PHAGOCYTES

Influenza A Virus Accelerates Neutrophil Apoptosis and Markedly Potentiates Apoptotic Effects of Bacteria

By Maria Luisa Colamussi, Mitchell R. White, Erika Crouch, and Kevan L. Hartshorn

Influenza A virus (IAV) infection induces apoptosis of cultured epithelial cells (e.g., MDCK or HeLa cells) and of human peripheral blood monocytes. IAV also has been reported to induce apoptosis of airway epithelial cells and lymphoid cells in vivo. Whether IAV induces apoptosis of neutrophils has not previously been reported. We have studied extensively the interactions of IAV with neutrophils because there is evidence that neutrophils may play a role in initial containment of IAV infection, but also because IAV-induced depression of neutrophil functions is a likely contributory factor to the development of bacterial superinfections during IAV infection. Such bacterial superinfections constitute a major cause of morbidity and mortality during IAV epidemics. Defects in neutrophil and monocyte chemotactic, oxidative, and bacterial killing functions have been documented in IAV infections. Moreover, it is been shown in animal models that there is a clear correlation between impairment of functions of these cells and predisposition to bacterial superinfections. We have shown that IAV has complex effects on neutrophil function. IAV causes depression of the ability of these cells to mount respiratory burst responses to the bacterial peptide, fMLP, or the phorbol ester, PMA. In addition, the virus itself induces activation of neutrophils as evidenced by stimulation of phosphoinositide and calcium metabolism and generation of H2O2. The current studies were undertaken to determine if IAV also induces apoptosis of neutrophils.

We also aimed to determine whether incubation of neutrophils with both IAV and bacteria would result in an enhancement of apoptosis compared with effects of either IAV or bacteria alone. Data regarding the effects of bacteria on neutrophil apoptosis are conflicting. Watson et al reported that ingestion of serum-opsonized, heat-killed Escherichia coli induces neutrophil apoptosis. In contrast, Baran et al reported that ingestion of Staphylococcus aureus promoted apoptosis of monocytes while inhibiting apoptosis of neutrophils. In any case, demonstration of an interaction between IAV and bacteria in induction of apoptosis could have relevance to bacterial superinfection of IAV infected subjects.

We report that IAV and E coli independently accelerate neutrophil apoptosis, and that the combination of E coli and IAV caused markedly greater apoptosis than either pathogen alone. The mechanisms of these effects are elucidated. The effects of opsonins on IAV or E coli–induced apoptosis are examined as well.

MATERIALS AND METHODS

Reagents. Dextran, sodium citrate, RPMI 1640, propidium iodide (PI), Dulbecco’s phosphate-buffered saline with Ca2+ and Mg2+, Trypan blue stain, Wright–Giemsas stain, scopolatin, and horseradish peroxidase-type II were purchased from Sigma Chemicals Co (St Louis, MO). Ficoll-Paque was obtained from Pharmacia Biotech (Piscataway, NJ). Dulbecco’s phosphate-buffered saline without Ca2+ and Mg2+ was purchased from GIBCO-BRL (Grand Island, NY). Organic solvents were purchased from Fisher Scientific (Fairlawn, NJ). The phycoerythrin-labeled anti-CD95 murine monoclonal antibody and isotype control were obtained from PharMingen (San Diego, CA). Rabbit polyclonal antibodies against the Fas antigen (C-20) and Fas ligand (C-20) and from Sigma. Recombinant human pulmonary surfactant protein D (hSP-D) is a pulmonary surfactant collectin that binds to, and increases neutrophil uptake of, both IAV and E coli. hSP-D was prepared as described.

Neutrophil preparation. Neutrophils from healthy volunteer donors were isolated to greater than 95% purity, as previously described, by using dextran precipitation, followed by a Ficoll-Hypaque gradient separation for removal of mononuclear cells and hypotonic lysis to eliminate contaminating erythrocytes. Neutrophils were used within 2 hours of isolation.

Virus preparation. IAV strain H3N3 A/Bangkok/79 (Bangkok 79; gracious gift of Robert Webster, St Jude’s Hospital, Memphis, TN) was grown in the choriocallantoic fluid of 10-day-old embryonated hens’ eggs.
Bacterial preparations. E coli (K-12 strain, unlabeled), fluorescein isothiocyanate (FITC)-labeled E coli (K-12 strain), and E coli-opsonizing reagent (polyclonal rabbit IgG) were purchased from Molecular Probes (Eugene, OR). Additionally viable E coli (K-12) were obtained from American Type Culture Collection (Rockville, MD), grown in our laboratory, and fixed with formalin for use in experiments. All bacteria used in experiments described in this report were killed. E coli suspensions in phosphate-buffered saline (PBS) were sonicated (for three 20-second intervals) to eliminate bacterial aggregates and washed three times in PBS before each experiment.

Measurement of bacterial uptake by neutrophils. E coli uptake by neutrophils was measured incubating FITC-labeled bacteria with neutrophils, followed by evaluation of cell-associated fluorescence using a flow cytometer. For fluorescence measurements, 250 µg/mL (or 1.2 x 10^6 bacteria/mL) of FITC-labeled E coli was incubated with neutrophils (ratio 24 bacteria per neutrophil), alone or in presence of various concentrations of IAV. After allowing E coli and neutrophils to interact for 1 hour at 37°C, the neutrophils were washed, resuspended in E coli–free PBS, and fixed with 2% paraformaldehyde. Cell-associated fluorescence was measured on a Becton Dickinson FACScan (for three 20-second intervals) to eliminate bacterial aggregates and washed three times in PBS before each experiment.

Assessment of apoptosis. Neutrophil apoptosis was assessed by analysis of the percent of cells with hypoploid nuclei by exposing the cells to the DNA-binding dye PI. The method was performed using a slight modification of the method described by Nicoletti et al. Neutrophils (5 x 10^6/mL) were incubated for 1 hour with IAV, and/or E coli and/or rHSP-D at 37°C; washed; resuspended in RPMI 1640 supplemented with 4% human serum, 20 mM/L HEPES buffer, 1% L-glutamine, 1% penicillin, and streptomycin pH 7.4; and left to incubate at 37°C. This incubation was carried in slowly rotating 15-mL conical, polypropylene tubes. At various periods of incubation, 200 µL of cell suspensions were centrifuged at 200g for 10 minutes, and the pellets were fixed in 1 mL of ice-cold 70% ethanol at 4°C for 60 minutes at least. Fixed cells were then washed with cold PBS with Ca^{2+} and Mg^{2+} and gently resuspended in 1 mL of PI solution (40 µg/mL in PBS with Ca^{2+} and Mg^{2+}). The cells were finally incubated in the dark at room temperature for 15 minutes and analyzed on a Becton Dickinson FACScan 2. Forward and side scatter of neutrophils were simultaneously acquired. The red fluorescence due to the PI staining of red blood cells was eliminated using the Lysis II program (Becton Dickinson, Mountain View, CA) in presence of 0.02% of Trypan blue to quench extracellular fluorescence.

Assay for neutrophil expression or release of Fas or Fas-ligand. Fas antigen expression was assessed using either the phycoerythrin-labeled CD95 monoclonal or unlabeled rabbit polyclonal anti-Fas (C-20) antibodies (followed by Fab, fragments of FITC-labeled goat-anti-rabbit IgG; Jackson Laboratories, West Grove, PA). Fas Ligand expression on neutrophils was assessed using a rabbit polyclonal anti-Fas ligand preparation (anti-Fasl C-20). During incubation of neutrophils with rabbit polyclonal anti-Fas or anti-Fasl, antibodies, nonspecific rabbit immunoglobulin was added to the buffer to inhibit Fc-receptor–mediated binding. Release of Fas ligand into the culture supernatant was measured by allowing neutrophil culture supernatants to incubate in 96-well plates overnight (to allow proteins to adhere to plastic), followed by washing and incubation with biotinylated rabbit polyclonal anti-Fas ligand antibody. Bound antibody was detected by incubation with streptavidin coupled to horseradish peroxidase, followed by peroxidase substrates (TMB substrate; BioRad, Hercules, CA), sulfuric acid to terminate the reaction, and assay of OD450 on a plate-reading device.

Statistical methods. Statistical significance was determined using Student’s paired t-test.
84 ± 5% of cells were able to exclude Trypan blue, as compared with 93 ± 2% of control cells; \( P < .05 \). At other concentrations of \( E\ coli \) (or other time points), the \( E\ coli \)-treated samples did not show significantly greater lysis than control cells. Therefore, the extent of apoptosis measured using the PI assay greatly exceeded the extent of lysis as determined by Trypan blue dye exclusion.

As shown in Table 1, opsonization of \( E\ coli \) with polyclonal IgG increased the extent of apoptosis resulting from \( E\ coli \) treatment. We have previously shown that opsonization with this antibody preparation or with rHSP-D increases neutrophil uptake of \( E\ coli \). Opsonization of \( E\ coli \) with rHSP-D did not significantly alter apoptosis induced by the bacteria (Table 1). Table 1 also shows that treatment of neutrophils with \( E\ coli \) significantly enhanced neutrophil Fas antigen expression. Opsonization of \( E\ coli \) with IgG, or with one concentration of rHSP-D, caused greater increase in Fas antigen expression than \( E\ coli \) alone. The anti–\( E\ coli \) IgG preparation alone did not increase neutrophil apoptosis or Fas antigen expression (Table 1).

**Bangkok 79 IAV potentiates the apoptotic effect of \( E\ coli \) (and vice versa).** As shown in Fig 4A, no significant increase in apoptosis was seen when neutrophils were treated with various concentrations of \( E\ coli \) for 6 hours. However, when the cells

**Fig 1. Neutrophil apoptosis induced by IAV.** Neutrophil apoptosis assessed by PI-stained hypodiploid nuclei and flow cytometry (A) and Wright-Giemsa stain to detect the morphologic characteristics of apoptotic cells (B). A total of \( 5 \times 10^6 \)/mL neutrophils were incubated with control buffer or 2, 10, and \( 20 \mu g/mL \) of Bangkok 79 IAV stock at 37°C. At indicated times, aliquots of each sample (1 \times 10^6 and 1 \times 10^5 cells for PI stain and Wright-Giemsa stain respectively) were assessed for apoptosis. Data are expressed (A) as percent of cells with hypodiploid DNA (counted 5,000 cells) or (B) as percent of cells with apoptotic characteristics with respect to the total number of cells counted (at least 400 in five different fields) and represent the mean ± SEM of at least four separate experiments. (*) Indicates where IAV significantly increased apoptosis (\( P < .05 \)).

**Fig 2. Effect of rHSP-D on IAV-induced neutrophil apoptosis.** Various amounts of rHSP-D were preincubated alone or with 25 \( \mu g/mL \) of Bangkok 79 IAV for 30 minutes at 37°C before incubation (for 20 minutes at 37°C) with neutrophils (5 \times 10^6/mL). At indicated times the samples were assessed for apoptosis using the PI flow cytometry assay. Data are expressed as percentage of cells with hypodiploid nuclei and represent mean ± SEM of at least four separate experiments. As indicated by the (*), Bangkok 79 IAV alone caused significant (\( P < .05 \)) apoptosis. Samples treated with IAV and rHSP-D together did not cause significantly greater apoptosis than did IAV alone. rHSP-D alone did not cause significant apoptosis.

**Fig 3. Neutrophil apoptosis induced by \( E\ coli \).** The indicated concentrations of \( E\ coli \) were incubated with neutrophils as described in Materials and Methods. (The ratios of bacterial particles to neutrophils were between 20 and 460 bacteria/neutrophil.) Results are expressed as percentage of cells with hypodiploid DNA as measured by the PI assay and represent the mean ± SEM of three separate experiments. (*) Indicates where \( E\ coli \) alone caused significantly greater apoptosis than control buffer.
Table 1. Effects of Opsonizing Antibodies or rHSP-D on Neutrophil Apoptosis Induced by E coli

<table>
<thead>
<tr>
<th>Neutrophil Treatment</th>
<th>Apoptotic Neutrophils (%)</th>
<th>Fas Antigen Expression</th>
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<tbody>
<tr>
<td>Control buffer</td>
<td>5 ± 1</td>
<td>5 ± 0.4</td>
</tr>
<tr>
<td>E coli alone</td>
<td>26 ± 5*</td>
<td>10 ± 2*</td>
</tr>
<tr>
<td>E coli + 18 µg/mL IgG</td>
<td>36 ± 8†</td>
<td>16 ± 2†</td>
</tr>
<tr>
<td>E coli + 36 µg/mL IgG</td>
<td>38 ± 8†</td>
<td>19 ± 1†</td>
</tr>
<tr>
<td>36 µg/mL IgG alone</td>
<td>4 ± 1</td>
<td>4.5 ± 1</td>
</tr>
<tr>
<td>E coli + 0.13 µg/mL rHSP-D</td>
<td>23 ± 4*</td>
<td>10 ± 2*</td>
</tr>
<tr>
<td>E coli + 0.26 µg/mL rHSP-D</td>
<td>20 ± 5*</td>
<td>12.5 ± 2†</td>
</tr>
<tr>
<td>E coli + 1 µg/mL IAV</td>
<td>28 ± 4*</td>
<td>12 ± 2*</td>
</tr>
<tr>
<td>E coli + 2 µg/mL IAV</td>
<td>24 ± 1*</td>
<td>10 ± 2*</td>
</tr>
</tbody>
</table>

Results represent mean ± SEM for five experiments (ie, 5 individual blood donors). Percentages of apoptotic neutrophils were assessed using PI as described in Fig 1. Fas antigen expression was assessed by flow cytometry using polyclonal rabbit anti-Fas antibodies. Fas antigen results are expressed in fluorescence units (ie, mean channel fluorescence of 5,000 cells). All assays were done after 20 hours of incubation of neutrophils with control buffer, E coli alone, or E coli that had been preincubated with either opsonizing IgG or rHSP-D as indicated. Opsonizing IgG alone (in absence of E coli) did not cause any increase in percent of apoptotic cells compared with control (n = 3; data not shown).

* P  0.05 as compared with neutrophil treated with buffer alone.
† Indicates P  0.05 as compared with neutrophils treated with E coli alone (ie, no opsonizer agent).

Using rhodamine-labeled E coli and fluorescent microscopy, we found that rHSP-D caused aggregation of E coli as previously described (data not shown). When rhodamine-labeled E coli and FITC-labeled IAV were incubated simultaneously with rHSP-D it was evident that bacterial aggregates also contained IAV (ie, both red and green fluorescence was present in the aggregates). Based on these results we tested whether IAV caused apoptosis. As shown in Fig 7, the additive effect of incubating neutrophils with IAV and E coli together was again evident in these experiments. Addition of IAV caused a modest but statistically significant further increase in apoptosis after 8 or 24 hours. The effect of IAV was minimal possibly due to the marked extent of apoptosis already induced by the combination of IAV and E coli alone.

Neutrophil uptake of E coli. As shown in Fig 8A, incubation of neutrophils with a fixed concentration of IAV enhanced binding of various concentrations of FITC-labeled E coli to

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![Fig 4. Neutrophil apoptosis induced by E coli alone or E coli in combination with 40 µg/mL of IAV. The degree of apoptosis induced by E coli alone or E coli in combination with 40 µg/mL of IAV after 6 (A) or 24 (B) hours of incubation are shown. Results are expressed as percentage of cells with hypodiploid DNA and represent the mean ± SEM of three separate experiments. At 24 hours all concentrations of E coli alone caused significantly more apoptosis than control buffer (P  0.05). (*) Indicates where the combination of E coli and IAV caused significantly greater apoptosis than E coli alone. (**) Indicates where the combination of E coli and IAV caused significantly greater apoptosis than either E coli or IAV alone.](image-url)
these cells. In these experiments the neutrophils were allowed to incubate with IAV and E coli simultaneously for 1 hour at 37°C and then to incubate with Trypan blue to quench extracellular fluorescence. The results reflect mainly the amount of E coli that internalized by the cells (see Hartshorn et al23 for validation of this method). Figure 8B (upper curve) shows the effect of incubating neutrophils with a fixed concentration of E coli and increasing concentrations of IAV. All concentrations of virus significantly increased uptake of E coli. The maximum enhancement of E coli uptake was seen at a concentration of 7 µg/mL of IAV (neutrophil fluorescence was significantly greater at this concentration of IAV than at lower or higher concentrations of the virus). However, all concentrations of IAV tested (including 0.36 µg/mL) significantly increased E coli binding. Figure 8B (lower curve) also shows the effect of preincubating neutrophils with various concentrations of IAV, followed by washing off unbound virus and then incubating the cells with E coli. Using this method, the virus still enhanced binding of E coli to neutrophils, although only at the highest concentration of IAV shown.

Neutrophil hydrogen peroxide production in response to E coli and IAV alone or in combination. As shown in Fig 9, neutrophils that were treated with the combination of E coli and IAV produced H2O2 at a more rapid rate than cells treated with either IAV or E coli alone. The results shown are representative of four experiments in which the combination of IAV and E coli consistently elicited more H2O2 than did either E coli or IAV alone. The mean ± SEM rate of H2O2 production (in nmol/L/3
min/4 x 10^6 cells) was 0.84 ± 0.16 for the combination of IAV and E coli as compared with 0.26 ± 0.05 and 0.19 ± 0.03, respectively, for IAV or E coli alone (P = 0.02 for the combination as compared with either IAV or E coli alone). Rates of H_2O_2 production elicited by E coli alone were not significantly greater than rates of production by untreated neutrophils (data not shown). Addition of rHSP-D to the combination of IAV and E coli resulted in production of 1.16 ± 0.13 nmol/L/3 min of H_2O_2, which was significantly greater than IAV or E coli alone, but not significantly greater than the rate of H_2O_2 produced by the combination of IAV and E coli.

Effect of IAV and/or E coli on neutrophil expression of Fas antigen or Fas ligand. As shown in Table 2, incubation with IAV for 3 or 20 hours increased neutrophil expression of Fas antigen as assessed using either the CD95 monoclonal antibody or rabbit polyclonal antibodies directed against Fas. To rule out the possibility that these results reflected a general increase in binding of antibodies, the effect of IAV on binding of either isotype control monoclonal antibody or nonspecific rabbit IgG was tested as well. Although a slight increase in isotype control antibody did result from IAV treatment, this increase was insufficient to account for the increase in binding of CD95 monoclonal antibodies. IAV did not alter binding of rabbit polyclonal IgG (data not shown). Also, the IAV-induced increase in binding of polyclonal anti-Fas antibodies was abrogated by preincubation of the antibodies with blocking peptide.

E coli also increased expression of Fas antigen (as assessed using either antibody) after 20 hours. Although the combination of IAV and E coli appeared to increase Fas antigen expression to a greater extent than either IAV or E coli alone, these results are more difficult to interpret because there was substantial variability in results between donors and because the combined treatment caused additive increases in isotype binding. Also note that the blocking peptide only partially eliminates the enhanced binding of Fas polyclonals in the case of neutrophils treated with IAV and E coli. The marked degree of neutrophil apoptosis and lysis occurring after 20 hours in samples treated with IAV and E coli may have precluded definitive determination of whether additive increases in Fas expression occurred.

Fas ligand expression on neutrophils was significantly (al-
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through modestly) increased after 20 hours of treatment with IAV (see Table 3). There was a substantially, and statistically significant, increase in release of Fas ligand into the culture supernatant in neutrophils treated with IAV for 20 hours.

**DISCUSSION**

IAV induces apoptosis of epithelial cells and peripheral blood monocytes. IAV has been shown to upregulate Fas antigen expression in MDCK and HeLa cells providing one possible mechanism for induction of apoptosis. It has also been found that MDCK cells transfected with bcl-2 are resistant to IAV-mediated apoptosis and indeed show greater resistance to IAV replication as compared with control cells. These studies have generally found that inactivated IAV does not induce apoptosis and indicate an association between active viral replication and apoptosis. Pahl and Baeuerle have found, however, that expression of the IAV hemagglutinin alone activates transcription factor NF-κB in HeLa and 293 cells through a mechanism that involves cellular oxidant production. Activation of NF-κB could lead to elaboration of cytokines (e.g., tumor necrosis factor) that in turn could accelerate apoptosis.

In this report we show for the first time that IAV accelerates neutrophil apoptosis. Cassidy et al have reported that IAV causes only abortive infection of neutrophils such that viral proteins are produced but infectious viral particles are not released into the supernatant. The time course of apoptosis induction by IAV (first clearly observable effect at ~20 hours’ incubation) is compatible with virus-mediated induction of either cellular (e.g., Fas antigen, cytokines) or viral protein expression. IAV increased expression of neutrophil Fas antigen to a moderate extent (see Table 2). Neutrophil surface Fas ligand expression and release of Fas ligand into the culture supernatant were also increased by treatment with IAV (Table 3). Other factors may also be involved in IAV-induced neutrophil apoptosis. We have reported that IAV acts as a stimulus for neutrophil activation in its own right and also causes depression of neutrophil responsiveness to other stimuli. Our prior studies have shown the IAV-induced respiratory burst response occurs predominantly at an intracellular location, which could contribute to apoptosis.

We have also reported that preincubation of IAV with SP-D enhances neutrophil binding of the virus and increases the respiratory burst response triggered by the virus. In addition SP-D is able to protect neutrophils against the depressing effects of the virus on neutrophil respiratory burst responses to other stimuli. We anticipated that SP-D would also be found to...
alter the ability of IAV to cause neutrophil apoptosis. However, in experiments reported in this study, we show no such effect for reasons that remain unclear to us.

We also show that unopsonized E coli accelerates neutrophil apoptosis in a dose-related and time-dependent fashion, and that the degree of neutrophil apoptosis is enhanced when E coli is preincubated with opsonizing antibodies. Although we have reported that rHSP-D enhances neutrophil uptake of E coli to an extent similar to opsonizing antibodies, the effects of rHSP-D on E coli–related apoptosis were much more subtle (see Table 1). Watson et al16 have also found that antibody-opsonized E coli accelerates apoptosis of neutrophils. Because inactivated bacteria were used in both studies, it is clear that bacterial replication is not necessary for induction of apoptosis. Watson et al16 showed that acceleration of neutrophil apoptosis by E coli was inhibited by addition of antioxidants. We show that E coli moderately increased neutrophil expression of Fas antigen after 20 hours, which may also have contributed to apoptosis. Fas antigen expression was increased to a greater extent by opsonized, rather than by unopsonized, E coli.

A striking finding of this report was that simultaneous incubation of neutrophils with IAV and E coli caused a substantial and significant enhancement (both in terms of rate and total extent) of apoptosis as compared with that induced by either pathogen alone. The addition of rHSP-D caused a modest but statistically significant further increase in apoptosis induced by the combination of IAV and E coli. Even in the absence of opsonizing agents, however, the combination of IAV and E coli was so profound as to lead to loss of the majority of neutrophils by 24 hours.

Our studies also provide insight into the mechanism of the cooperative acceleration of apoptosis by IAV and E coli. First, IAV was shown to cause increased neutrophil uptake of E coli. Uptake of E coli was enhanced most strongly when neutrophils were incubated simultaneously with E coli and IAV (ie, the same conditions used in the apoptosis experiments). We have found that treatment of neutrophils with IAV causes a marked and rapid upregulation of CEA-related antigens on the neutrophil surface (K.L. Hartshorn, unpublished data, March 1998). Because these CEA-related antigens (also called nonspecific crossreacting antigens or CD66 and CD67) have been shown to be important binding sites for E coli on neutrophils,32 this may partly account for the enhancement of E coli uptake. Clearly further studies will be needed to clarify the mechanism of IAV-induced enhancement of E coli uptake, since this could be a contributory factor to the cooperative acceleration of apoptosis induced by the combination of IAV and E coli. Note that opsonizing antibodies both enhance E coli uptake and the degree of apoptosis in E coli–treated neutrophils.

Not only did IAV enhance binding of E coli, but it also increased the respiratory burst response elicited by the bacteria. This was unexpected since, as noted above, treatment of neutrophils with IAV depresses respiratory burst responses to fMLP or PMA.13 The respiratory burst elicited by the combination of IAV and E coli significantly exceeded that produced by either pathogen alone. This cooperative induction of the neutrophil respiratory burst response may, therefore, be a contributory factor in the marked acceleration of apoptosis caused by the combination of IAV and E coli.

Finally, IAV induced increases in surface expression of Fas antigen and Fas ligand on neutrophils and release of Fas ligand into the supernatant. Neutrophil surface Fas antigen has been shown to contribute to spontaneous neutrophil apoptosis, whereas soluble Fas ligand may mediate effects on neighboring cells.33 We cannot reach any firm conclusion regarding whether IAV and E coli cooperate in inducing Fas antigen or Fas ligand expression or release, due to nonspecific changes seen with isotype controls. Possible cooperative effects of IAV and E coli in production of cytokines were not examined but could be involved in acceleration of apoptosis. For instance, Nain et al34 have reported synergistic induction of tumor necrosis factor release from monocytes treated with both IAV and lipopolysaccharide.34 Clearly further studies will be needed to sort out these possibilities. It will be of interest to determine if monocytes or macrophages (which also have been reported to undergo apoptotic changes after exposure to influenza virus35) also show enhanced expression of Fas antigen or release of Fas ligand in response to IAV.

In any case, our findings may have relevance to IAV infection, or to bacterial superinfection during IAV infection, in vivo. Neutrophils have been shown to contribute to the early inflammatory response to IAV infection1,6 and are also likely to be recruited to the airway in cases when bacterial superinfection occurs during IAV infection. IAV-induced apoptosis may be beneficial in the sense that inflammatory responses may be minimized during clearance of the virus. Alternatively, accelerated apoptosis of neutrophils could impair bacterial clearance in patients with IAV infection. Further studies will be needed to determine if IAV has cooperative apoptotic effects with more common respiratory bacterial pathogens.

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REFERENCES

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