Neutrophil Deactivation by Influenza A Viruses: Mechanisms of Protection After Viral Opsonization With Collectins and Hemagglutination-Inhibiting Antibodies

By Kevan L. Hartshorn, Kenneth B.M. Reid, Mitchell R. White, Jens C. Jensenius, Shirley M. Morris, Alfred I. Tauber, and Edmond Crouch

Bacterial superinfections are a major cause of morbidity and mortality during influenza A virus (IAV) epidemics. Depression of phagocyte functions resulting from attachment of the IAV hemagglutinin (HA) to cell surface sialo-glycoproteins is a likely contributory cause of these infections. We have proposed that the group of collagenous lectins (termed collectins) present in blood and pulmonary surfactant play a role in initial host defense against IAV. We used here several recombinant human surfactant protein D (RhSP-D) preparations to determine the mechanism through which opsonization of IAV with collectins protects neutrophils against the deactivating effects of IAV on cellular respiratory burst responses in vitro. RhSP-D was markedly more potent than antibodies that inhibited viral hemagglutination activity (anti-HA antibodies) at protecting neutrophils in this assay. Unlike the anti-HA antibodies, RhSP-D was protective at concentrations that minimally inhibited viral hemagglutination activity. Two related features of SP-D—the degree of multimerization and the ability to cause aggregation of IAV particles—were critical determinants of the ability of SP-D to protect neutrophils against deactivation. Similarly SP-D-induced viral aggregate formation resulted in enhanced IAV binding to neutrophils and potentiated the ability of the virus itself to trigger neutrophil respiratory burst responses. In contrast to the case of IAV-antibody complexes, SP-D-IAV complexes attached to and activated neutrophils through a neuraminidase-sensitive mechanism (ie, similar to unopsonized IAV). These results indicate that collectin-mediated viral aggregation per se may be an important host defense mechanism not only by virtue of reducing the number of infectious viral particles, but also by promoting phagocyte responsiveness.

© 1996 by The American Society of Hematology.

MATERIALS AND METHODS

Reagents. Formyl-methionyl-leucyl-phenylalanine (FMLP), cytochalasin B, horseradish peroxidase-type II, scopoletin, superoxide dismutase (SOD), cytochrome-C, neuraminidase-type X (protease activity, <0.002 U/mg protein), ficoll, dextran, sodium citrate, citric acid, and Staph protein A were purchased from Sigma Co (St Louis, MO) and Hycap was obtained from Winthrop Pharmaceuticals (Des Plaines, IL). Organic solvents were purchased from Fisher Scientific (Fairlawn, NJ). Dulbecco's phosphate-buffered saline was purchased from Flow Laboratories (Costa Mesa, CA). Phospholipase C was purchased from Boehringer Mannheim (Indianapolis, IN). The monoclonal antibodies (MoAbs) 3G8 (Fab2) and IV3 (Fab) directed respectively against the FcRIII and FcRII subtypes of neutrophil surface Fc receptors were purchased from Medarex Inc (West Lebanon, NH).

Collectin preparations. RhSP-Ds were expressed in CHO K1 from the Departments of Medicine and Biochemistry, Boston University School of Medicine, Boston, MA; the Department of Biochemistry, University of Oxford, Oxford, UK; the Department of Immunology, University of Aarhus, Aarhus, Denmark; and the Department of Pathology, Washington University School of Medicine, St Louis, MO.

Submitted May 26, 1995; accepted November 28, 1995.

Supported by National Institutes of Health Grants No. AI92550-03 and AI34897 (K.L.H.) and HL44015 (E.C.).

Address reprint requests to Kevan L. Hartshorn, MD, Boston University School of Medicine, S-301, 80 E Concord St, Boston, MA 02118.

© 1996 by The American Society of Hematology.

From www.bloodjournal.org by guest on August 30, 2017. For personal use only.
cells (ATCC CCL-61) and purified as previously described.\textsuperscript{29,30} Soluble SP-D was purified by affinity chromatography on maltosyl-agarose, followed by gel filtration chromatography on 4% agarose (Bio-Rad A-15M; Bio-Rad, Hercules, CA) in the presence of 10 mmol/L EDTA. Three fractions of RsSP-D were purified and used in these experiments. One fraction eluted near the void volume and made up approximately one-third of the total immunoreactive SP-D present. This fraction (hereafter called RsSP-D multimers) contained a large proportion of very high order multimers of SP-D (up to 32 arms), as determined by electron microscopy.\textsuperscript{30} An additional fraction consisted predominantly of dodecamers of similar appearance to native and recombinant rat SP-D.\textsuperscript{30} Finally, a third fraction (termed hereafter RsSP-D single arms) eluted at a position equivalent to that previously shown for trimeric or single arm forms of SP-D.\textsuperscript{30} These recombinant SP-D preparations contained \( \leq 100 \) pg/mL of endotoxin.

Another recombinant SP-D preparation consisting of the carbohydrate recognition domain (CRD) and adjacent hinge region of bovine SP-D fused to glutathione-S-transferase (GST) was also used. This preparation was made by expression in Escherichia coli of expression plasmid containing GST and the coding sequences for amino acids 197 through 349 of bovine SP-D. The preparation was purified by sequential use of GST and maltose affinity columns, as described.\textsuperscript{32} Inclusion of the neck region allows spontaneous formation of homotrimers of CRD. As described,\textsuperscript{32} this protein (termed hereafter GST-SP-D/CRD) consists predominantly of trimers of the CRD lacking the collagen domain or N-terminus of the molecule. Lacking the SP-D N-terminus (which contains cysteine residues), this molecule is not able to form further multimeric structures in the usual sense. However, a limited degree of dimerization of through GST domain cannot be excluded.

Native human MBP was purified from mannan Sepharose and anti-IgG columns.\textsuperscript{33} After purification, human serum albumin (HSA) was added to the native MBP preparation to enhance stability of the protein. Conglutinin was purified from bovine serum as described\textsuperscript{34,35} and graciously provided by Dr Y.M. Lee (formerly of Applied Immune Sciences, Menlo Park, CA). All of the collectin preparations used in these studies were free of other contaminating proteins as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis, except in the case of native human MBP, which contained HSA, as mentioned.

**Virus preparation.** IAVs were grown in the chorioallantoic fluid of 10-day-old embryonated hens' eggs and purified on a discontinuous sucrose density gradient as previously described.\textsuperscript{11} Virus stocks were dialysed against phosphate-buffered saline (PBS), aliquoted, and stored at \(-70°C\) until used. HA titers were determined by titration of virus samples in PBS followed by addition of thoroughly washed human type 0 red blood cells. The A/Texas 77/H3N2 (Texas 77) was the gracious gift of Dr Jon Abramson (Bowman-Gray School of Medicine, Winston-Salem, NC). The Mem71B-Bel, strain of IAV was used in prior studies.\textsuperscript{27} Neutrophil preparation. Neutrophils from healthy volunteer donors were isolated to greater than 95% purity, as previously described, using dextran precipitation, followed by a Ficoll-Hypaque gradient separation for removal of mononuclear cells and hypotonic lysis to eliminate contaminating erythrocytes.\textsuperscript{31} Cell viability was greater than 98%, as determined by trypan blue staining, and cells were used within 5 hours of isolation. To desialate neutrophil surface proteins, \(5 \times 10^7\) neutrophils were incubated with 0.128 U/mL of neuraminidase at \(37°C\) for 1 hour with constant mixing.\textsuperscript{25} Cells were subsequently washed three times and resuspended in PBS.

**Measurement of neutrophil activation.** \( \Delta \)O production was measured by the oxidation of scopoletin, and \( \Delta \)O was assessed by the continuous monitoring of the SOD inhibitable reduction of cytochrome C.\textsuperscript{20} Deactivation was assessed by incubating neutrophils with IAV for various periods of time followed by measurement of superoxide production in response to FMLP. Superoxide was assessed using the superoxide dismutase inhibitable reduction of ferricytochrome-C.\textsuperscript{11}

**Measurement of viral binding to neutrophils.** Viral binding to neutrophils was measured by preparing fluorescein isothiocyanate (FITC)-labeled virus and incubating this preparation with neutrophils, followed by evaluation of cell associated fluorescence using a flow cytometer. FITC stock was prepared at 1 mg/mL in 1 mol/L sodium carbonate, pH 9.6. The FITC-labeled virus was prepared by incubating concentrated virus stocks with FITC (10:1 mixture by volume of virus in PBS with FITC stock) for 1 hour, followed by dialysis of the mixture for 18 hours against PBS. Neutrophils were preincubated with various priming agents, followed by washing in PBS and the addition of 10-μL aliquots of fluorescent viral samples to neutrophils (\(10^6\) cells in 100 μL PBS). After allowing virus and neutrophils to interact for 15 minutes at 4°C, the neutrophils were washed, resuspended in virus-free PBS, and fixed with 2% paraformaldehyde. Cell-associated fluorescence was measured on a Becton Dickinson FACScan 2 and analyzed using the Lysys II program (Becton Dickinson, Mountain View, CA). In some experiments, flu-
OREX SINE VINCERE HUMAN MBP was also found to protect neutrophils to FMLP was control neutrophils that were not exposed to IAV. As shown or Bangkok 79 strain of IAV markedly inhibited the ability shows that, although either RhSP-D multimers or RhSP- attachment of the ability of these virus-treated cells to produce 0; responses of virus-treated IAV samples used in (A), (B), and (C) were tested for their ability to cause red blood cell hemagglutination. The results are expressed as the mean ± SEM reduction in HA titer as compared with unopsonized IAV controls. *P ≤ .05 comparing the percentage of O₂- or HA assays comparing results obtained with SP-D- or MoAb-treated samples to those obtained with unopsonized virus.

Electron microscopy. Neutrophils were incubated with opsonized or unopsonized Bangkok 79 IAV at 4°C for 20 minutes, as described in the preceding paragraph. After this, the unbound virus was washed off and the cells were fixed for 2 hours in cold 1% glutaraldehyde buffered with 0.1 mol/L sodium cacodylate. After fixation, cells were rinsed in cacodylate buffer containing 1% sucrose, postfixed for 1 hour in 1% osmium tetroxide, dehydrated through a graded series of ethyl alcohols, and embedded in Polybed (Polysciences, Inc, Warrington, PA). Thin sections were cut with a diamond knife on an LKB ultramicrotome (LKB, Stockholm, Sweden) and mounted on copper grids. The sections were stained with uranyl acetate and lead citrate and examined with a Phillips 300 electron microscope (Mahwah, NJ).

Statistics. Statistical comparisons were made using the Student’s paired t-test.

RESULTS

Effects of HA inhibitory antibodies or collectins on IAV-induced neutrophil deactivation. To further characterize the mechanisms through which opsonization with SP-D protects effects against IAV-induced neutrophil deactivation, we tested in parallel the activity of two RhSP-D preparations that differ only in their degree of multimerization. RhSP-D multimers consist of fractions of RhSP-D containing highly multimerized forms, whereas RHSP-D single arms consist of fractions containing trimeric forms (see Materials and Methods). The assay for IAV-induced neutrophil deactivation entailed treatment of neutrophils with IAV alone (or the same amount of IAV that had been preincubated with either antibodies or collectins as indicated), followed by measurement of the ability of these virus-treated cells to produce O₂- in response to FMLP. The O₂- response of virus-treated cells was compared with that of parallel control neutrophils that were not exposed to IAV. As shown in Fig 1, treatment of neutrophils with either the Texas 77 or Bangkok 79 strain of IAV markedly inhibited the ability of the cells to generate O₂- in response to FMLP. Figure 1A shows that, although either RhSP-D multimers or RhSP-D single arms protected neutrophils against the depressing effects of the virus on O₂- responses to FMLP, the RhSP-D multimers were significantly more potent in this regard. Native human MBP was also found to protect neutrophils against IAV-induced deactivation, although (as in previous experiments) approximately 10-fold higher concentrations of MBP were required to achieve a degree of protection similar to that obtained with RhSP-D (data not shown).

We also tested the effect of preincubating IAV with a preparation termed GST-SP-D/CRD that consists of CRDs of bovine SP-D linked to GST but without the collagen domain, and hence without the ability to form higher order multimeric structures (beyond the basic trimer that forms the globular CRD region). Substantially higher concentrations of GST-SP-D/CRD (Fig 1B) did not reduce IAV-induced neutrophil deactivation.

It was necessary to preincubate IAV with SP-D to achieve maximal protection against neutrophil deactivation. RhSP-D multimers incubated with neutrophils before or after adding IAV did not significantly enhance O₂- responses of virus-treated cells to FMLP. Also, the addition of RhSP-D to neutrophils in the absence of IAV had no significant enhancing effect on O₂- production (data not shown).

As shown in Fig 1C, preincubation of either Texas 77 or Bangkok 79 strains of IAV with strain-specific HA-neutralizing MoAbs before adding the virus to neutrophils was also protective against IAV-induced neutrophil deactivation. As in the case of RhSP-D, the ability of the MoAbs to restore FMLP-stimulated O₂- responses depended on preincubation of IAV with the MoAbs before addition of the virus-MoAb complex to neutrophils. If the virus was allowed to bind to neutrophils first, followed subsequently by the addition of MoAbs, no enhancement of FMLP-stimulated O₂- responses was observed (data not shown).

Effects of anti-HA MoAbs or collectins on IAV HA activity. Because IAV-induced deactivation is mediated by binding of the viral HA to neutrophil surface sialo-glycoproteins, we initially thought that inhibition of HA binding to the cell alone might account for the protective effects of the antibodies or collectins. The need to preincubate IAV with MoAbs or collectins to achieve protection against deactivation (see above) suggested that prevention of the initial attachment of the viral HA to the cell surface was critical.

Figure 1E, F, and G also illustrates the degree of reduction in HA titers found in the opsonized viral samples used in the deactivation assays described above. The relative efficacy of the two MoAbs to protect against neutrophil deactivation
not inhibit HA activity or inhibited by ≤50%. (Note that full inhibition of viral HA activity could be obtained using higher concentrations of SP-D [data not shown and Hartshorn et al]). RhSP-D single arms did not protect against deactivation to the same degree as did RhSP-D multimers, even at concentrations that caused a similar degree of HA inhibition. GST-SP-D/CRD did not protect against deactivation despite inhibiting HA activity to an equal or greater extent than RhSP-D. Therefore, whereas inhibition of HA activity may largely account for the protective effect of IAV opsonization with anti-HA antibodies, this is not the case for SP-D. Also, the degree of multimerization of SP-D is an important determinant of its ability to protect against deactivation.

**Ability of collectins to cause aggregation of IAV particles.** We next examined the ability of collectins to cause aggregation of IAV particles as measured by decreases in light transmission through a stirred viral suspension. Figure 2 shows the comparative IAV aggregating ability of RhSP-D multimers, RhSP-D single arms, and the GST-SP-D/CRD preparation. RhSP-D multimers were extremely potent at inducing aggregation (comparable to results previously obtained with native hSP-D or unfractionated RhSP-D). RhSP-D single arms were significantly less active on a protein concentration basis at inducing aggregation in this assay. Neither GST-SP-D/CRD nor GST alone caused significant aggregation. MBP caused viral aggregation at approximately 10-fold greater concentrations (ie, ~5 μg/mL) than the optimal aggregating concentrations of RhSP-D multimers (data not shown). HA neutralizing MoAbs directed against Bangkok 79 and Texas 77 IAV also did not cause measurable aggregation of these viral strains using this assay (data not shown).

**Effects of anti-HA MoAbs or collectins on IAV binding to neutrophils.** Figure 3A shows that RhSP-D multimers enhanced FITC-labeled IAV binding to neutrophils at the concentrations that protected the cell against deactivation. In contrast, preincubation of IAV with RhSP-D single arms did not significantly enhance viral binding (Fig 3B). A moderate enhancement in virus binding was observed with GST-SP-D/CRD or GST alone. Preincubation of IAV with RhSP-D multimers, GST-SP-D/CRD, or GST alone did not enhance IAV binding, although at higher concentrations GST-SP-D/CRD significantly reduced viral binding as compared with binding of unopsonized IAV or IAV treated with GST alone. Preincubation of IAV with 40 or 100 μg/mL of MoAb 73/1 modestly enhanced viral binding, whereas higher MoAb concentrations actually reduced binding (Fig 3C). Addition of the MoAb after first allowing the virus to bind to neutrophils did not significantly affect binding.

Figure 4 depicts the light and fluorescent microscopic appearance of neutrophils treated with unopsonized IAV or IAV opsonized with RhSP-D multimers. Whereas binding of unopsonized IAV resulted in a faint, diffuse fluorescence on the surface of neutrophils, binding of SP-D-IAV complexes was evidenced by large aggregates of cell-associated fluorescence. Figure 5 shows electron micrographs that confirm the presence of large aggregates of IAV particles attached to the surface of neutrophils treated with IAV opsonized with RhSP-D multimers.

To test whether collectin-opsonized IAV binds to neutrophils, we...
**Significantly reduced binding as compared with control.**

Significantly increased binding of opsonized IAV samples to neutrophils was measured. In IC, FITC-labeled IAV was preincubated with HA inhibitory MoAb (n = 5; data not shown). Treatment of neutrophils with phospholipase C (PLC) reduced binding of the anti-FcRIII MoAb to 22% ± 6% of control (n = 4; P = 0.05), but did not significantly reduce binding of IAV alone or of viral-MoAb complexes (data not shown). Hence, these data suggest that a proportion of binding of IAV-MoAb complexes is mediated by attachment in a neuraminidase-insensitive manner to FcRII receptors.

**Effect of opsonization with collectins or IgG on ability of IAV to stimulate neutrophil respiratory burst responses.** We have previously reported that preincubation of several strains of IAV with either MBP,26 conglutinin,27 or SP-D28 leads to marked enhancement of neutrophil H2O2 production as compared with responses elicited by unopsonized IAV. In those studies, the concentrations of SP-D and conglutinin required to optimally enhance H2O2 production correlated with optimal concentrations for eliciting aggregation of IAV particles. As shown in Fig 7, preincubation of IAV with RhSP-D multimers or RhSP-D single arms also enhanced neutrophil H2O2 responses to the virus, although the RhSP-D multimers did so to a significantly greater extent. In similar experiments, preincubation of IAV with 134 μg/mL of GST-SP-D/CRD did not cause any enhancement of IAV-stimulated H2O2 production (mean H2O2 production rates, 0.64 ± 0.20 and 0.69 ± 0.16 nmol/min, respectively, for neutrophils stimulated with IAV alone or IAV opsonized with GST-SP-D/CRD; n = 3). Overall, these data are again consistent with the hypothesis that collectin-mediated enhancement of IAV-induced H2O2 production results (at least in part) from viral aggregation.

Despite markedly enhanced H2O2 production, IAV opso-
Fig 4. Effect of RhSP-D on IAV binding to neutrophils as assessed by fluorescent microscopy. Neutrophils were allowed to adhere to plastic coverslips followed by incubation of the cells with either FITC-labeled Bangkok 79 IAV alone (top panels) or IAV that had been preincubated with either 1.6 (middle panels) or 3.2 pg/mL (bottom panels) of RhSP-D multimers for 15 minutes at 4°C. After incubation, the coverslips were washed, fixed with 1% paraformaldehyde, and mounted. The left panels show phase contrast microscopy and the right panels show fluorescence microscopy of the same fields. Results depicted are representative of more than three separate experiments.

nized with either MBP, conglutinin, or SP-D did not elicit neutrophil O₂ production in numerous experiments (data not shown). In addition, as shown in Fig 8, preincubation of neutrophils with neuraminidase caused nearly complete blockade of the ability of these cells to produce H₂O₂ in response to IAV opsonized with SP-D. Parallel experiments were performed in which neuraminidase-treated neutrophils were stimulated with IAV opsonized with either MBP or conglutinin, yielding similar results. Neuraminidase-treated cells produced 5% ± 5% and 11% ± 8% as much H₂O₂ in response to IAV opsonized with conglutinin or MBP, respectively, as did control cells (n = 4; P < 0.05 for each
Fig 5. Electron micrographs of neutrophils treated with either IAV alone or IAV opsonized with RhSP-D. (A) shows a neutrophil that had been treated with unopsonized IAV (Bangkok 79 strain) for 20 minutes at 4°C, followed by thorough washing to remove unbound IAV and preparation of cells for EM as described. (B) shows neutrophils treated similarly with the same concentration of IAV that had been preincubated with RhSP-D multimers (3.2 μg/mL). (Original magnification ×8,090 in both panels.)
set of experiments). These results contrast with those obtained with IAV opsonized with anti-HA MoAbs (Table 1 and Fig 8).

As shown in Table 1, complexes of either Texas 77 or Bangkok 79 IAV with their respective HA-inhibiting MoAbs elicited markedly increased H₂O₂ production as compared with the unopsonized viruses alone. Whereas neuraminidase treatment of neutrophils inhibited H₂O₂ responses of neutrophils to unopsonized virus, it had no effect on the responses elicited by IAV opsonized with high concentrations of anti-HA MoAb. In (A), binding of IAV alone or IAV opsonized with all tested concentrations of RhSP-D or conglutinin was significantly reduced by treatment of neutrophils with neuraminidase. In (B), binding of IAV or IAV preincubated with 100 µg/mL of the MoAb was significantly reduced (P < .05) by neuraminidase treatment; that of IAV preincubated with either 200 or 400 µg/mL of the MoAb was not. *Binding of collectin or MoAb preincubated IAV to control cells significantly enhanced compared with binding of unopsonized IAV to these cells (P < .05). **Binding of MoAb preincubated IAV to control cells significantly reduced compared with binding of unopsonized IAV to these cells (P < .05).

**Fig 6. Binding of IAV alone or IAV that had been preincubated with collectins or anti-HA MoAb to control or neuraminidase-treated neutrophils. Neutrophils were preincubated with either 0.128 U/mL of neuraminidase (90 minutes at 37°C; ■) or control buffer (□), followed by assessment of binding of FITC-labeled Bangkok 79 IAV to these cells (as described in Fig 3). IAV was preincubated with various concentrations of RhSP-D, conglutinin, or anti-HA MoAb. In (A), binding of IAV alone or IAV opsonized with all tested concentrations of RhSP-D or conglutinin was significantly reduced by treatment of neutrophils with neuraminidase. In (B), binding of IAV or IAV preincubated with 100 µg/mL of the MoAb was significantly reduced (P < .05) by neuraminidase treatment; that of IAV preincubated with either 200 or 400 µg/mL of the MoAb was not. *Binding of collectin or MoAb preincubated IAV to control cells significantly enhanced compared with binding of unopsonized IAV to these cells (P < .05). **Binding of MoAb preincubated IAV to control cells significantly reduced compared with binding of unopsonized IAV to these cells (P < .05).**

is of interest that, whereas unopsonized IAV did not elicit any O₂ response (data not shown), the viral-MoAb complexes did. Neutrophils treated with Bangkok 79 IAV complexed with 400 µg/mL of anti-HA MoAb produced O₂ at a rate of 5.3 ± 1 nmol/L/min/10⁶ cells (mean ± SEM; n = 4). Similar results were obtained with Texas 77 IAV complexed with its specific anti-HA MoAb (data not shown).

**DISCUSSION**

These studies show that RhSP-D is substantially more potent than anti-HA IgG MoAbs on a protein concentration basis at protecting neutrophils against the deactivating effects of IAV. Several lines of evidence suggest that the propensity of SP-D to cause viral aggregation is an important determinant of its ability to modulate IAV-neutrophil interactions. Whereas the ability of anti-HA antibodies to protect against IAV-induced deactivation of neutrophils depended on nearly complete inhibition of IAV HA activity, SP-D...
was protective at concentrations that did not inhibit or only modestly inhibited IAV HA activity. The ability of the various SP-D preparations to protect against deactivation paralleled their ability to cause aggregation of IAV particles. For instance, RhSP-D multimers were significantly more active than were RhSP-D single arms at protecting against deactivation. Furthermore, the GST-SP-D/CRD preparation (which cannot form multimers beyond the basic trimer composing the CRD globular head) was not protective at all against IAV-induced deactivation even at concentrations that reduced HA activity to a greater extent than RhSP-D.

**Table 1. Effect of Preincubating IAV With Antiviral MoAbs on IAV-Induced Neutrophil H2O2 Responses**

<table>
<thead>
<tr>
<th></th>
<th>IAV</th>
<th>IAV + MoAb</th>
<th>IAV + MoAb/Prot A</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Texas 77</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Control cells</td>
<td>0.80 ± 0.24</td>
<td>1.40 ± 0.20*</td>
<td>0.03 ± 0.031</td>
</tr>
<tr>
<td>B. Neuraminidase- treated cells</td>
<td>0.14 ± 0.07</td>
<td>1.30 ± 0.08</td>
<td></td>
</tr>
<tr>
<td><strong>II. Bangkok 79</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Control cells</td>
<td>0.05 ± 0.05</td>
<td>0.56 ± 0.21*</td>
<td>0.05 ± 0.05†</td>
</tr>
<tr>
<td>B. Neuraminidase- treated cells</td>
<td>0</td>
<td>1.09 ± 0.53</td>
<td></td>
</tr>
</tbody>
</table>

Results shown are the mean ± SEM (n = 3 to 4 experiments) neutrophil H2O2 response (in nanomoles per minute per 4 × 10⁶ cells) to stimulation with either Texas 77 or Bangkok 79 IAV alone or to aliquots of these strains of IAV that had been preincubated with 800 or 400 pg/mL, respectively, of the 81/4 or 73/1 antiviral MoAbs. Preincubation with MoAbs was performed as described in Fig 1 C. Where indicated (ie, IAV + MoAb/Prot A), MoAbs complexed with Staph protein A (as described in the Materials and Methods) before incubation with the virus. An equivalent concentration of Staph A to that used in these experiments was also used in the absence of MoAbs (eg, H2O2 response to Texas 77 IAV in presence of Staph A was 0.86 ± 0.16 nmol/min/4 × 10⁶ cells). Where indicated, neutrophils were either preincubated for 90 minutes in control buffer or buffer containing neuraminidase (final concentration, 0.128 U/mL) before stimulation with IAV preparations.

* P < .05 compared with neutrophils stimulated with IAV alone.
† P < .05 compared with neutrophils stimulated with IAV complexed with MoAb in the absence of Staph protein A.

Son et al recently showed that simple aggregates of IAV particles formed by mechanical means did not cause neutrophil deactivation, whereas nonaggregated IAV preparations did. Hence, SP-D-induced alterations in IAV particle size alone may be critical in protecting neutrophils against deactivation.

Note that, although MBP was also protective against IAV-induced neutrophil deactivation, approximately 10-fold higher concentrations of this serum collectin were required both to achieve a protective effect similar to those obtained with SP-D and to induce a comparable degree of aggregation. It is possible that this discrepancy results from the smaller molecular size of MBP as compared with SP-D (ie, hexameric or smaller forms of MBP predominate; MBP collagen domain approximately one-third the size of those of SP-D or conglutinin). In any case, these data also support the conclusion that collectin-induced viral aggregation contributes to protection against neutrophil deactivation.

The importance of collectin-induced IAV aggregation was also underscored by our studies of the comparative mechanisms through which collectins and anti-HA IgG alter IAV binding to and activation of neutrophils. Opsonization with IgG altered the mechanism of IAV binding in that binding of antibody-IAV complexes was partially or fully insensitive to neuraminidase treatment of neutrophils and partially inhibitable by blockade of FcRII receptors. After preincubation of FITC-labeled IAV with lower concentrations of antibody (ie, those that did not fully inhibit viral HA activity), FITC-labeled binding to neutrophils was modestly enhanced, whereas at the higher antibody concentrations binding was...
ha molecules on the surface of collectin-IAV aggregates involved engagement of Fc receptors and alteration of the stoichiometry of the resulting respiratory burst such that IgG and the wide separation of CRD domains, it is plausible gates may be available for binding. The average span of the collectins to elicit O2 production will require further testing. We acknowledge the assistance of Dr Ken Zaner in performing the fluorescent microscopy experiments.

REFERENCES

10. Mufson MA, Chag V, Gill V, Wood SC, Romansky MJ,
NEUTROPHIL DEACTIVATION BY INFLUENZA A VIRUS

45. Abramson JS, Hudnor HR: Role of the sialoglycoprotein (CD43) receptor in mediating influenza A virus-induced polymorphonuclear leukocyte dysfunction. Blood 85:1615, 1995
Neutrophil deactivation by influenza A viruses: mechanisms of protection after viral opsonization with collectins and hemagglutination-inhibiting antibodies

KL Hartshorn, KB Reid, MR White, JC Jensenius, SM Morris, AI Tauber and E Crouch