumerated by flow cytometry. Additionally, several cell functions were

assayed, including natural killer (NK) activity, and in vitro proliferation of mononuclear cells in response to phytohemagglutinin (PHA) or RV-39.

Preparation of peripheral blood mononuclear cells. Peripheral blood mononuclear cells (PBMNC) were obtained by centrifugation of heparinized venous blood on Ficoll-HiPaque gradients. PBMNC were recovered from the gradients, washed in RPMI 1640 (Gibco, Grand Island, N.Y.), and counted in the presence of trypan blue dye. Fresh PBMNC were used for NK cytotoxicity and flow cytometry studies. For proliferation assays PBMNC were suspended at the cell concentration of 5 to $10 \times 10^6/\text{ml}$ in RPMI 1640 medium containing 20% (vol/vol) fetal calf serum and 10% dimethyl sulfoxide, cryopreserved in a controlled-rate liquid nitrogen freezer (Cryomed, New Baltimore, Md.), and stored in liquid nitrogen vapors for assays at a later time.

Flow cytometry. For two-color flow cytometry, cells were adjusted to a concentration of $0.5 \times 10^6/\text{ml}$ in phosphate-buffered saline solution containing 0.1% sodium azide. Cells were stained with fluorescein- and phycoerythrin-labeled monoclonal antibodies specific for T cell-associated antigens CD3, CD4, and CD8; activation antigens interleukin (IL)-2 receptor (CD25) and HLA-DR; NK cell-associated antigens CD56 and CD16; and a B-cell antigen, CD19. All monoclonal antibodies were purchased as labeled reagents from Becton Dickinson, Mountain View, Calif. Staining and two-color flow cytometry were performed as described earlier.¹³ Controls included isotype monoclonal antibodies, phosphate-buffered saline solution, and a CD14/CD45 monoclonal antibody combination.

Lymphocyte transformation assays. Proliferation microassays were done with cryopreserved PBMNC. The samples were batched so that all samples obtained from a given subject were tested in the same assay. After being defrosted PBMNC were checked for viability with trypan blue, washed in medium, and plated in wells of Terasaki plates at $2 \times 10^6/\text{ml}$. The cell suspension was added to the wells first ($10 \mu\text{l}/\text{well}$), followed by PHA-P mitogen (Gibco) at 10, 5, and 2.5 $\mu\text{g}/\text{ml}$, or a RV-39 antigen (ATCC, 10 $\mu\text{l}/\text{ml}$) obtained by freeze-thaw of MRC5 cells and used at 1:10, 1:5, and 1:2.5 dilutions of the supernatants. The cells were incubated in the presence of stimulators for 3 days (with PHA) or 5 days (with RV-39). On day 3 or 5, respectively, [^3H]-thymidine, 1 $\mu\text{Ci}/\text{well}$ (NEN, Boston, Mass.; specific activity of 6 Ci/mmol per liter), was added for the last 18 hours of incubation. The plates were incubated in a water-jacketed carbon dioxide incubator at 37° C. The cultures were harvested with a Brandel microharvester designed specifically for use with Terasaki plates. After being harvested on filter disks, the disks were transferred to counting vials, dried, and counted in a liquid scintillation counter. The results were expressed as the sum of net counts per minute (cpm) for three different concentrations or dilutions of each stimulating agent used. The sum of these

net cpm is proportional to the area under the dose-response curve generated for mitogen or antigen concentrations used. Banked PBMNC of three normal persons were defrosted and used as a measure of interassay variability each time the assay was performed.

NK cell assay. NK activity was measured in 4-hour chromium 51-release assays as described previously.¹⁴ K562, a chronic myelogenous leukemia cell line, was used as a target cell. All assays were performed at effector/target ratios ranging from 1:50 to 1:6 in triplicate. The data are presented as log lytic units (LU)₂₀ per 10^7 effector cells, with one LU of NK activity defined as the number of effector cells required for 20% lysis of 5×10^3 target cells.

Statistical methods

For analysis of symptoms the total symptom score (sum of eight interview symptom scores) and the nasal symptom score (sum of rhinorrhea, congestion, and sneezing interview scores) were considered. The methods used for analysis of these variables were described previously.¹

Immunologic data are presented as changes from baseline values. Each subject's data obtained for days 4 or 7 and for day 23 after challenge were compared with his or her own prechallenge baseline and plotted as median changes from baseline for AR and nonallergic subjects. The box plots that are used to display the data give a median value (a horizontal line) and lower 25th and upper 75th percentile range for each parameter.

Lymphocyte or lymphocyte subpopulation counts are presented as absolute numbers, which were transformed into log₁₀ values. The results of lymphocyte proliferation assays are expressed as the square root of the sum of the net cpm. NK activity is presented as log₁₀ LU. These transformations were made to obtain approximately normal distributions to satisfy the assumptions of the statistical procedures.

Analysis of variance and *t* tests were used to compare immune parameters between cohorts and allergic status groups. Where the normality assumption of these tests was not met, Kruskal-Wallis and Wilcoxon rank sum tests were used.¹⁵

The nominal type I error rate for all statistical tests was 0.05. Because of the large number of tests performed, the true type I error rate was greater than that figure. The *p* values within each cohort or allergic status group comparison were therefore adjusted with a Bonferroni correction.¹⁶

RESULTS

Documentation of infection and illness

The descriptive clinical and laboratory parameters related to the RV-39 challenge were presented previously.¹ All subjects were infected as indicated by virus shedding and/or seroconversion. Fifteen of 18 nonallergic (83%) and 17 of 20

TABLE I. Immune cell number and function at baseline in allergic (AR) and nonallergic subjects

Cell parameter	AR (n = 20)	Nonallergic (n = 18)
WBCs (cells/mm ³)	5263 ± 1456	5677 ± 1412
Lymphocytes (cells/mm ³)		
Total	1405 ± 357	1671 ± 494
T cells	1021 ± 290	1350 ± 408*
DR + (activated) T cells	51 ± 30	55 ± 28
Helper T cells	640 ± 207	883 ± 342*
CD25 + (activated) helper T cells	124 ± 66	154 ± 50*
Suppressor T cells	434 ± 145	522 ± 151
CD25 + (activated) suppressor T cells	13 ± 13	11 ± 12
Helper/suppressor T ratio	1.6 ± 0.6	1.7 ± 0.6
B cells	109 ± 66	114 ± 69
NK cells	246 ± 106	222 ± 84
DR + (activated) NK cells	24 ± 16	24 ± 27
NK/T cell ratio	0.2 ± 0.1	0.1 ± 0.1
Activated NK cell/activated T cell ratio	0.5 ± 0.3	0.4 ± 0.4
Lymphocyte function		
PHA stimulation†	50.5 ± 9.3	57.0 ± 12.0
RV-39 stimulation†	1.9 ± 1.5	3.6 ± 2.6*
NK activity (LU ₂₀ /10 ⁷ cells)	144 ± 95	124 ± 59

Values expressed as mean ± SD.

*Statistically significant difference ($p < 0.05$) between AR and nonallergic subjects.

†Sum of net cpm divided by 1000.

AR subjects (85%) had a cold on the basis of the modified Jackson criteria. All six subjects without a cold were in cohort II. Symptoms showed a slight increase on day 1, a peak on day 2 or 3, and a return to the baseline values by days 6 to 10. Mucus production increased on day 2 and peaked on day 3.¹

Prechallenge (baseline) immune parameters

Effect of cohort assignment. The effect of cohort assignment on lymphocyte number and function was examined separately for AR and nonallergic subjects. Of the 17 different parameters examined for each allergy status group, statistically significant ($p < 0.05$) cohort differences were noted for only three immunologic parameters. Thus the absolute number of CD8+ T suppressor cells (mean ± SD) was 379 ± 158 and 513 ± 90 for AR subjects in cohorts I and II, respectively. The CD56+ NK cell/CD3+ T-cell ratio was 0.2 ± 0.1 and 0.1 ± 0.02 for nonallergic subjects in cohorts I and II, respectively. The absolute number of CD3+DR+ activated T cells was significantly different in cohorts I and II for both AR and nonallergic groups: 40 ± 15 and 64 ± 40 in AR subjects, and 36 ± 15 and 64 ± 20 for nonallergic subjects, respectively. Although cohort assign-

ment was associated with these unexpected differences, the data for the two cohorts were combined in subsequent comparisons between AR and nonallergic subjects. We believed this was justified because the differences at baseline were infrequent (in 3/17 parameters), relatively small, largely confined to one or the other allergy status groups, and without any known clinical relevance.

Effect of allergy status. The baseline levels of lymphocyte number and function are presented for AR and non AR subjects in Table I. Compared with nonallergic subjects, AR subjects had significantly fewer circulating CD3+ T cells ($p = 0.01$), CD4+ helper T cells ($p = 0.02$), and CD4+CD25+ activated helper cells T ($p = 0.04$). Also, RV-39-induced lymphocyte proliferation was significantly ($p = 0.01$) decreased in AR compared with nonallergic subjects. Differences between AR and nonallergic subjects were not observed for any of the other measured immune parameters at baseline.

Postchallenge immune parameters

Effect of allergy status. The postchallenge differences from baseline for AR and nonallergic subjects were not significantly different except for two immune parameters: (1) NK cell activity on

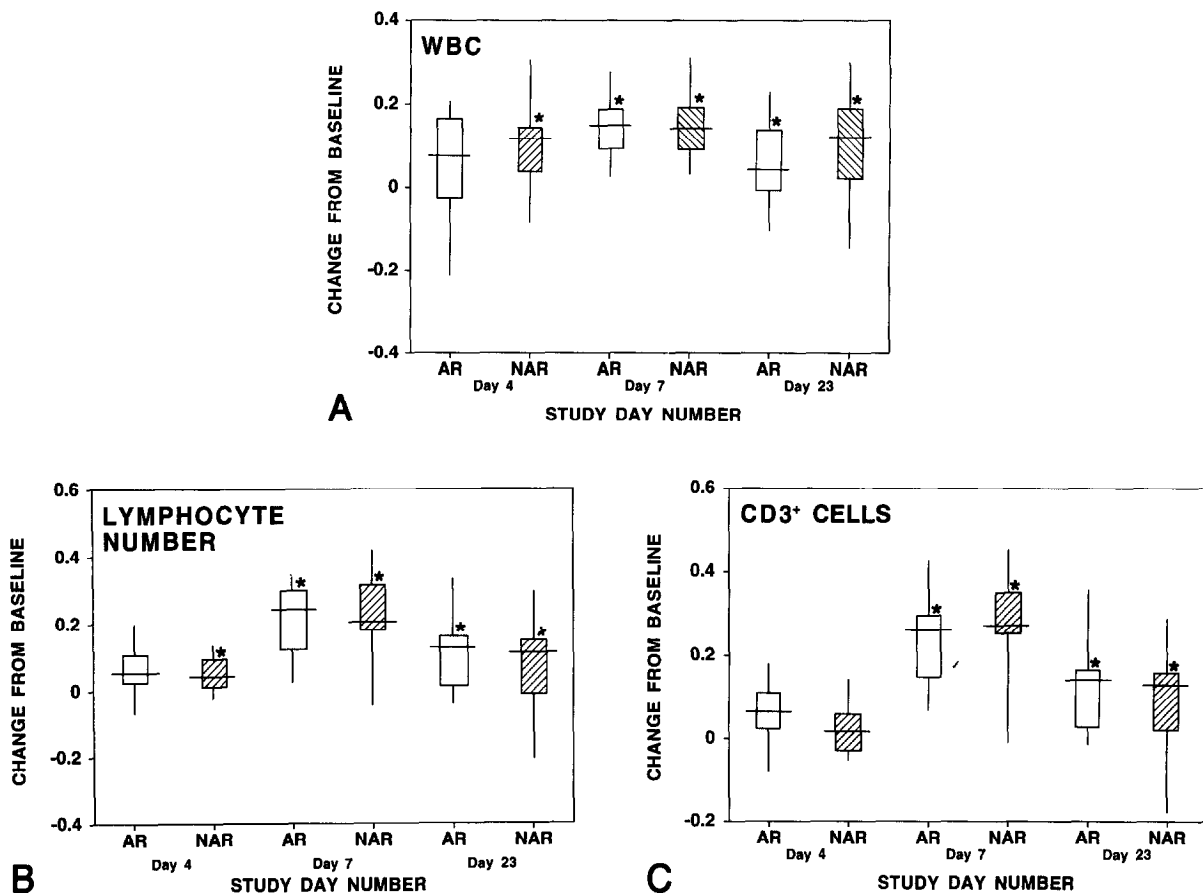


FIG. 1. Box plots for differences from baseline in $\log_{10}$ counts of WBCs (A), lymphocytes (B), and CD3+ T lymphocytes (C) by allergy status and day of study. Horizontal lines at top and bottom of boxes represent 75th and 25th percentiles of sample, respectively. Horizontal line within box represents median value. Vertical lines extending upward and downward from box indicate maximum and minimum values of sample, respectively, and can extend up to 1.5 times interquartile range. Significant differences from baseline are indicated by asterisks, and p values are given in text for this and all other figures. NAR, Nonallergic subjects.

day 7, which decreased significantly more in non-allergic than in AR subjects ($p = 0.004$) and (2) RV-39-induced lymphocyte proliferation on day 4, which also decreased significantly more in non-allergic than in AR subjects ($p < 0.0015$). These changes after treatment from baseline values are shown in Figs. 3, B, and 4, A, respectively.

Effect of RV-39 challenge on immune parameters. Because of the relatively few postchallenge differences between AR and nonallergic subjects, the data for the two groups were combined for analysis to examine the overall effect of the RV-39 challenge on the immune system parameters. Exceptions to pooling included NK cell activity and lymphocyte proliferation to RV-39, where postchallenge differences from baseline for AR and nonallergic subjects were significantly different on one or more of the postchallenge study days (Fig.

3, b, and 4, a). When a significant difference from baseline was noted for a given variable, each of the postchallenge days and allergy status groups was analyzed separately.

As shown in Fig. 1, A, a significant overall RV-39-induced increase was found in the number of white blood cells (WBCs) ($p = 0.0003$) in both AR and nonallergic groups. This increase was significant for nonallergic subjects only on postchallenge day 4 ($p = 0.03$) but was most dramatic, and was observed in both AR and nonallergic groups on days 7 and 23 ($p = 0.0001$ and $p = 0.0002$, respectively). An identical pattern was observed for the total number of lymphocytes (Fig. 1, B) and for the number of CD3+ T lymphocytes (Fig. 1, C), except that for total lymphocytes the overall difference from baseline on day 4 was more striking ($p = 0.004$) and that

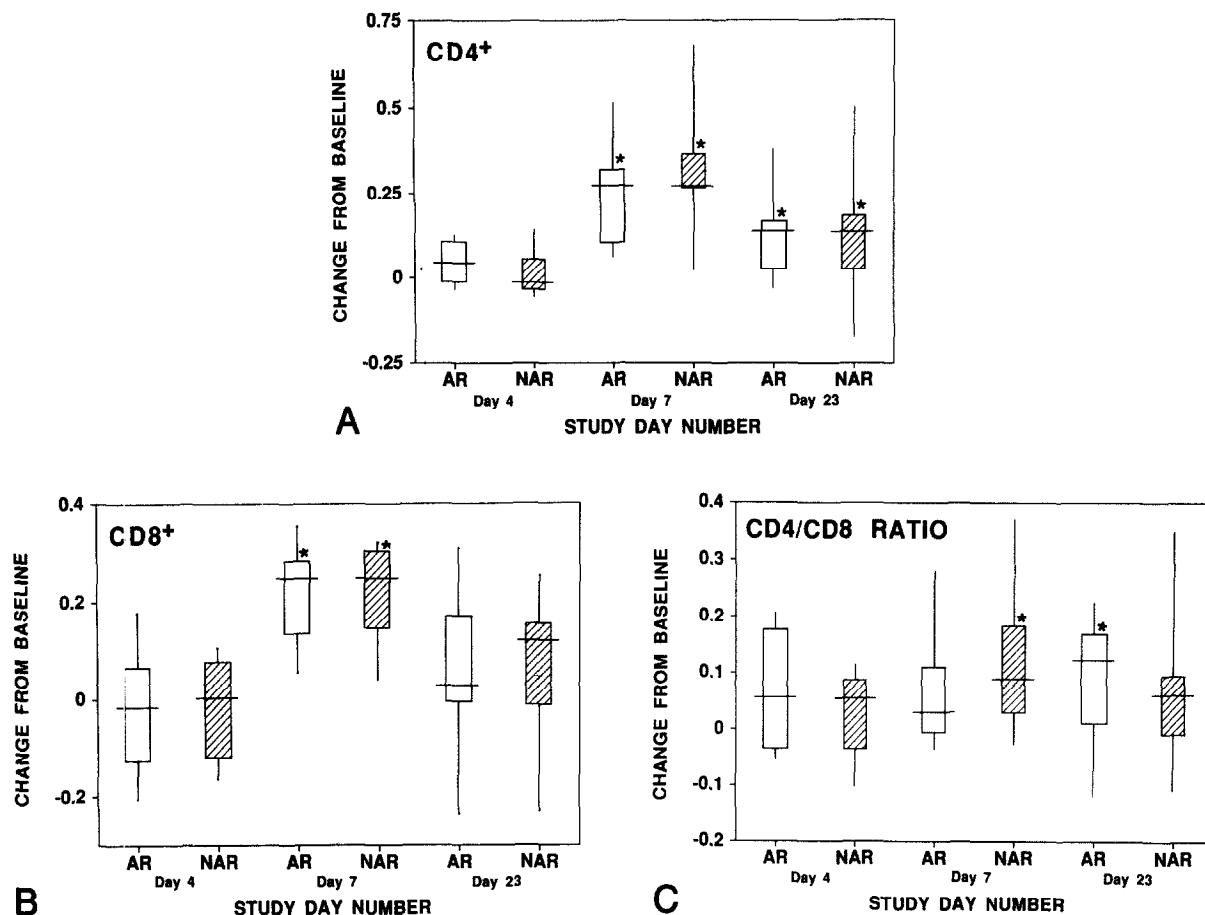


FIG. 2. Box plots for differences from baseline in $\log_{10}$ CD4+ helper T lymphocytes (A), CD8+ suppressor T lymphocytes (B), and CD4+/CD8+ ratio (C) by allergy status and day of study. Description of box plot is same as that in Fig. 1.

for CD3+ T lymphocytes the differences from baseline on day 4 were not statistically significant ($p = 0.06$). Additionally, significant RV-39-induced increases in the numbers of circulating CD3+DR+ activated T lymphocytes were observed on day 7 for both AR and nonallergic subjects ($p = 0.03$).

Not only the number of total T lymphocytes but also that of both CD4+ helper T and CD8+ suppressor T lymphocyte subsets increased significantly in comparison with baseline values in both the AR and non-AR group after the RV-39 challenge ($p = 0.0003$ and $p = 0.0009$, respectively) (Fig. 2, A and B). For CD4+ T cells this effect was observed on days 7 ($p = 0.0001$) and 23 ($p = 0.0001$), and for CD8+ T cells on day 7 only ($p = 0.0003$). An overall increase in the CD4+/CD8+ ratio was also observed ($p = 0.003$), but this effect was confined to nonallergic subjects on day 7 and AR subjects on day 23 (Fig. 2, C). In terms of CD4+CD25+ and

CD8+CD25+ (IL-2 receptor-positive, activated helper T and suppressor T lymphocytes, respectively) no significant differences from baseline were observed with the exception of a moderate but significant ($p = 0.03$) decrease in the latter on day 7 in the AR group only. Additionally, no significant RV-39-induced changes were observed for CD19+ B lymphocytes.

Fig. 3, A shows the effect of RV-39 challenge on the absolute number of CD3-CD56+ NK cells. A significant increase compared with baseline values was observed on day 4 in the nonallergic group only ($p = 0.02$), with a return to baseline levels by day 7. Such an increase was not evident in the AR group until day 7 and was also present in this group on day 23 ($p = 0.04$). This observation indicated that the AR group had a delayed and possibly sustained increase in the number of circulating NK cells in response to RV-39 challenge. On the other hand, the number

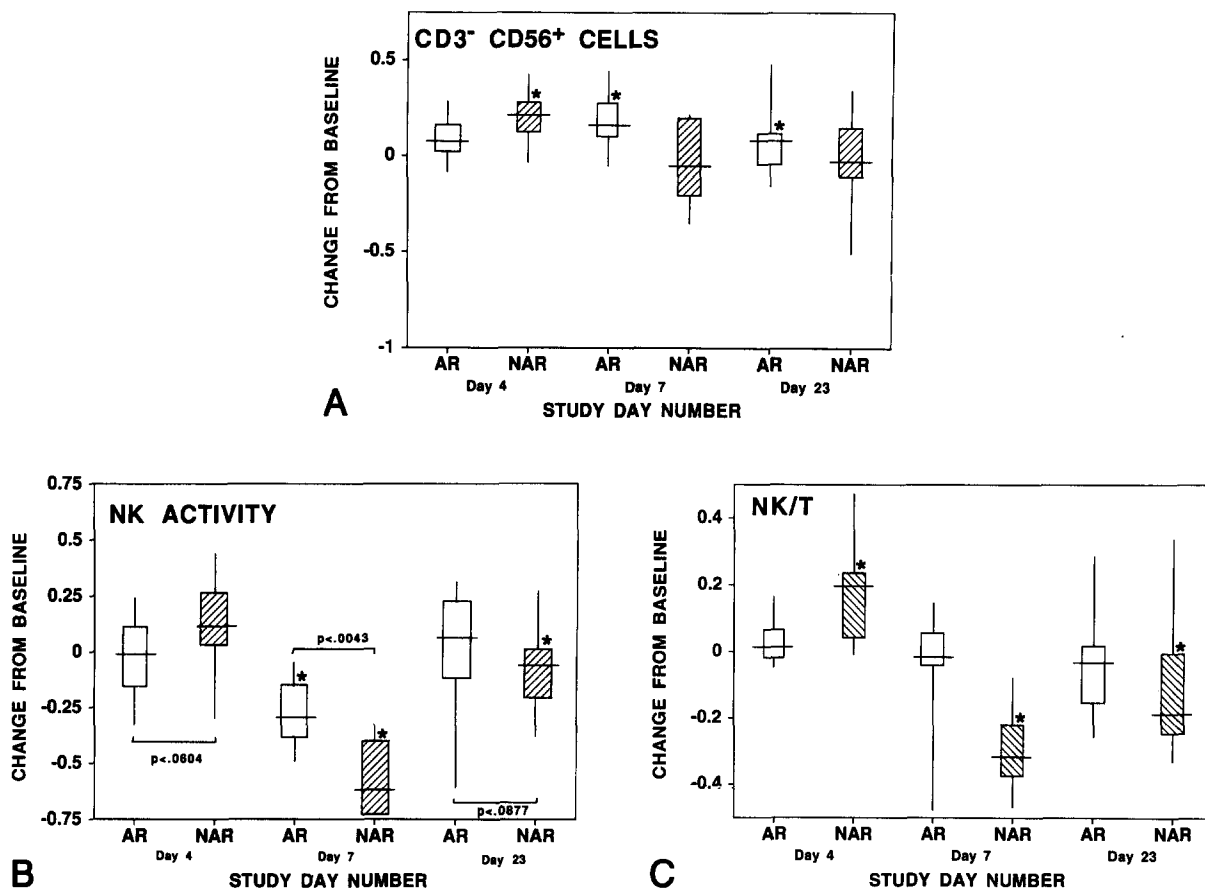


FIG. 3. Box plots for differences from baseline in log₁₀ CD3-CD56+ NK cell numbers (A), NK cell LU (B), and NK/T cell ratio (C) by allergy status and day of study. Description of box plot is same as that in Fig. 1.

of CD56+DR+ activated NK cells decreased significantly ($p = 0.02$) on day 23 in both AR and nonallergic subjects (data not shown). A similar trend was observed for NK cells expressing the CD16 phenotype, even though the overall change in numbers of CD16+ NK cells induced by RV-39 was not significant ($p = 0.1$). As shown in Fig. 3, A, no significant differences in absolute numbers of NK cells were observed between AR and nonallergic groups during postchallenge follow-up. However, the patterns of changes from baseline values in each group paralleled those in NK activity (as shown in Fig. 3, B).

Because changes in absolute numbers of both T and NK lymphocytes were observed after challenge with RV-39, we also analyzed changes in the NK/T cell ratio (Fig. 3, C). This ratio was significantly increased on day 4 ($p = 0.01$) and decreased on days 7 ($p = 0.01$) and 23 ($p = 0.02$).

These changes from baseline values were significant only for nonallergic subjects. The initial increase in the ratio was likely to be due to an early increase in NK cells, whereas the subsequent decreases on days 7 and 23 were clearly a result of the increased numbers of circulating T cells (see Fig. 1, C). When only activated cells were considered in calculating the NK/T cell ratio, significant decreases were observed on days 7 ($p = 0.005$) and 23 ($p = 0.002$). The day 7 change was most likely due to an increased number of activated T cells, whereas the day 23 change was explainable by a decreased number of activated NK cells.

Not only changes in the absolute number of NK cells but also in systemic NK activity were observed after intranasal challenge with RV-39 (Fig. 3, B). An early rise in NK activity was observed in the nonallergic group on day 4, but this change from the baseline value was not statistically sig-

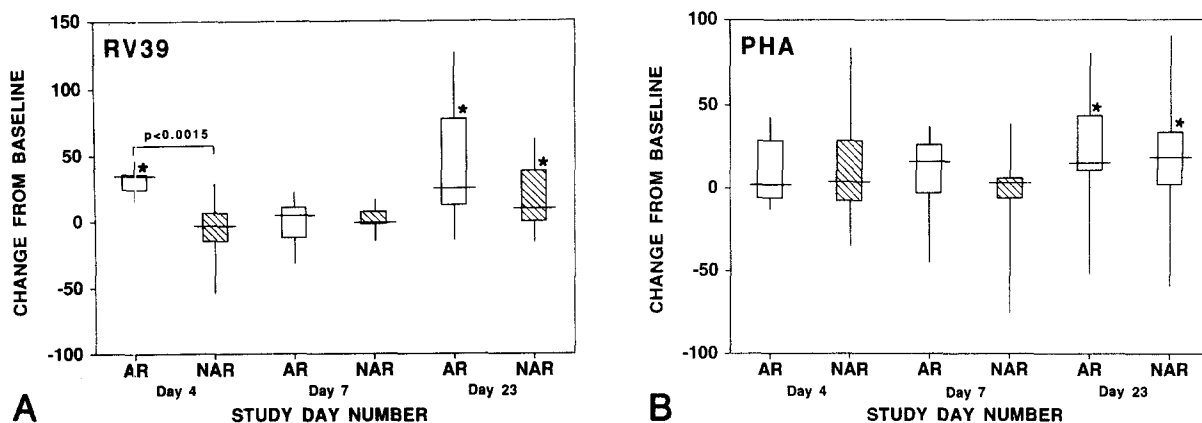


FIG. 4. Box plots for differences from baseline in square root of sum of net cpm of ³[H]-thymidine incorporation by lymphocytes stimulated with RV-39 antigen at 1:10, 1:5, and 1:2.5 dilutions (**A**) or with PHA at 10, 5, and 2.5 μg/ml (**B**) by allergy status and study day. Description of box plot is same as that in Fig. 1.

nificant ($p = 0.08$). A significant decrease in NK activity occurred, however, on day 7 for both AR ($p = 0.002$) and nonallergic subjects ($p = 0.004$). As mentioned previously and as shown in Fig. 3, *B*, this decrease on day 7 was significantly greater in nonallergic than in AR subjects. NK activity returned to baseline levels on day 23 in AR subjects but remained depressed in nonallergic subjects ($p = 0.04$).

The effect of RV-39 challenge on lymphocyte proliferation to RV-39 is illustrated in Fig. 4, *A*. The data show a significant increase from baseline values on day 4 in AR subjects ($p = 0.004$) and increases on day 23 in both AR and nonallergic groups ($p = 0.0001$ and $p = 0.01$, respectively). As mentioned previously, the increase on day 4 was significantly greater in AR versus nonallergic subjects. In contrast to the RV-39-specific proliferative response, the lymphocyte response to a mitogen, PHA, increased significantly only by day 23 ($p = 0.0002$, Fig. 4, *B*). Also, unlike the RV-39 response, the PHA response was not different in the AR versus the nonallergic group.

Relationship to clinical parameters

The relationship between immune parameters and the presence or absence of a cold in the subjects participating in this study was examined. In this analysis the baseline and day 23 immune parameters of subjects with colds ($n = 32$) and without colds ($n = 6$) were compared. No significant differences between the two groups were

present except for baseline values for total WBCs (mean $\pm$ SD, 6940 ± 1150 for no cold, 5219 ± 1336 for cold; $p = 0.01$) and CD16+DR+ cells (mean $\pm$ SD, 19.4 ± 9.6 for no cold, 9.2 ± 12.1 for cold; $p = 0.04$). No differences between the two groups were noted on day 23.

DISCUSSION

The presence of upper airway allergic disease may be a factor complicating both the screening of candidates for experimental RV-39 trials and the treatment of patients with infections of the upper airways. Compared with nonallergic subjects, AR subjects (in vivo) or their cells (in vitro) have been reported to respond differentially to various stimuli, including allergens, mediators, and viruses.^{2, 7, 17-22} The types of responses were physiologic (airway responses to histamine or methacholine,¹⁹⁻²⁰ biochemical (adenosine 3':5'-cyclic phosphate response to cellular adrenergic stimulation^{11, 21}), and immunologic (enhanced production of immunoglobulins and NK activity^{8, 12, 22}).

The relationship between upper respiratory allergy and infection has recently been studied with the experimental RV-39 intranasal challenge model. This human model yields a well-defined array of symptomatic and physiologic abnormalities.¹ The results of a series of studies with the same subjects showed that AR subjects hyperresponded to RV-39 challenge biochemically (enhanced level of vascular permeability²), but not physiologically (subjective and objective measurements of nasal and eustachian tube function¹).

With this same experimental model and the same subjects, the present study compared for the first time immunologic responses of AR and nonallergic subjects to upper respiratory tract viral infection. The study was not conducted during any of the relevant pollen seasons for these subjects.

Although many of the immune parameters measured before and after viral challenge indicated that AR and nonallergic subjects responded similarly to RV-39, the following differences were noted for AR subjects when compared with nonallergic subjects: (1) smaller increases in numbers of total WBCs and lymphocytes on day 4 after challenge, (2) an increase in the helper/suppressor T cell ratio only on day 23 and not on day 7, (3) a decrease in the number of IL-2 receptor-positive suppressor T cells on day 7, (4) an increase in the NK number on days 7 and 23 but not on day 4, (5) a lack of an increase on day 4 but a decrease on day 7 in the NK/T cell ratio, (6) a smaller decrease in NK activity on day 7 and normal levels of NK activity on day 23, and (7) an exaggerated increase in RV-39-induced lymphocyte proliferation on day 4. These differences were not confined to a single cell type or response but instead encompassed a broad spectrum of immune cells and responses. Although other explanations are possible, this array of differences in response to RV-39 challenge of AR versus nonallergic subjects might reflect or result from a preexisting dysregulation of the immune system in AR subjects.

One of the most interesting findings was the significantly greater increase in RV-39-induced lymphocyte proliferation observed in AR versus nonallergic subjects early on day 4. This swift response to RV-39 of AR subjects could perhaps be a reflection of their significantly lower baseline level of proliferative function as compared with nonallergic subjects. This study provided a unique opportunity to compare AR and nonallergic subjects for baseline (prechallenge) differences in other immune parameters. As summarized in Table I, AR subjects had significantly fewer T cells, fewer helper T cells, fewer activated helper T cells, and a smaller response of RV-39-induced lymphocyte proliferation than nonallergic subjects. As a group these comprise an interesting and potentially coherent array of immunologic findings that indicate the existence of immune dysregulation in the AR subjects. More specifically these data suggest the possibility of causality among the different dysregulation immune re-

sponses. This would apply most readily to the reduced number of activated helper T cells, which could translate into a relatively poor lymphocyte response to RV-39. Adequate T help is a prerequisite for the expression of a normal immune response, both *in vitro* and *in vivo*.^{23, 24}

Unexpectedly, nonallergic and AR subjects had similar prechallenge levels of NK activity. This result contrasted with that of the previously reported enhanced level of NK activity in association with AR.^{8, 9} However, when the results of different studies are compared, the time of year during which the blood sample was obtained, in relation to any confounding environmental variables, must be considered. The timing of phlebotomy and the temporal relationship to symptoms and pollen exposure have frequently been unreported in other studies comparing AR with nonallergic groups. In the current study blood samples were collected when the subjects were asymptomatic with regard to allergy and not exposed to relevant allergens. Relevant seasonal allergen exposure is well known to prime tissues of the respiratory tract to hyperrespond physiologically to various stimuli.²⁵ An equally plausible scenario could be proposed for the cells of the immune system, whereby altered levels of responsiveness could develop during periods of relevant allergen exposure.

In addition to the comparison of responses of AR and nonallergic subjects, this study also provided an opportunity to compare the current report with previous reports addressing immunologic changes induced by respiratory viruses. The conclusions that can be drawn from the analysis presented in this report, and indeed many of those in previous reports, are somewhat limited by the relatively infrequent collection of blood samples (baseline, day 4 or day 7 after challenge, and day 23 after challenge in the present study). Daily blood samples collected during the early stages of the infection would be more likely to yield more meaningful and complete sets of results. Nonetheless, early (day 4 or 7) postchallenge involvement of nonspecific immunity (NK), followed by the later (day 23) involvement of RV-39-specific immune responses (T cells), was expected.

The results of our study clearly show that experimental infection with RV-39 induced a systemic cellular immune response in RV-39-seronegative subjects. Effects on both the number and function of peripheral blood lymphocytes were noted after the RV-39 challenge. The main

changes were observed in the number of WBCs, including those of helper and suppressor T lymphocytes and NK. The functional changes included both nonspecific mitogenic responses and specific recalled antigenic responses, as well as non-MHC-restricted cytotoxicity.

Other investigators have reported changes in the number of WBC or lymphocytes during RV infections. However, not all previous reports are in agreement with the results of our study. Thus Levandowski et al.⁵ reported that RV-25 induced decreases in the absolute numbers of total lymphocytes, T lymphocytes, and helper T lymphocytes on day 3 after RV-25 challenge. Moreover, the decreased number of lymphocytes was associated with increased severity of symptoms.⁵ Reports from other investigators, including Henderson et al.,²⁶ also provided evidence of an early RV-induced reduction in WBC or lymphocytes. Bush et al.²⁷ reported RV-16-induced decreases in these cells, which were variable for total WBCs and lymphocytes but which were more consistent for T lymphocytes. Cate et al.²⁸ reported slight but apparently nonsignificant decreases in lymphocytes during the acute illness. Unfortunately, in all the above studies a small number of subjects on the protocol was a limiting factor ($n = 12$, $n = 14$, $n = 7$, and $n = 15$, respectively, vs $n = 38$ in the present study). The acute-phase decrease in the total lymphocyte number reported by these investigators contrasts sharply with the findings of both the present study, where decreases in these cells were not present on day 4 and where significant elevations were evident by day 7, and those reported by Hsia et al.⁴ for the Hanks RV, where increases in lymphocytes were present on day 4 after challenge. None of these previous studies differentiated AR and nonallergic subjects and none tested peripheral blood lymphocytes for cell activation markers such as those used in the current study.

The baseline lymphocyte counts observed in persons participating in the present study (see Table I) were somewhat lower than those reported by others in experimental viral challenge protocols. Overall, 19 of 36 subjects (53%) studied had baseline WBC counts less than 1500. However, these counts were not in the lymphopenic range. Moreover, these lower baseline counts are not likely to be responsible for the increased number of WBCs seen on day 7 after challenge. The numbers of WBCs, lymphocytes, and T cells all increased after challenge with

RV-39 in subjects whose baseline WBC counts were less than 1500 or greater than 1500. Thus it appears that these changes were related to viral challenge and not to baseline WBC counts.

Functional changes in lymphocytes have also been reported to occur after experimental RV challenges. An increased level of viral antigen-stimulated blastogenesis has been reported on postchallenge day 21 for Hanks virus,³ postchallenge day 15 for RV-39,²⁶ and 6 weeks after challenge for RV-4, which was serotype specific.²⁹ In the current study a gradual rise of RV-39-specific proliferative responses was observed after viral challenge, which resulted in a significant increase on day 23. Overall, a rise in virus-specific proliferation was a consistent immunologic response after local administration of the RV. Lymphocyte responsiveness to a mitogen, PHA, increased in parallel with that to RV-39 antigen, suggesting that both virus-specific and nonspecific lymphocyte responses were similarly induced by RV-39 challenge.

NK cells have been reported to play a potentially important role in viral infections, including (1) elimination of virally infected cells³⁰⁻³²; (2) production of a spectrum of biologically active cytokines,³³ including IL-1, IL-2, and especially interferon- γ ; and (3) changes in NK activity in response to modulation by stress-associated hormones and peptides.^{34, 35} Thus changes in NK cell numbers and function after RV challenge were anticipated. Additionally, because NK cells are considered to be the first line of antiviral defense, an early involvement was expected. Indeed, RV-39 did induce marked changes in both NK cell number and activity. However, the changes from baseline in these two parameters were not necessarily in the same direction. For example, the marked increase in the NK cell number in AR subjects on day 7 was accompanied by a marked decrease in the levels of NK cell activity. This divergence in the number and function of NK cells in AR subjects was somewhat unexpected, and it suggests that RV-39 preferentially induced a relatively hyporeactivity of NK cells in these persons as compared with nonallergic subjects.

In support of an early involvement of NK cells in antiviral defense, Hsia et al.^{3, 4} reported that both the NK cell number and cytotoxicity were increased on day 4 after Hanks RV challenge. In the present study it is likely that very early postchallenge increases (i.e., on day 1 or day 2) in NK activity also occurred, as judged by the higher

than baseline levels of NK activity seen on day 4 in nonallergic subjects, although by postchallenge day 7 NK activity decreased to significantly less than the baseline level. These findings could indicate that NK involvement occurred earlier than day 4 in the course of RV-39 infection. The decrease in NK activity was observed after the peak of symptoms, which suggests that NK cells were potentially involved in the early host response to the virus and that increased consumption of NK cells or their selective migration from blood to tissues could be responsible for this decrease. Alternatively it could also be explained by the production of immunoinhibitory factors by the virally infected cells during the early phases of the RV-39 infection.

The results of this study suggest the possible existence of a relationship between the immune cell functions and clinical and physiologic manifestations of RV-39 infection. The strongest evidence for the existence of this relationship was provided by a comparison of immune parameters in subjects in whom colds did and did not develop in response to the RV-39 challenge. Subjects in whom colds did not develop had higher numbers of WBC and higher numbers of activated NK cells at baseline. Even though a relationship between these immune parameters and the development and expression of colds was suggested and was supported by the work of other investigators,³ the nature of this relationship is currently unclear and its definition awaits the results of further investigation. However, several possibilities deserve further consideration. The development of cold symptoms may be related directly to the degree of viral cytopathology (and therefore is independent of the immune response) or alternatively may be the direct result of the antiviral immune response. Thus the lack of development of a cold could potentially be due to (1) a lesser degree of susceptibility conferred by relatively heightened systemic immune parameters at baseline (to protect against viral cytopathology) or (2) a less robust early immune response.

Overall, our results suggest that specific and nonspecific immune responses may be involved not only in clearing viral infections, but also in determining baseline susceptibility to infection. The results also highlight the importance of monitoring such immunologic parameters during future viral challenge protocols, especially those which include subjects with differential degrees of viral susceptibility, as determined by serum neutralizing antibody titers and possibly by the baseline immune cell number and function.

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