Antibody Enhancement of Respiratory Syncytial Virus Stimulation of Leukotriene Production by a Macrophagelike Cell Line

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The clinical and epidemiologic features of respiratory syncytial virus (RSV) infections suggest that RSV-specific antibody may sometimes contribute to the disease process. Recently, it has been demonstrated that virus-specific antibody can enhance RSV infection of macrophagelike cells in vitro. We evaluated the possibility that antibody might also enhance RSV stimulation of the bronchoactive mediator of inflammation leukotriene C-4 (LTC4) in a macrophagelike cell line, U937. The addition of RSV led to little increase in LTC4 production, but addition of RSV plus anti-RSV antibody increased production to a level similar to that achieved with calcium ionophore, a known stimulator of LTC4 production. The antibody-enhanced increase in LTC4 production occurred rapidly (within 15 min), peaked at 60 min, and achieved levels 1.5- to 3.0-fold above that for cells or cells plus virus. RSV plus anti-RSV antibodies in the form of polyclonal serum, monoclonal antibodies, or F(ab')2 fragments and parainfluenza virus types 1 and 3 plus their respective antibodies all increased LTC4 levels over that for the virus alone. These results demonstrate that antibody plus the corresponding virus or protein can increase leukotriene production. This phenomenon could contribute to diseases, such as RSV bronchiolitis, that appear to be caused by an interaction between the virus (or antigen) and host immunity.

Respiratory syncytial virus (RSV) is the single most important cause of severe lower respiratory tract disease in infants and young children. The most distinctive clinical feature of RSV disease in infants and young children is bronchiolitis, a syndrome in which the child develops bronchospasm similar to that seen with asthma (26). The fact that the peak age for RSV bronchiolitis, 2 to 6 months of age, is also a time when the infant has persistent maternal anti-RSV antibodies suggests that these antibodies may contribute to the disease process (19). Several studies have also demonstrated that high-titer maternally acquired neutralizing antibody sometimes protects against serious disease (17, 18, 30). Antibody-mediated enhancement (AME) of infection is one mechanism by which antibody could contribute to protection in some infants and disease in others (29). Two groups have now demonstrated that AME of RSV infection occurs in vitro in macrophagelike cells (16, 24). In those studies, the yield of infectious virus was low and led us to postulate that AME might contribute to disease by mechanisms other than lytic infection of cells.

One possibility is that the virus or virus plus antibody, when it interacts with host cells, stimulates the release of mediators of inflammation or cytokines that in turn contribute to the disease process. Some have suggested that RSV-specific immunoglobulin E (IgE) is present in patients with RSV bronchiolitis and might cause disease through an intermediate hypersensitivity-type reaction (7, 44–46). One group noted that RSV antigen-antibody complexes stimulate the production of thromboxane in neutrophils, and thromboxane can cause smooth-muscle contraction (14). Recently, the concentration of leukotriene C4 (LTC4) in respiratory secretions has been associated with RSV bronchiolitis (40, 41). Leukotrienes are products of arachidonic acid metabolism via the 5-lipoxygenase pathway, are potent mediators of allergic and inflammatory responses (9, 27, 33), and can cause constriction of the small airways of the lung (47). Insoluble immune complexes with IgE and aggregates of IgG have been shown to stimulate LTC4 in macrophages, eosinophils, and basophils (15, 36–38, 48). We felt that leukotrienes were particularly promising as potential contributors to RSV bronchiolitis and chose to evaluate this possibility by examining the effect of RSV plus RSV-specific antibody on LTC4 production in vitro in a macrophagelike cell line. Our findings suggest that virus plus virus-specific antibody increases the production of leukotrienes, a process that may contribute to RSV disease.

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MATERIALS AND METHODS

U937 cells. The U937 cell line was chosen for these studies because it has macrophagelike characteristics and worked well in studies of AME of RSV infection in our laboratory. This hematopoietic cell line is derived from a patient with generalized histiocytic lymphoma (39). Like most monocytes, U937 cells show strong esterase activity that is inhibited by NaF. More than 30% of the cells were positive for CD4 and Fc surface receptors on the basis of flow cytometric analysis. U937 cells as well as monocytes produce leukotrienes (12).

The U937 cells were grown in RPMI 1640 supplemented with 10% fetal calf serum, 100 μg of streptomycin per ml, 100 U of penicillin per ml, and 1% glutamine at 37°C and 5% CO2. Just before leukotriene stimulation studies, cells were harvested, pelleted at 1,200 × g for 10 min at 4°C, and washed three times in Hanks balanced salt solution without calcium or phenol red. Cell number and viability were determined by trypan blue exclusion. The appropriate num-

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ber of viable cells were resuspended in Hanks balanced salt solution with calcium for experiments of less than 2 h; for longer experiments, the cells were incubated in RPMI 1640 medium as described above.

**Preparation of adherent blood monocytes.** Adherent monocytes were obtained from the buffy coat of peripheral blood of healthy human volunteers by centrifugation over lymphocyte separation medium as recommended by the supplier (Oxorgan Teknika, Durham, N.C.). The mononuclear leukocyte layer was collected in a centrifuge tube, mixed with an equal volume of balanced salt solution, and centrifuged at 200 × g for 10 min (at room temperature) to sediment the cells. The pellet was washed twice with phosphate-buffered saline (PBS) and then resuspended in RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.); the pellet was incubated in tissue culture dishes (Falcon; Becton Dickinson, Lincoln Park, N.J.) for 2 h at 37°C, and the nonadherent cells were aspirated off. The cells were further incubated for 48 h at 37°C under 5% CO₂, detached with a rubber policeman, washed with Hanks balanced salt solution, counted by trypan blue exclusion, resuspended at 10⁵ cells per tube, and stimulated with the various reactants.

**Viruses.** The RSV strain used in this study was A2 (25) grown in HEp-2 cells, the parainfluenza virus type 1 strain was C-35 grown in HUT 292 cells (10), and the parainfluenza virus type 3 strain was C-243 grown in HUT 292 cells. Influenza titers were determined for RSV in HEp-2 cells and parainfluenza virus types 1 and 3 in HUT 292 cells by a previously described tissue culture enzyme immunoassay (EIA) (3, 4). The endpoint dilution was calculated by the Reed-Muench method and was considered to contain one 50% tissue culture infective dose of virus (TCID₅₀).

The titer of antigen in the respective virus preparation was determined by previously described time-resolved fluoroimmunoassays (20). For parainfluenza virus types 1 and 3, the hemagglutination titer was determined by using guinea pig erythrocytes (21).

**Antibodies.** The RSV-positive serum pool consisted of eight adult serum specimens that were positive for IgG and negative for IgA and IgM RSV antibodies. The RSV-nega
tive serum was from an infant with pneumonia. One serum was negative for RSV IgG, IgA, and IgM antibodies by antibody capture EIA (13). The human anti-parainfluenza virus type 1 and 3 sera were convalescent-phase specimens from children with parainfluenza virus type 1 and 3 infections, respectively. The seven RSV monoclonal antibodies (MAbs) used in this study have been previously described (2, 5, 6) and include three anti-F protein (131-2a, 133-1h, and 143-6c), three anti-G protein (130-6d, 130-5f, and 232-1f), and one anti-N protein (130-12h) monoclonal.

The neutralizing antibody titer for the positive human serum specimens was determined by a previously described tissue culture EIA (4). Virus-specific IgG, IgE, and F(ab)₂ titers were determined by reacting serial dilutions of antibody against virus-infected and uninfected tissue culture material adsorbed to 96-well microtiter plates (Costar, Cambridge, Mass.), washing the plates, adding peroxidase-conjugated anti-human IgG, anti-human F(ab)₂, or anti-human Fc antibodies (Pierce, Rockford, Ill.), washing the plates, adding substrate (tetramethylbenzidine), and reading A₄₅₀. A reaction was considered positive if the absorbance reading for infected material was more than three standard deviations above the mean of uninfected material.

**RSV IgG and F(ab)₂ preparations.** The RSV IgG was purified from the RSV-positive human serum pool described above on a DEAE-sephacel column (32). Part of the result-

ant RSV IgG was stored at −20°C for IgG studies, and the remainder was cleaved with immobilized pepsin according to the manufacturer’s instructions (Pierce). After the pepsin cleavage, the F(ab)₂ fragments were separated from the Fc fragments and unreacted IgG by elution through protein A. The F(ab)₂ was dialyzed overnight in three changes of 0.01 M PBS (32) and concentrated with a 30-pm-pore-size Amid
colon filter.

**LTC₄ stimulation.** The reactants (for example, uninfected HEp-2 cells, RSV, antibody, and RSV plus antibody, or 10 nM calcium ionophore A23187) were diluted in RPMI 1640 and incubated for 30 min at 37°C in polypropylene test tubes or siliconized glass tubes with shaking. This mixture was then incubated with 2 × 10⁵ cells at a total reaction mixture volume of 0.5 ml at 37°C for the specified period of stimulation.

**LTC₄ assay.** LTC₄ is an intermediate metabolite in the leukotriene metabolism and can be measured by a commercially available radioimmunoassay (RIA) kit. We used LTC₄ production as a proxy for overall leukotriene production. LTC₄ was extracted from the cell supernatant by a modification of a previously published procedure (34). Briefly, at the end of the stimulation period, the cells and supernatant were put on ice for 15 min and centrifuged for 10 min at 1,200 × g at 4°C, and the supernatant was decanted into clean siliconized glass tubes. Two milliliters of methanol-acetonitrile (50%, vol/vol) was added to deproteinize the sample. Next, the sample was centrifuged at 1,500 × g for 10 min at 4°C, the pellet was discarded, and the supernatant was collected and either lyophilized or vacuum evaporated to dryness. The dried samples were stored at −70°C under argon until tested for LTC₄ by a competition RIA (LTC₄ radioimmunoassay kit; New England Nuclear, Boston, Mass.). To perform the assay, the samples were resus
pended in 0.1 ml of the assay buffer supplied as part of the kit. The RIA was performed as recommended by the manufacturer and as described by Aeringhaus et al. (1). LTC₄ standards and LTC₄-negative control were included in each run. The negative control was not adsorbed with charcoal. A standard curve constructed for each run was used to calculate sample concentration. The LTC₄ levels in the sample. The LTC₄ RIA gave consistent results, with a 95% confidence interval for five replicates of less than or equal to ±15% of the mean value for a given experiment. A 50% increase in LTC₄ levels was ≥2 and usually ≥3 standard deviations above the mean and was considered a significant increase. The anti-LTC₄ antibody cross-reacts with LTD₄ and LTE₄, and thus the RIA also detects these two leukotrienes but with less sensi
tivity than LTC₄.

**Reverse-phase HPLC analysis.** Supernatants from RSV-
only and RSV-plus-IgG stimulations were concentrated and partially purified by using C₁₈-Sep-Pak columns (Waters Associates, Milford, Mass.). The columns were equilibrated with methanol and deionized water before the samples were loaded. After the samples were loaded, the column was washed with deionized water containing methanol (60% water and 40% methanol) and eluted with 100% methanol. The eluates were evaporated to dryness under vacuum and then resuspended in 100 μl of 30% methanol in deionized water for high-performance liquid chromatography (HPLC) separation. The HPLC used was a Waters dual-piston pump with a C₁₈ Nova-Pak (Waters Associates) reverse phase column; UV peaks were monitored at 280 nm on a Waters detector. The mobile phase consisted of 60% methanol, 40% water, and acetic acid containing 1.0 mM EDTA adjusted to pH 5.4 with ammonium hydroxide. The flow rate was 1
ml/min. The UV peaks were collected separately and pooled. The fractions corresponding to the separate peaks were dried under vacuum, resuspended in the RIA buffer, and stored at −20°C under argon until tested.

RESULTS

Characteristics of LTC₄ production. An increase in LTC₄ levels occurred rapidly after the addition of 10 nM calcium ionophore A23187. An increase in LTC₄ levels over that after the addition of media was detected within 5 min and peaked at about 15 min (Fig. 1). In contrast, RSV alone gave minimal increase in LTC₄ levels above that for media, and the RSV-positive serum pool gave levels of LTC₄ similar to that with RSV alone and with a similar time course (data not shown). The RSV-positive serum pool plus RSV markedly increased LTC₄ levels with a time course similar to that for calcium ionophore (Fig. 1). This increase was noted within 15 min of stimulation and peaked at about 60 min. We chose to use 60-min stimulation times in the studies described below.

The status of the cells at the time of stimulation proved important for antibody enhancement of LTC₄ production. The best results were achieved when the U937 cells were at log phase of growth at the time of stimulation (data not shown). In time course experiments, fresh 2 × 10⁵ U937 cells per ml were at log phase of growth after 30 to 40 h in culture. For the remaining studies, 2 × 10⁵ cells per ml were incubated for 36 to 40 h, washed, and resuspended at 2 × 10⁵ cells per ml before stimulation.

A wide range of virus and antibody dilutions gave increased levels of LTC₄. For example, a 10⁻³ dilution of the RSV-positive serum pool mixed with 50 to 1,000 TCID₅₀ of RSV significantly increased LTC₄ levels over that for RSV alone (Fig. 2A). Similarly, 100 TCID₅₀ of RSV mixed with 10⁻² to 10⁻⁶ dilutions of the RSV-positive serum pool significantly increased levels over that for virus alone (Fig. 2D). LTC₄ levels were always higher after stimulation with RSV plus the RSV-positive serum pool than after stimulation with virus alone. In 10 experiments, this increase averaged 2.7-fold ± 0.4-fold (range of 1.5- to 3.3-fold). For comparison, in 12 experiments, calcium ionophore stimulation gave an average increase in LTC₄ levels of 3.0-fold ± 0.4-fold (range of 2.4- to 3.3-fold) over that for virus alone. In contrast, stimulation with virus alone (12 experiments) gave an average increase of 1.20-fold ± 0.10-fold (range of 0.83- to 1.34-fold) over that for media, and stimulation with the RSV-positive serum pool (12 experiments) gave an average increase of 1.11-fold ± 0.08-fold (range of 0.73- to 1.12-fold). The addition of uninfected HEp-2 cells and uninfected HEp-2 cells plus the RSV-positive serum pool gave LTC₄ levels similar to that for RSV alone.

Specificity of antibody-enhanced LTC₄ stimulation. We used HPLC fractionation to evaluate the specificity of antibody-enhanced LTC₄ production detected by RIA. In these experiments, we looked at two measures of the effect of antibody on RSV stimulation of LTC₄, area under the curve of the eluted peaks and RIA-determined LTC₄ levels for the respective peaks. We identified the peaks corresponding to LTC₄ and LTD₄ by HPLC analysis of purified commercial standards (Sigma Chemical Co., St. Louis, Mo.). LTC₄ elutes at peak 3, and LTD₄ elutes at peak 4 (Table 1). When we compared results for samples after RSV stimulation with the positive serum pool to those with RSV alone, we found that the greatest increase occurred at peak 3 by both measures. This supports the specificity of the antibody-enhanced increase in LTC₄ detected by RIA.

To demonstrate that the increase in LTC₄ levels was specific for the antigen-antibody interaction, we tested a variety of control reactants as summarized in Fig. 3. The negative controls included tissue culture material, antibodies alone, and virus plus antibody (serum or monoclonal) without detectable anti-RSV antibodies. None of the controls gave LTC₄ levels significantly above that achieved with the virus alone. In contrast, RSV plus the RSV-positive serum pool or RSV MAbs increased LTC₄ to levels similar to that
with calcium ionophore. All the anti-RSV MAbs mixed with RSV significantly increased LTC₄ over that for RSV alone (data not shown). These included MAb's against the F, G, and N proteins and of subclasses IgG1, IgG2a, and IgG2b. This increase occurred over dilutions of 10⁻² to 10⁻⁸ and to levels >2.0-fold above that for virus alone with all monoclonal antibodies.

The antibody enhancement of LTC₄ production, however, was not restricted to RSV. Two other respiratory viruses, parainfluenza virus types 1 and 3, gave results similar to RSV. As with RSV, virus alone did not significantly increase LTC₄ levels above that produced by cells (mean of 1.1-fold ± 0.1-fold for parainfluenza virus type 1 [n = 3] and 1.1-fold ± 0.3-fold for parainfluenza virus type 3 [n = 3]). Neither the parainfluenza virus type 1 serum nor the parainfluenza virus type 3 serum significantly increased LTC₄ production (data not shown). However, with the addition of virus-specific polyclonal antibody, LTC₄ levels were increased to an average of 1.6-fold ± 0.3-fold for parainfluenza virus type 1 (n = 3) and 2.3-fold ± 0.5-fold for parainfluenza virus type 3 (n = 3). Antibody-enhanced LTC₄ production occurred at a lower range of antibody dilutions for parainfluenza virus types 1 and 3 (10⁻³ to 10⁻⁷) than for RSV (10⁻² to 10⁻⁶) (Fig. 2B and C). This difference occurred despite similar neutralizing titers of the respective antibodies (Table 2). Antibody enhancement of LTC₄ production occurred at 10 to 100 TCID₅₀ of input parainfluenza virus. Parainfluenza virus type 1 and 3 MAb's plus the corresponding viruses signifi-

**TABLE 1. HPLC analysis of the supernatant from stimulated cells**

<table>
<thead>
<tr>
<th>Peak</th>
<th>Retention time (min)</th>
<th>%AREA⁺</th>
<th>Area ratio⁺</th>
<th>RIA results (pg/ml)</th>
<th>RIA ratio⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RSV</td>
<td>RSV + IgG</td>
<td>RSV</td>
<td>RSV + IgG</td>
</tr>
<tr>
<td>1</td>
<td>0.94</td>
<td>1.31</td>
<td>2.02</td>
<td>1.54</td>
<td>459</td>
</tr>
<tr>
<td>2</td>
<td>1.22</td>
<td>97.33</td>
<td>95.82</td>
<td>0.98</td>
<td>920</td>
</tr>
<tr>
<td>3</td>
<td>4.08</td>
<td>0.76</td>
<td>1.56</td>
<td>2.04</td>
<td>690</td>
</tr>
<tr>
<td>4</td>
<td>4.63</td>
<td>0.40</td>
<td>0.60</td>
<td>1.50</td>
<td>459</td>
</tr>
</tbody>
</table>

⁺ On the basis of the retention time of purified commercial LTC₄ and LTC₄. LTC₄ should elute at peak 3, and LTA should elute at peak 4.

⁻ Retention time by HPLC on a C₁₈ RP column with methanol-water-acetic acid (60%-40%-0.1%) (pH 5.4) mobile phase at 1.0 ml/min for 1 h.

⁺⁺ Area = area of the peak/total area of all peaks.

⁻⁻ Ratio of value for RSV plus IgG to that for RSV.
Para 3

LTC₄ production by purified RSV F and G proteins. Since the enhancement of LTC₄ production occurred rapidly, before virus replication could occur, we sought to determine whether the viral proteins alone might also be effective stimulators. Proteins purified by affinity chromatography, kindly provided by S. Hildreth and P. Paradiso, Praxis Biologics, Rochester, N.Y. (42, 43), were tested with and without antibody. The individual proteins stimulated levels of LTC₄ higher than that achieved with virus alone (1.89-fold for F and 3.56-fold for G protein) (Fig. 4). We felt that the increased levels of LTC₄ produced with the individual proteins might have occurred because of antibody that coeluted off the affinity column with the proteins. To test this hypothesis, we evaluated LTC₄ levels with the affinity-purified proteins after adsorption with protein G Sepharose (Pharmacia LKB Biotechnology, Piscataway, N.J.) and F and G proteins partially purified from cell lysate by column chromatography. The affinity-purified proteins adsorbed with protein G did not give increased levels of LTC₄, but the addition of antibody to the adsorbed proteins did significantly increase the levels of LTC₄ produced (Fig. 4). Similarly, the column-purified proteins did not give increased levels of LTC₄. The two peaks from the column that contained F and G proteins by EIA gave levels of LTC₄ 0.75- and 0.67-fold above that for RSV alone, while the addition of RSV-positive serum pool at a 1:1,000 dilution increased

![Graph](image)

**FIG. 3.** Specificity of antibody enhancement of RSV stimulation of LTC₄. The negative controls include the following reactants added to U937 cells: media (Media), uninfected HEp-2 cells (HEp2), RSV A2 (RSV), two RSV MAb (131-2a and 232-1f) (rsMAb), the RSV MAb plus uninfected HEp-2 cells (rsMAb/HEp2), serum (Serum), serum plus uninfected HEp-2 cells (Serum/HEp2), a parainfluenza virus type 3 MAb (211-6c) plus RSV (p3MAb/RS), or serum negative for RSV antibodies plus RSV (Neg.Serum/RS). The positive control is 10 mM calcium ionophore (A23187). The test specimens are RSV plus the anti-RSV serum pool (Serum/RS) or RSV plus the two RSV MAb (rsMAb/RS).

The data, from one experiment, are representative of two experiments. Note that the negative controls all gave levels of LTC₄ similar to or below that for RSV alone and that the test specimens gave levels significantly higher than and comparable to those with A23187.

<table>
<thead>
<tr>
<th>Virus*</th>
<th>Host cell</th>
<th>Serum preparation neutralization titer*</th>
<th>Virus preparation infectivity titer*</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV</td>
<td>HEp-2</td>
<td>1:320</td>
<td>10⁵⁺⁻⁺⁵⁺</td>
</tr>
<tr>
<td>Para 1</td>
<td>HUT 292</td>
<td>1:480</td>
<td>10⁵⁺⁻⁵⁺</td>
</tr>
<tr>
<td>Para 3</td>
<td>HUT 292</td>
<td>1:320</td>
<td>10⁴⁻⁴⁺</td>
</tr>
</tbody>
</table>

* Para 1 is parainfluenza virus type 1, and Para 3 is parainfluenza virus type 3.
* Neutralization titer by tissue culture EIA.
* Infectivity titer (TCID₅₀/0.1 ml) by tissue culture EIA.

**TABLE 2.** Characteristics of serum and virus preparations.
LTC₄₄ levels for the two peaks to 1.91- and 2.17-fold above that for RSV alone. The specificity of antibody-enhanced LTC₄₄ is further supported by the fact that a G protein MAb plus the affinity-purified F protein gave no enhancement (0.88-fold compared with that for F alone), while an F protein MAb plus the affinity-purified F protein gave enhancement of 1.65-fold compared with that for F alone.

**Fc receptors and LTC₄₄ production.** To investigate the possibility that antibody enhancement of LTC₄₄ production occurred by way of the Fc receptor in a fashion similar to AME of infection, we looked at the ability of purified anti-RSV IgG from the serum pool and F(ab')₂ fragments to enhance LTC₄₄ production. The F(ab')₂ preparation was free of whole IgG by polyacrylamide gel electrophoresis and Coomassie brilliant blue staining; a heavy band was seen at 110 kDa [consistent with F(ab')₂], and no band was seen at 160 kDa (consistent with IgG). The F(ab')₂ fragment had a titer of 1:128 against RSV with an anti-F(ab')₂ detector and a titer <1:2 with the anti-Fc detector. In comparison, purified IgG had a titer of 1:512 with both the anti-F(ab')₂ and anti-Fc detectors. The F(ab')₂ enhanced LTC₄₄ production to a level similar to that for purified anti-RSV IgG (Fig. 5 and Table 3). In contrast, the purified IgG gave RSV AME, while the F(ab')₂ gave no AME (Fig. 5). Preincubating cells with anti-Fc receptor 1 and anti-Fc receptor 3 antibodies did not affect LTC₄₄ levels produced by RSV plus antibody, while anti-Fc receptor 2 antibodies at dilutions of (10⁻⁴ to 10⁻⁵) did appear to decrease levels of LTC₄₄ from about 2-fold to 1.2-fold over that for virus alone.

**LTC₄₄ production in human peripheral blood monocytes.** In initial studies to determine whether antibody enhancement of leukotriene production might contribute to RSV disease, we tested the ability of antibody to increase LTC₄₄ production by RSV in peripheral blood monocytes from five volunteers (Table 4). The level of antibody enhancement of LTC₄₄ for peripheral blood monocytes from four donors was similar to that seen with U937 cells, 2.4- to 3.4-fold above that for virus alone. With cells from one volunteer, the levels were increased only by 1.2-fold with the addition of specific antibody.

**DISCUSSION**

This study demonstrates that virus-specific antibody can enhance in vitro stimulation of LTC₄₄ production by RSV and parainfluenza virus types 1 and 3. In U937 cells, the addition of virus was associated with LTC₄₄ levels similar to that with cells alone, but the addition of virus plus virus-specific antibody was associated with a 1.5- to 3.0-fold increase in LTC₄₄ levels. This level of stimulation was similar to that achieved with a known stimulator of leukotrienes, calcium ionophore A23187 (37). Conversely, serum lacking the virus specific antibody or a MAb against a different virus and a variety of other controls did not increase leukotriene levels above that produced when medium was added to cells. Thus, the increase in levels of LTC₄₄ appeared to require the corresponding virus-specific antibody. We also demonstrated antibody enhancement of LTC₄₄ levels with RSV F and G proteins plus their corresponding specific antibodies.

The potential range of antigens plus the specificity of the antibody-antigen interaction make this phenomenon an intriguing potential mechanism for a number of disease processes that involve both viruses or foreign antigens and host immunity. RSV bronchiolitis could well be explained in part by this type of virus and immune interaction. In infants, high levels of maternally acquired antibody are associated with
FIG. 5. IgG and F(ab')2 enhancement of RSV stimulation of LTC₄ production and of RSV infection. Fold enhancement is picograms of LTC₄ per ml after addition of RSV plus IgG (IgG LTC-4) or F(ab')2 [F(ab')2 LTC-4] divided by picograms of LTC₄ per ml after addition of RSV alone. Fold enhancement of infection is percent RSV-positive U937 cells 48 h after inoculation with RSV plus IgG (IgG AME) or RSV plus F(ab')2 [F(ab')2 AME] over percent positive cells after inoculation with RSV alone. Extent of RSV infection was identified by adding biotinylated anti-RSV MAbs to the cells, adding fluorescein-conjugated streptavidin to the cells, and determining percent antigen-positive cells with a fluorescein-activated cell sorter as previously described (11). The data, from one experiment, are representative of three experiments. Note that LTC₄ levels were significantly (>1.5-fold) increased with the addition of IgG or F(ab')2 to RSV, but levels of RSV-positive cells were increased only after addition of IgG but not after addition of F(ab')2.

but do not ensure protection from disease (17, 18, 30). If host immunity fails to prevent spread of virus to the lower respiratory tract, then antibody plus virus or antibody plus viral proteins (presumably in the form of complexes) would be present in the bronchioles, where their reaction with macrophages or other cells could release leukotrienes and cause bronchospasm. Leukotrienes have also been proposed as mediators of some of the symptoms associated with allergic rhinitis and asthma (7, 23). Recently, a leukotriene inhibitor has been shown to sometimes decrease symptoms of allergic rhinitis (23). Foreign antigens plus the host’s specific antibodies increasing cellular release of leukotrienes or other mediators of inflammation might explain the contribution of host immunity to these disease processes. Studies to explore the potential link between antibody enhancement of leukotriene production and diseases such as RSV bronchiolitis are in progress.

We chose to measure LTC₄ as a proxy for leukotriene production because an RIA was available commercially. LTC₄ is produced from LTA₄ and is metabolized to LTD₄, which is then metabolized to LTE₄, LTC₄, LTD₄, and LTE₄ have bronchospastic activity, while LTB₄, which is metabolized by another pathway, does not (26, 31). Since LTC₄ is an intermediate in the metabolic pathway of leukotrienes, we expect the results for LTC₄ to be representative of those for the other leukotrienes. Further study, however, is needed to confirm this assumption. It is also possible that other mediators of inflammation or cytokines might be stimulated by antigen-antibody complexes.

One concern about our results is their applicability to other cells, especially those in vivo. The U937 cell line has the characteristics of a macrophage precursor cell and not those of a fully differentiated macrophage. Our results with adherent peripheral blood monocytes, however, suggest that antibody enhancement of leukotrienes is not limited to U937 cells and may occur in vivo.

It was surprising to find that the mechanism for antibody enhancement of leukotriene production was apparently distinct from that for AME. Digesting IgG to F(ab')2, as expected, did not eliminate the ability of the antibody to react with RSV but did eliminate its ability to enhance RSV infection in the U937 cells. In contrast, it did not eliminate its

<table>
<thead>
<tr>
<th>Antibody</th>
<th>10⁻²</th>
<th>10⁻³</th>
<th>10⁻⁴</th>
<th>10⁻⁵</th>
<th>10⁻⁶</th>
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<tbody>
<tr>
<td>F(ab')₂</td>
<td>2.0</td>
<td>2.0</td>
<td>1.6</td>
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</tr>
<tr>
<td>IgG</td>
<td>1.9</td>
<td>2.1</td>
<td>1.9</td>
<td>1.5</td>
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</tbody>
</table>

a Fold enhancement = LTC₄ levels after stimulation with the respective antibody plus virus divided by LTC₄ levels after stimulation with virus alone.
TABLE 4. Antibody enhancement of leukotriene production by adherent human peripheral blood monocytes

<table>
<thead>
<tr>
<th>Donor</th>
<th>LTC4 (pg/ml) per reactant</th>
<th>Medium</th>
<th>RSV</th>
<th>Serum</th>
<th>RSV + serum</th>
</tr>
</thead>
<tbody>
<tr>
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<td>750</td>
<td>980</td>
<td>850</td>
<td>3,300 (3.4)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>760</td>
<td>800*</td>
<td>905</td>
<td>1,002 (1.3)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>780</td>
<td>1,130</td>
<td>1,500</td>
<td>2,960 (2.6)</td>
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</tr>
<tr>
<td>4</td>
<td>1,028</td>
<td>1,306</td>
<td>920</td>
<td>3,150 (2.4)</td>
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</tr>
<tr>
<td>5</td>
<td>1,170</td>
<td>1,400</td>
<td>700</td>
<td>3,500 (2.5)</td>
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</tr>
</tbody>
</table>

* Adherent peripheral blood monocytes were collected as described in Materials and Methods, incubated for approximately 48 h, stimulated with medium, 100 TCID50 of RSV A2, a 1:1,000 dilution of the RSV-positive serum pool, and RSV plus serum; LTC4 in the supernatant was measured 1 h after stimulation as described in the Materials and Methods. Fold enhancement = LTC4 levels after stimulation by RSV plus anti-RSV serum pool divided by LTC4 levels after stimulation with RSV alone.

ability to enhance RSV stimulation of leukotriene production. It is possible that undigested RSV IgG, IgA, or IgM was present in the F(ab')2 preparation. However, we did not detect IgG by gel electrophoresis in the F(ab')2 preparation or RSV-specific IgA or IgM by EIA (13) in the original preparation. In blocking experiments, on the other hand, anti-Fc receptor 2 antibodies did decrease levels of LTC4 associated with addition of antibody to RSV. This could have occurred by blocking the Fc receptor directly or possibly by attachment at the Fc receptor affecting another receptor. The apparent disparate results with F(ab')2 and blocking experiments make the role of Fc receptors in this phenomenon unclear but do suggest that their role, if any, is distinct from that for AME of infection.

We have not yet identified a promising alternate receptor. It is known that arachidonic acid, the precursor of leukotrienes, is stored in the cell membrane as a phospholipid ester that is removed and made available to the cell through receptor-mediated, calcium-dependent activation of specific lipases (8, 12, 28). The receptors on the cell surface that are activated by unopsonized zymosan, calcium ionophore, and bacterial lipopolysaccharide are known to be capable of stimulating leukotriene production but are not known to bind antibody-antigen complexes (22). Previous studies have demonstrated that LTC4 or LTB4 levels produced by eosinophils, basophils, or macrophages can be increased by adding aggregates of IgG, IgA, and IgE; IgG or IgE reacted against antigen-coated beads; or large immune complexes of IgE and albumin (15, 35, 38, 48). It has been suggested but not clearly shown that this occurs through immune complex binding to the Fc receptor. Our data suggest that soluble antigen-IgG antibody complexes can also stimulate leukotriene production and that if this occurs through the Fc receptor, it does so independently of the Fc fragment of the antibody. Further study is required to clarify this issue.

This study demonstrates that increases in leukotrienes by the interaction of antibody with antigen are not limited to insoluble immune complexes of IgE antibodies but can occur with the type of IgG-mediated immune complexes that might form in the lungs or other organs. Their presence in the vicinity of resident monocytes could in turn cause the release of leukotrienes and contribute to the disease process in that organ, for example, bronchospasm in RSV bronchiolitis or asthma.

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REFERENCES


