Suppressed Expression of ICAM-1 and LFA-1 and Abrogation of Leukocyte Collaboration after Exposure of Human Mononuclear Leukocytes to Respiratory Syncytial Virus In Vitro

Comparison with Exposure to Influenza Virus

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Abstract

Human mononuclear leukocytes (MNL) exposed to respiratory syncytial virus (RSV) produce net IL-1 inhibitor bioactivity with the anticipated consequences of cell cycle arrest, suppressed virus-specific proliferation, and reduced expression of activation markers. These studies were undertaken to investigate effects of exposure and resultant net IL-1 inhibitor activity on the expression of the intercellular adhesion molecule-1 (ICAM-1), and its ligand the lymphocyte function-associated antigen (LFA-1). MNL collected at 1, 4, and 24 h after exposure to influenza virus (which induces net IL-1 bioactivity) showed enhanced expression of ICAM-1 and LFA-1 relative to sham-exposed MNL and exhibited cell clustering. In contrast, exposure to RSV was associated with suppressed expression of both ICAM-1 and LFA-1 and with minimal detectable cell clustering throughout the culture period. Influenza virus-exposed MNL produced significantly more IL-1 and IFN-γ (which require cell–cell collaboration for optimal production) than did RSV-exposed MNL. These data raise the possibility that exposure of MNL to RSV fails to elicit or blocks the early events necessary for cellular collaboration, contributing to early suppression of the clonal expansion of RSV-specific lymphocytes. (*J. Clin. Invest.* 1991. 88:505–511.) Key words: immunoregulation • interferon-gamma • interleukin-1 • lymphocyte function associated antigen-1 • intercellular adhesion molecule-1

Introduction

Cell–cell interaction between antigen presenting cells (APC)[1] and T lymphocytes is an essential process in the generation of immunological responses (1–4). This interaction is thought to consist of both a specific signal, namely recognition of the MHC-antigen complex and the occupied T cell receptor (5, 6), and nonspecific or accessory signals, such as expression of costimulatory molecules or production of IL-1 (7, 8). The complete repertoire of accessory signals is yet to be established. Nonetheless, factors that interfere with either specific signals or accessory signals may be expected to result in an abrogated immune response (9).

Several adhesion molecules have been identified that are thought to play a central role in the interaction of the APC with the T lymphocyte (10). Intercellular adhesion molecule-1 (ICAM-1) serves as the major ligand for lymphocyte function-associated antigen-1 (LFA-1) (11, 12). The expression and/or avidity of ICAM-1 is augmented upon cell activation and in the presence of inflammatory cytokines, such as IL-1 and IFN-γ (13). In contrast, the expression of LFA-1 has not been demonstrated to be cytokine sensitive (14). However, LFA-1 is thought to increase in expression and/or avidity for ICAM-1 upon cell activation (15). Complete T lymphocyte activation, demonstrated by IL-2 production and DNA synthesis, has been shown to occur with simultaneous cross-linking of LFA-1 and CD3 (16). Therefore, modulation of the expression and/or avidity of ICAM-1 and LFA-1 would affect initiation and progression of the immune response.

We have shown previously that human mononuclear leukocytes (MNL) that have been exposed to respiratory syncytial virus (RSV) produce net IL-1 inhibitor bioactivity (17, 18). The anticipated consequences of such net IL-1 inhibition were demonstrated: namely, cell cycle arrest, suppressed proliferation, and a decrease in the expression of cell surface markers indicative of activation and/or proliferation (19). These observations suggested that net IL-1 inhibitor activity could result in abrogation or delay of RSV-specific anamnestic immune responses by inhibiting the early clonal expansion of RSV-specific lymphocytes. The well established ability of RSV to reinfect individuals who have preexisting cellular and humoral evidence of immunity to the virus (20–22) may represent an in vivo correlate of such in vitro observations.

These studies were undertaken to determine whether exposure to RSV and induction of net IL-1 inhibitor activity affects the early collaborative events leading to emergence of RSV-specific MNL responses, events that include expression of ICAM-1 and/or LFA-1 and leukocyte clustering. The studies also examined production of the cytokines IL-1 and IFN-γ, which require cell–cell collaboration for optimal production (23, 24). In all experiments, autologous MNL were sham exposed or exposed to influenza virus (which induces net IL-1 bioactivity) for comparison.

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1. *Abbreviations used in this paper:* APC, antigen presenting cells; ICAM-1, intracellular adhesion molecule-1; LFA-1, lymphocyte function-associated antigen-1; MNL, mononuclear leukocytes; MOI, multiplicity of infection; RSV, respiratory syncytial virus.

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Methods

Cell collection and exposure to viruses. Standard techniques were used to produce and measure viral inocula. RSV (Long strain; American Type Culture Collection, Rockville, MD) was grown in HEp-2 cells, and influenza A/AA/Marton/43 H1N1 was grown in allantoic cavities of 10-d-old embryonated hen's eggs (17, 25). Each virus was stored at −70°C until used. Both RSV and influenza virus pools titered 10^8 plaque forming units/ml using HEp-2 cells or MDCK cells in assays, respectively (25). Previous studies showed that neither HEp-2 culture fluid nor allantoic fluid affect the proliferative response or the expression of activation markers by MNL (19).

Peripheral blood collected from healthy adult donors was separated by Ficoll-Hypaque sedimentation (26). In all experiments, comparisons were made between aliquots of autologous cells examined concomitantly. Aliquots of cells were sham-exposed at 37°C for 1 h in serum-free Eagle's minimum essential medium or exposed to influenza virus or RSV at a multiplicity of infection (MOI) of one (17–19). Previous studies have shown that, at equal MOI, the percentage of MNL infected by influenza virus or RSV is relatively similar (27, 28) (Roberts, N. J., Jr., and J. E. Nichols, unpublished observations). The MNL cultures were then supplemented with 10% heat-inactivated FCS and incubated for 1, 4, 24, and 72 h before analysis. Cell-free supernatant fluid was collected by centrifugation at 24 and 72 h after exposure for analysis of IL-1 and IFN-γ activity. Cells were collected at 1, 4, and 24 h for analysis by flow cytometry or, in other experiments described below, were stained in situ and examined by light microscopy. For all experiments, viability was assayed by trypan blue exclusion. Viability (always > 95%) was similar after exposure to the different viruses.

IL-1β and IFN-γ assays. Supernatant fluids were obtained from sham-exposed, influenza virus-infected or RSV-infected MNL as described above. Cell-free fluids were collected at 1 and 3 days after infection and stored at −70°C until assayed. Quantitative measurement of IL-1β, which is the soluble form produced by monocytes-macrophages after activation (29), was obtained by ELISA (Cistron, Pine Brook, NJ). IFN-γ was measured using a solid phase radioimmunoassay (Centocor, Malvern, PA).

Microscopic analysis. The extent of cell clustering after exposure was determined by culturing cells over plastic cover slips. Cover slips from the cultures were retrieved at 1, 4, and 24 h after sham-exposure or exposure to influenza virus or RSV. Cells were stained by the Diff-Quick method (Dade Diagnostics, Aquada, PR) immediately after collection of coverslips and examined for cell clustering using a Zeiss microscope.

Analysis of expression of cell adhesion molecules. Expression of cellular adhesion molecules was measured using indirect immunofluorescent labelling and flow cytometry. MNL were also analyzed by fluorescence microscopy to verify that fluorescence was associated with a cell-surface pattern of staining. MNL were either sham-exposed or exposed to influenza virus or RSV. After further incubation, the live MNL were washed and stained with either murine monoclonal anti-ICAM-1 or anti-LFA-1 (AMAC, Inc., Westbrook, ME) at 4°C for 30 min. The cells were then washed and stained at 4°C for 30 min with FITC-conjugated goat anti-mouse antibody (Tago, Inc., Burlingame, CA) (13, 30). Analysis of stained MNL (monocytes-macrophages and lymphocytes) was performed on a Becton-Dickinson FACScan® flow cytometer (Mountain View, CA) by gating on the respective populations using forward and side light scatter (28) (Fig. 1 *a*). In all experiments, the cursor for the negative population of cells was set using a mouse isotype (IgG_1) control monoclonal antibody (Becton-Dickinson). Representative histograms (and cursor settings) for control MNL are shown in Fig. 1, *b–e* and described in Results.

Data analysis. Results represent the mean±SEM for five or more experiments. Determination of significant differences (paired *t* test) was performed using the commercially available software program Faststat (Systat, Inc., Evanston, IL).

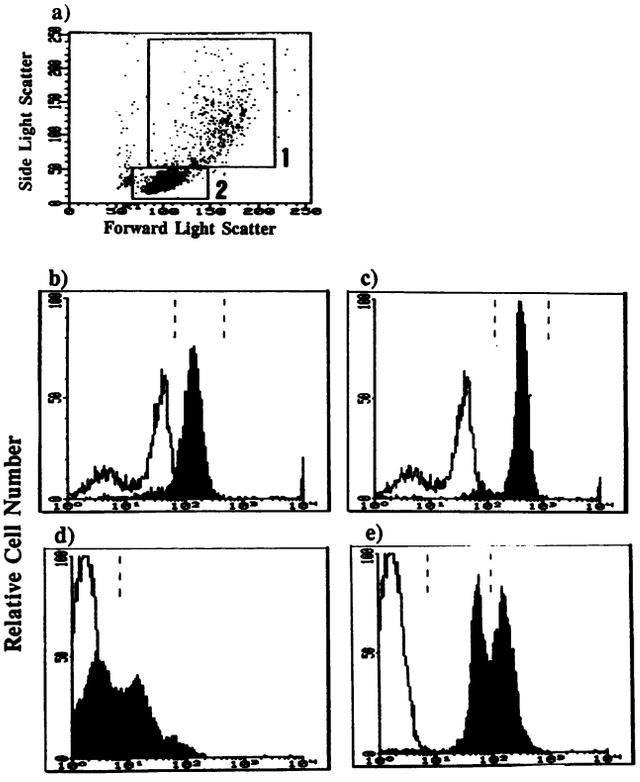

Figure 1. Cell population gatings for flow cytometry analyses. Control MNL are shown. (*a*) Monocyte-macrophage and lymphocyte gates using forward and side light scatter characterization: *1*, monocytes-macrophages; *2*, lymphocytes. (*b–e*) Histograms of monocytes-macrophages (*b* and *c*) and lymphocytes (*d* and *e*) stained for expression of ICAM-1 (*b* and *d*) and LFA-1 (*c* and *e*). The clear histograms depict isotype control antibody staining. The solid histograms depict staining with the respective murine monoclonal anti-ICAM-1 and anti-LFA-1 antibodies. Negative (nonstained), "dim" (intermediate staining, except d), and "bright" (intensely stained) populations are separated by the cursors.

Results

Microscopic analysis. Cell–cell interaction is a sentinel event in the induction and progression of the immunological response. The formation of cellular clusters was examined at 1, 4, and 24 h after human MNL were sham-exposed or exposed to influenza virus or RSV. As early as 1 h, and extending through the period of culture, MNL exposed to influenza virus showed increased cluster formation (Fig. 2). In contrast, sham-exposed MNL or MNL exposed to RSV showed minimal clustering.

Cytokine production. Earlier studies showed that RSV-exposed MNL did not proliferate in response to the virus (17, 19). The observation that RSV-exposed MNL showed a diminished or absent clustering pattern prompted us to investigate the production of IL-1 and IFN-γ, both of which require cellular collaboration (but not cellular proliferation) for optimal expression (23, 24, 31). Determination of IL-1β activity demonstrated that there was a minimal amount of IL-1 produced by control cells by 24 and 72 h (Fig. 3). Both influenza virus and

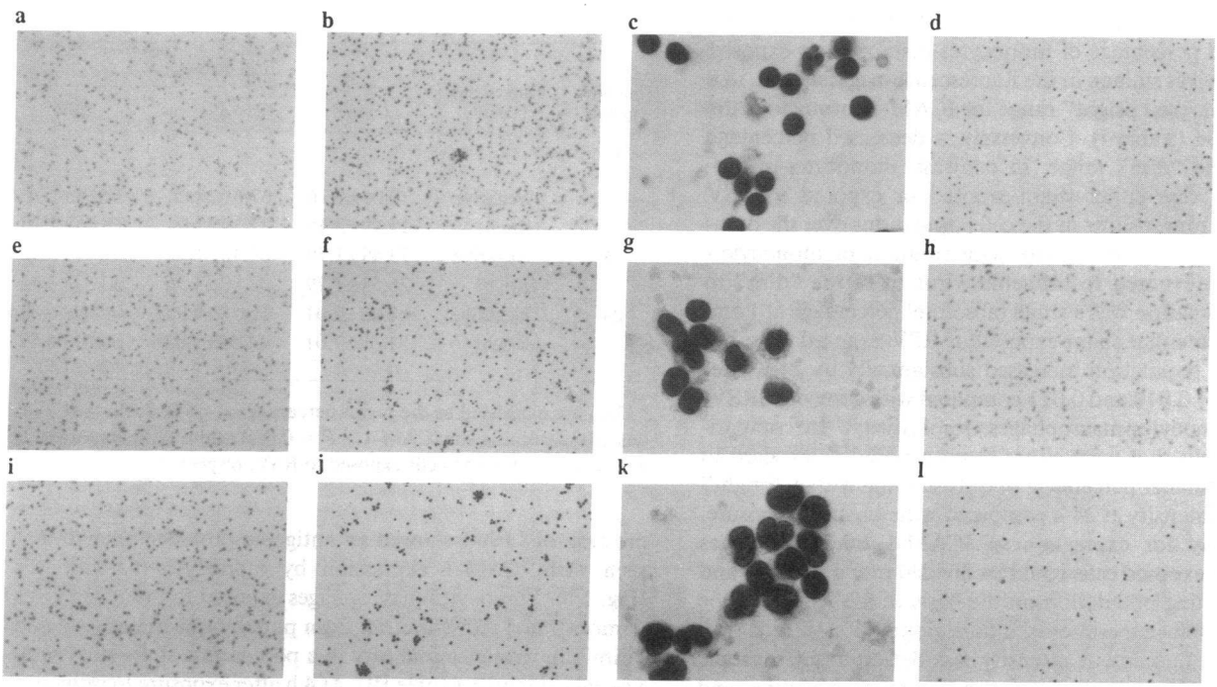

Figure 2. Cell cultures stained for analysis of cell cluster formation. Cover slips from MNL cultures were stained 1 h (*a–d*), 4 h (*e–h*), and 24 h (*i–l*) after sham exposure (Control; *a, e, i*) or exposure to influenza virus (*b, f, j* and *c, g, k*) or RSV (*d, h, l*). Representative 10× photomicrograph fields are shown, with photomicrographs *c, g,* and *k* showing clusters from influenza virus-exposed cultures viewed under oil at 100×.

RSV induced IL-1 production by 24 h, with a small difference (P = 0.058) in the magnitude of response to the viruses. In contrast, by 3 d after exposure the quantity of IL-1 produced by influenza virus-exposed cells continued to increase (P = 0.026), while that of RSV-exposed cells decreased to a level below that of day 1.

The production of IFN-γ by MNL exposed to influenza virus was substantial by one day in culture (P = 0.034 and 0.046 versus control and RSV-exposed MNL, respectively) (Fig. 4). MNL that were sham-exposed or exposed to RSV produced minimal IFN-γ. 3 d after exposure, fluids from sham-exposed cells showed little additional accumulation of IFN-γ, but IFN-γ was produced by MNL in response to RSV (P = 0.005) or influenza virus (P = 0.008). Although IFN-γ was detectable in fluids from RSV-exposed cells by three days in culture, the titers were still several-fold lower than those produced by cells exposed to influenza virus (P = 0.050).

Cell surface expression of ICAM-1 and LFA-1. The above and earlier studies suggested that cell collaboration, essential for both cytokine production and lymphocyte proliferative responses, is suppressed at an early stage after exposure of MNL to RSV. Alteration of the expression and/or avidity of ICAM-1/LFA-1 by RSV-exposed MNL could be one mechanism contributing to diminished leukocyte clustering and collaboration, and expression of these molecules was examined. Cells were exposed and cultured as MNL, with separate analysis by appropriate gating (Fig. 1) during flow cytometry. Analysis of MNL by fluorescence microscopy showed a rim of fluorescence indicative of cell surface staining. Profiles of ICAM-1/LFA-1 expression by control MNL were similar in regard to intensity and percentage of positive cells to those of previous reports (30, 32, 33) (see Fig. 1), and such results were compared with profiles of virus-exposed MNL.

Expression of ICAM-1 by monocytes-macrophages showed that three monocyte-macrophage populations could be identified based on fluorescence intensity. A fluorescence-negative population, a population with intermediate (dim) fluorescence intensity, and a brightly stained population of monocyte-ma-

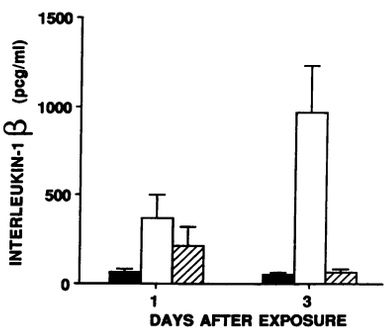

Figure 3. Interleukin-1 β production by control MNL (*solid bars*), influenza virus-exposed MNL (*open bars*), and RSV-exposed MNL (*hatched bars*). Results represent mean titers±SEM.

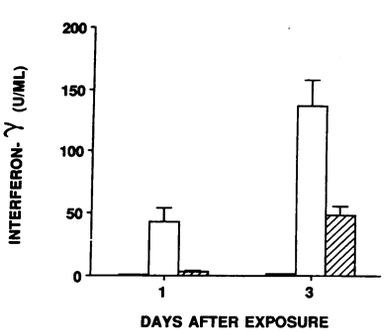

Figure 4. Interferon-γ production by control MNL (*solid bars*), influenza virus-exposed MNL (*open bars*), and RSV-exposed MNL (*hatched bars*). Results represent mean titers±SEM.

crophages were identified after staining for ICAM-1 (Fig. 1 *b*). An increased percentage of monocytes-macrophages exposed to influenza virus stained in the fluorescence-negative and, to a greater extent, the "bright" range for ICAM-1 throughout the culture period (Table I). Conversely, a decreased percentage stained in the "dim" range. In contrast, monocytes-macrophages that were either sham-exposed or exposed to RSV showed only minor shifts in the cell populations over the duration of culture (Table I). The redistribution of monocytes-macrophages exposed to influenza virus from the "dim" to "bright" population was a small (absolute) percentage at 1 and 4 h relative to either sham-exposed or RSV-exposed cells, but the "bright" population increased substantially by 24 h after exposure ($P = 0.018$ and 0.021 compared with control or RSV-exposed monocytes-macrophages, respectively). In comparison, those cells that were either sham-exposed or exposed to RSV had a smaller percentage of cells staining with a "bright" fluorescent intensity at 24 h compared with 4 h after exposure.

The assay for expression of ICAM-1 on lymphocytes showed that exposed cells could be divided into a negative and positive staining population on the basis of the $IgG_{(1)}$ isotype control. In some experiments, a third population of cells with intermediate fluorescent intensity was detected (for example see Fig. 1 *d*). However, this was an inconsistent finding, and this intermediate subset of cells was therefore not separated for analysis. At 1 and 4 h after exposure to influenza virus, there was an absolute but small increase in the number of lymphocytes expressing ICAM-1 relative to either the control or RSV-exposed cells (Table II). By 24 h, however, there was a large increase in the percentage of influenza virus-exposed lymphocytes expressing ICAM-1 ($P = 0.022$ and 0.023 compared with control and RSV-exposed lymphocytes, respectively). In contrast, lymphocytes exposed to RSV showed a minimal increase in their expression of ICAM-1 from 1 to 4 h in culture. From 4 to 24 h after exposure there was no appreciable change in the expression of ICAM-1 by RSV-exposed lymphocytes. At 24 h of culture, expression of ICAM-1 was slightly below that observed with sham-exposed cells.

Analysis of the monocyte-macrophage population for expression of LFA-1 showed an antigenic profile similar to that seen with ICAM-1 expression by monocytes-macrophages (Fig. 1 *c*). Monocytes-macrophages exposed to influenza virus demonstrated redistribution from populations of negative and "dim" fluorescence intensity to a population of "bright" fluorescence intensity (Table III). At 4 h after exposure to influenza virus, the percentage of "dim" cells was significantly decreased compared with the control population ($P = 0.014$). Simultaneously the number of monocyte-macrophages with "bright" fluorescence intensity was increased compared with control ($P = 0.006$) and RSV-exposed cells ($P = 0.035$). Examination of influenza virus-exposed monocytes-macrophages after 24 h showed that redistribution of cells into the "bright" range had remained stable from 4 to 24 h of culture ($P = 0.030$ and 0.035 compared with control and RSV-exposed cells, respectively). There was a concomitant decrease in the percentage of cells in the fluorescence-negative and "dim" ranges ($P = 0.017$ and 0.032 compared with control and RSV-exposed monocytes-macrophages, respectively) (Table III). RSV-exposed cells did not differ from control cells in expression of LFA-1.

Staining for expression of LFA-1 on lymphocytes showed a

*Table I. Expression of ICAM-1 by Monocytes-Macrophages**

Time after exposure	Fluorescence intensity	Cell exposure		
		Control	Influenza virus	RSV
h				
1	Negative	3.49±1.35	3.35±0.94	3.62±0.69
	Dim	91.62±1.57	91.32±1.81	92.45±1.60
	Bright	4.89±1.83	5.33±1.68	3.93±1.07
4	Negative	3.29±0.75	4.28±1.69	4.09±1.05
	Dim	92.12±2.61	88.14±3.62	89.56±2.87
	Bright	4.59±1.90	7.58±2.09	6.35±2.14
24	Negative	3.43±1.52	5.70±2.02	3.45±1.23
	Dim	92.90±1.47	69.30±5.86‡	92.46±1.59
	Bright	3.67±0.061	25.00±6.84§	4.09±0.71

* Data are expressed as the mean percentage±SEM of cells with the described staining for ICAM-1. ‡ $P = 0.018$ and 0.021, and § $P = 0.032$ and 0.029 compared with control cells and cells exposed to RSV, respectively.

*Table II. Expression of ICAM-1 by Lymphocytes**

Time after exposure	Fluorescence intensity	Cell exposure		
		Control	Influenza virus	RSV
h				
1	Negative	70.01±3.26	67.61±4.04	71.34±2.70
	Bright	29.99±3.26	32.39±4.04	28.66±2.70
4	Negative	73.49±1.79	67.40±4.56	69.41±3.42
	Bright	26.51±1.79	32.60±4.56	30.59±3.42
24	Negative	68.42±7.01	55.58±9.93	69.09±6.52
	Bright	31.58±7.01	44.42±9.93‡	30.91±6.52

* Data are expressed as the mean percentage±SEM of cells with the described staining for ICAM-1. ‡ $P = 0.022$ and 0.023 compared with control cells and cells exposed to RSV, respectively.

*Table III. Expression of LFA-1 by Monocytes-Macrophages**

Time after exposure	Fluorescence intensity	Cell exposure		
		Control	Influenza virus	RSV
h				
1	Negative	3.89±1.16	3.65±1.24	3.79±1.08
	Dim	93.49±1.88	91.80±2.67	93.71±1.88
	Bright	2.62±0.98	4.55±1.60	2.50±0.93
4	Negative	3.31±1.03	3.53±1.54	3.82±1.29
	Dim	91.34±2.51	86.15±3.24‡	90.52±3.25
	Bright	5.35±1.68	10.32±2.24§	5.66±2.84
24	Negative	2.21±0.79	2.05±0.79	2.26±1.19
	Dim	93.94±1.37	87.55±2.88‖	94.54±1.72
	Bright	3.85±1.28	10.40±2.82¶	3.20±1.21

* Data are expressed as the mean percentage±SEM of cells with the described staining for LFA-1. ‡ $P = 0.014$ and 0.118, § $P = 0.006$ and 0.035, ‖ $P = 0.017$ and 0.032, and ¶ $P = 0.035$ and 0.03 compared with control cells and cells exposed to RSV, respectively.

bimodal distribution (Fig. 1 *e*). 1 h after exposure, there was an increase in the percentage of influenza virus-exposed lymphocytes in the "bright" population, and a concomitant decrease in the "dim" population, in comparison with control and RSV-exposed cells (Table IV). 4 h after exposure, both the influenza virus-exposed and RSV-exposed lymphocytes showed movement of cells from the fluorescence-negative and "bright" populations into the "dim" population. Nonetheless, influenza virus-exposed lymphocytes continued to show a relative increased number of lymphocytes in the "bright" region. Control lymphocytes showed a slight increase in the percentage of negative cells along with a decrease in LFA-1 "bright" cells and a larger percentage of cells in the LFA-1 "dim" region with time in culture ($P = 0.050$ compared with influenza virus-exposed cells). After 24 h of culture, there was a redistribution of influenza virus-exposed lymphocytes from the LFA-1 "dim" ($P = 0.004$ and 0.008 compared with control and RSV-exposed lymphocytes, respectively) to the LFA-1 "bright" ($P = 0.004$ and 0.020 compared with control and RSV-exposed cells, respectively) range, with only a minor shift of RSV-exposed lymphocytes.

To eliminate the possibility that increasing fluorescence intensity was due to an increase in cell size rather than increased density of expression of the molecules, cells were analyzed further by forward light scatter. At these early times after exposure, there were no significant differences in size between cells that were sham-exposed, exposed to influenza virus, or exposed to RSV (data not shown).

Discussion

These studies allow us to extend our understanding of the immunopathogenesis of recurrent RSV infection with several potentially important observations. (*a*) Suppression of ICAM-1/LFA-1 expression by MNL was evident within 1 h after exposure to RSV (relative to MNL exposed to influenza virus in parallel experiments) and was maintained throughout the culture period; (*b*) ICAM-1/LFA-1 expression was correlated with the extent of leukocyte cluster formation; and (*c*) decreased ICAM-1/LFA-1 expression and cell clustering were associated with curtailed production of IL-1β and IFN-γ.

*Table IV. Expression of LFA-1 by Lymphocytes**

Time after exposure	Fluorescence intensity	Cell exposure		
		Control	Influenza virus	RSV
h				
1	Negative	1.10±0.56	1.26±0.70	1.40±0.76
	Dim	50.21±3.57	46.66±5.17	51.63±5.37
	Bright	48.69±3.19	52.08±4.64	46.97±4.75
4	Negative	1.26±0.78	0.47±0.17	0.77±0.25
	Dim	70.81±3.95	60.53±5.03‡	63.82±7.22
	Bright	27.93±3.59	39.00±4.91	35.42±7.31
24	Negative	0.97±0.42	2.65±1.65	1.44±0.62
	Dim	65.74±5.71	51.60±4.24§	61.79±4.85
	Bright	33.29±5.43	45.75±4.42‖	36.77±4.67

* Data are expressed as the mean percentage±SEM of cells with the described staining for LFA-1. ‡ $P = 0.050$ compared with control cells. § $P = 0.004$ and 0.008, and ‖ $P = 0.004$ and 0.020 compared with control cells and cells exposed to RSV, respectively.

The alterations in expression of ICAM-1/LFA-1 by 1 h were not large. However, they could be correlated with changes in leukocyte cluster formation (Fig. 2). These data suggest that minor changes in the expression of ICAM-1/LFA-1 are associated with physiologically relevant changes in adhesion. The kinetics and vitality of APC-lymphocyte interaction after in vitro or in vivo viral challenge are not fully understood. Generation of an immune response does appear to require antigen-independent and antigen-dependent adhesion between the APC and T lymphocyte (10). Exposure of MNL to influenza virus resulted in an increased population of cells staining for "bright" fluorescence intensity of ICAM-1/LFA-1 after 1 h in culture, a time that may represent mainly antigen-independent leukocyte adhesion. Fluorescence intensity was maximal at 24 h after exposure, a time that may represent antigen-dependent adhesion. In contrast, RSV-exposed MNL did not show an increase in the population of MNL staining for "bright" expression of ICAM-1/LFA-1 during the entire culture period relative to control MNL. Overall, the level of expression of ICAM-1/LFA-1 at 1 h after in vitro exposure to influenza virus or RSV was predictive of the magnitude of expression and cell-cell interaction (as demonstrated by cell cluster formation) during the remainder of the culture period.

The mechanisms that contribute to the leukocyte responses described in this report are not known. Production of IL-1 inhibitor by MNL and abrogation of collaborative leukocyte responses appear to be associated with exposure of MNL to infectious but not inactivated RSV (19). MNL exposed to inactivated RSV produce net IL-1 activity and proliferate, just as do MNL exposed to infectious or inactivated influenza virus (17, 19, 34). Influenza virus infection of peripheral blood MNL appears to be abortive, with production of viral proteins but without release of free progeny virus (2, 28, 35). The occasional detection of RSV in culture fluids has been associated with MNL subpopulations that did not produce net IL-1 inhibitor activity in our studies, and not with monocyte-macrophage or lymphocyte populations that produced the inhibitory factor, even though producer and nonproducer populations of cells were both infected as determined by synthesis and expression of RSV F and G proteins (17, 27).

The differences between responses of MNL to influenza virus and RSV have been observed over a wide range of MOI, including MOI that would ensure that virtually all cells could be infected if susceptible. The data from immunofluorescent staining suggest that relatively similar proportions of monocyte-macrophages and lymphocytes are infected by the two viruses (27, 28; Roberts, N. J., Jr., and J. E. Nichols, unpublished data), but do not establish whether the same subsets of cells are infected. The differences in production of IL-1 and IL-1 inhibitors, and in cell–cell collaboration, are evident as early as synthesis and expression of influenza virus proteins are detected (2) and long before synthesis and expression of RSV proteins are detected (27). The differences in production of cytokines and cell–cell interactions persist even after RSV protein synthesis and expression are clearly detected, and after influenza virus protein synthesis is no longer detectable (2, 17, 28). The differences in response are so rapidly apparent that they are unlikely to be due to any delayed effect of RSV on host cell functions, although such effects also could exist.

The current data raise the possibility that early and sustained suppression of ICAM-1/LFA-1 expression and/or avidity by RSV-exposed MNL is a critical mechanism whereby exposure to the virus alters the kinetics, or abrogates the vigor, of an anticipated antiviral anamnestic immune response. In vitro evidence of the physiological consequences of suppressed leukocyte cluster formation may be provided by the results of IL-1β and IFN-γ assays in these studies, and the results of virus-specific and alternative lymphocyte proliferation assays reported earlier (17–19).

Antigen-specific lymphocyte proliferation requires adequate antigen presentation and the presence of IL-1 (7, 36). These studies also showed that the production of IL-1β by RSV-exposed MNL is relatively reduced over 3 d of culture (Fig. 3). In addition, as shown previously (17–19), IL-1 bioactivity was minimal or absent, reflecting concomitant (and excess) production of IL-1 inhibitor (data not shown). The level of IL-1 activity in fluids from RSV-exposed MNL at day three was $\sim$ 50% of the level present at day one, possibly reflecting the balance between production and utilization. In contrast, IL-1 activity was several-fold greater at day three compared with day one for influenza virus-exposed MNL. Monocyte-macrophage production of membrane-associated IL-1 may require physical contact with lymphocytes as well as accessory inducing signals from lymphocytes (23, 37). The data suggest that the early abrogation of cell–cell contact also results in diminished soluble IL-1β production. Further studies would be required to establish a role for IL-1 inhibitors specifically in these events.

Production of IFN-γ by T-lymphocytes requires monocyte-macrophage and lymphocyte collaboration (38), but does not require cell proliferation (31). IFN-γ production has been shown to augment the expression of ICAM-1 on human memory T lymphocytes in an autocrine fashion (32). This implies that ICAM-1 expression and IFN-γ production are associated with activation after rechallenge with a previously experienced antigen. In vitro exposure of MNL to influenza virus resulted in a prompt, substantial production of IFN-γ. In contrast, IFN-γ production by RSV-exposed MNL was delayed, and several-fold lower in magnitude (Fig. 4). Nonetheless, IFN-γ production is a measure of immune recognition (24), and its production to any extent supports the concept of preexisting RSV-specific immunity. Therefore, delayed and/or suppressed IFN-γ production may represent another in vitro correlate of an abrogated RSV-specific anamnestic response.

On the basis of these data and previous studies (17–19), it is possible to speculate on the sequence of events contributing to a diminished or delayed RSV-specific anamnestic response. Exposure to RSV induces both IL-1 inhibitor activity and produces suppression of ICAM-1/LFA-1 expression. The physiologic manifestation of this suppression, diminished leukocyte clustering, results in limited antigen presentation and the effective reduction or absence of costimulatory signals needed for the clonal expansion of RSV-specific lymphocytes. Inhibition of IL-1 activity affecting lymphocytes results in decreased expression of interleukin-2 receptors and transferrin receptors, and a block to lymphocyte proliferation (19, 39). Thus, these studies suggest that one possible mechanism for the very early inhibition of an RSV-specific response is suppression of ICAM-1/LFA-1 expression and cell clustering.

Such effects would explain, at least in part, the recurrence of RSV in individuals who should be immune. Exposure to RSV results in the abrogation of both proliferative and nonproliferative RSV-specific responses by human MNL that involve collaboration and would be critical for the induction of anamnestic defenses. The repeated challenge does not result in an expected limitation of virus replication, to levels less than would produce clinical illness, early after recurrent challenge. Such observations may also be relevant to the difficult and elusive goal of developing effective RSV-specific vaccines. That is, the events of subsequent natural challenge may be a formidable barrier for candidate vaccines that would rely heavily on MNL recruitment to respiratory defenses.

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