Apolipoprotein A-I decreases neutrophil degranulation and superoxide production

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Abstract Neutrophils participate in the acute phase response and are often associated with tissue injury in a number of inflammatory disorders. The acute phase response is accompanied by alterations in the metabolism of apolipoprotein A-I and high density lipoprotein (HDL). Structural considerations led to studies investigating the effect of purified HDL and apolipoprotein A-I on neutrophil degranulation and superoxide production. Apolipoprotein A-I but not HDL inhibited IgG-induced neutrophil activation by about 60% as measured by degranulation and superoxide production. This suggests that the lipid-associating amphipathic helical domains of apolipoprotein A-I mediate this effect. In support of this was finding inhibitory effects with two synthetic model lipid-associating amphipathic helix peptide analogs. Apolipoprotein A-I, containing tandem repeating amphipathic helical domains, was approximately ten times more effective than the two peptide analogs and inhibited neutrophil activation at well below physiologic concentrations. Competitive binding studies indicate that resting neutrophils have approximately 190,000 (K_d = 1.7 × 10^{-7}) binding sites per cell for apolipoprotein A-I, consistent with a ligand-receptor interaction. These observations suggest that apolipoprotein A-I may play an important role in regulating neutrophil function during the inflammatory response.—**Blackburn, W. D., Jr., J. G. Dohlman, Y. V. Venkatachalapathi, D. J. Pillion, W. J. Koopman, J. P. Segrest, and G. M. Anantharamaiah.** Apolipoprotein A-I decreases neutrophil degranulation and superoxide production. *J. Lipid Res.* 1991. **32:** 1911–1918.

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During the acute phase response there are profound changes in the structure and metabolism of high density lipoproteins (HDL) (1–12). The biological consequences of these dramatic changes are unknown. One major alteration in the composition of circulating acute phase HDL is the incorporation of serum amyloid A and depletion of apolipoprotein (apo) A-I (12). In vitro studies have shown that incubation of HDL with serum amyloid A displaces apoA-I from the HDL particle (6, 13). The fate of the displaced apoA-I is yet to be determined. However, incubation of acute phase HDL with neutrophils results in association of apoA-I with the neutrophil membrane (12). The functional consequence of this interaction has not been previously investigated, but it raises the possibility that during the acute phase response apoA-I may be liberated from HDL and become free to interact with inflammatory cells such as neutrophils, thereby altering their function.

ApoA-I contains amphipathic α-helical domains that have an affinity for lipid and therefore might interact with cell membranes (14, 15). ApoA-I is responsible for much of the structural and functional properties attributed to HDL. It consists of a single peptide chain of 243 amino acids. The carboxyl end consists of a 200-amino acid sequence of 22mer tandem repeating amphipathic helixes (15). The polar–nonpolar faces of the helical segments are aligned and the amphipathic α-helical segments are joined by prolyl residues, potentially making them more amenable to cellular interaction (15, 16). The amphipathic α-helical domains of apoA-I, like the helical domains of other apolipoproteins, are unique in their clustering of acidic amino acids at the center of the polar face and positively charged residues at the polar–nonpolar interface (16). The basic residues at the interface, when associated with phospholipid, may extend toward and nearly perpendicular to the polar face of the helix and insert the charged residues into aqueous milieu for aqueous solvation (16, 17). Similarities in the amphipathic helical domains of apoA-I and peptides, known to interact with cells (16–19), led to studies to determine whether apoA-I interacted with neutrophils and regulated their function. These studies were undertaken to test the hypothesis that free apoA-I, possibly through its lipid-associating domains, would diminish certain neutrophil biological responses.

Abbreviations: HDL, high density lipoprotein; FMLP, formyl-methionyl-leucyl-phenylalanine; PMA, phorbol myristate acetate; apo, apolipoprotein.

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METHODS

General methods

Purified human neutrophils at concentrations specified as described were subsequently incubated with human HDL, human apoA-I, or test peptides. After incubation, cells were then incubated in microtiter wells. The neutrophils were activated by the addition of FMLP or simply incubation in the wells that had been previously coated with IgG. After the cells were activated, the cell-free supernatants were assayed for the particular neutrophil product in question.

HDL and apoA-I purification

HDL was isolated by density gradient centrifugation according to previously described procedures (20). The density of HDL was between 1.06 and 1.21 g/ml. HDL_2 and HDL_3 were also isolated by density gradient centrifugation with densities of between 1.06 and 1.125 and between 1.125 and 1.21 gm/ml, respectively. Differences in the HDL subclasses were confirmed by analysis of each sample using non-denaturing PAGE (data not shown). ApoA-I was purified by reversed phase HPLC (20). The synthesis and physicochemical characterization of the amphipathic peptide analogues used in these studies have been previously described (21–23).

Neutrophil functional studies

Blood was drawn from normal human volunteers into a heparinized syringe containing 100 units of heparin per ml of blood, and neutrophils were purified as previously described (24). Briefly, the majority of the erythrocytes were removed by sedimenting the blood with 4.5% dextran for 20 min at room temperature. The leukocyte-rich upper layer was removed, washed three times in phosphate-buffered saline (PBS), and layered over Hypaque-Ficoll in a conical centrifuge tube. After centrifugation for 30 min at 300 *g*, the neutrophil-rich pellet was washed three times with PBS and residual erythrocytes were removed by hypotonic lysis. Cells were resuspended in HBSS and counted on a hemocytometer. In each experiment, greater than 95% (usually greater than 98%) of cells as determined morphologically were neutrophils. Purified normal human neutrophils were incubated for 15 min with either apoA-I or test peptides at 37°C. Two hundred microliters of the cell suspensions (1.25×10^6 cells/ml) was then either added to microtiter wells (96-well flat-bottom plates, Dynatech Laboratories, Chantilly, VA) that had been precoated with human IgG (0.1–0.5 μg/well) or incubated with FMLP. Superoxide production was measured by determining spectrophotometrically the superoxide dismutase inhibitable reduction of cytochrome C (25). After a 1-h incubation at 37°C, the cell suspension was aspirated, the cells were removed by centrifugation, and the cell-free supernatants were assayed for lactoferrin by radioimmunoassay (24). ApoA-I, HDL, and test peptides did not directly alter the sensitivity of the lactoferrin RIA when added to standard curves or the superoxide colorimetric assay.

ApoA-I depletion from serum

ApoA-I was depleted from normal heat-inactivated human serum by passage over a Sepharose column to which a polyclonal antibody (Chemicon, El Segundo, CA) with specificity to apoA-I had been coupled. The antibody was coupled to cyanogen bromide-activated Sepharose 4B according to the methods described in the package insert (Pharmacia Fine Chemicals). Depletion of apoA-I was assured by immunodotting the treated sera. The control sera was also placed over a Sepharose column to which anti-BSA had been coupled. Immunodotting confirmed that apoA-I was not removed from this serum.

Preparation of liposomes from neutrophil lipids

Neutrophil lipids were extracted with chloroform–methanol 2:1 and then sonicated in the presence of carboxyfluorescein dye. Dye-entrapped liposomes were separated from free dye by gel filtration using Sepharose CL-4B equilibrated with Tris-HCl, pH 7.4. Peptides were incubated with liposomes at a 1:6 (w/w) ratio. The concentration of the peptide in the mixture was 50 μg/ml. Interaction with liposomes was measured as a function of release of dye as detected by the increase in fluorescence intensity at 525 nm with an excitation frequency of 498 nm (26).

ApoA-I binding studies

Human neutrophils (2×10^6/334 μl), were incubated with 13 ng (39,000 cpm) ^{125}I-labeled apoA-I in 0.33 ml of phosphate-buffered saline, pH 7.4, containing 0.1% bovine serum albumin with or without with addition of unlabeled apoA-I. ApoA-I was labeled with ^{125}I using the lactoperoxidase method as previously described (27). Unlabeled peptides were added at the concentrations indicated. ApoA-I was insoluble at concentrations above 10^{-5} M. Samples were incubated for 1 h at 4°C and the reaction was stopped by centrifugation at 12,000 *g* for 3 min through a layer of oil (dibutyl-phthalate–dinonyl-phthalate 5:1). Radioactivity bound to the neutrophil pellet was counted after aspiration of the supernatant solution and the oil layer, followed by cutting the tip of the microfuge tube to minimize contamination with ^{125}I-labeled apoA-I bound to the tube. No significant radioactivity was noted in the oil layer indicating that apoA-I was not dissolving in the oil. Each data point was run in triplicate. The displacement curve data is expressed as percent maximal binding of ^{125}I-labeled apoA-I. Maximal binding ranged between 180 to 250 pg ^{125}I-labeled apoA-I per 2×10^6 cells. A Scatchard analysis was performed based on a representative displacement experiment by determining the ratio of bound to free apoA-I and plotting these data versus molar apoA-I bound using the program LIGAND (28).

RESULTS

Neutrophils were incubated with 10^{-6} M apoA-I for 15 min followed by incubation in wells coated with human IgG. Degranulation and superoxide production in response to surface-associated IgG were decreased by prior incubation with apoA-I (**Fig. 1**). In contrast, preincubation of cells with HDL did not decrease the response (Fig. 1). Studies were performed initially with HDL_2 and HDL_3 and neither decreased degranulation or superoxide production (data not shown); therefore, subsequent studies were performed with total HDL. Neither HDL nor apoA-I appeared to be toxic to the cell since neither increased the release of cytoplasmic lactate dehydrogenase nor decreased the ability of cells to exclude trypan blue (data not shown). To further determine whether apoA-I or HDL affected neutrophil viability, chemotaxis studies were performed. In a total of eight experiments no differences were seen when cells had been preincubated with apoA-I or HDL. In response to 10^{-8} M formyl-methionyl-leucyl-phenylalanine (FMLP), 157 ± 18 cells responded whereas when cells were incubated with apoA-I and HDL, 160 ± 18 and 140 ± 22, respectively, responded.

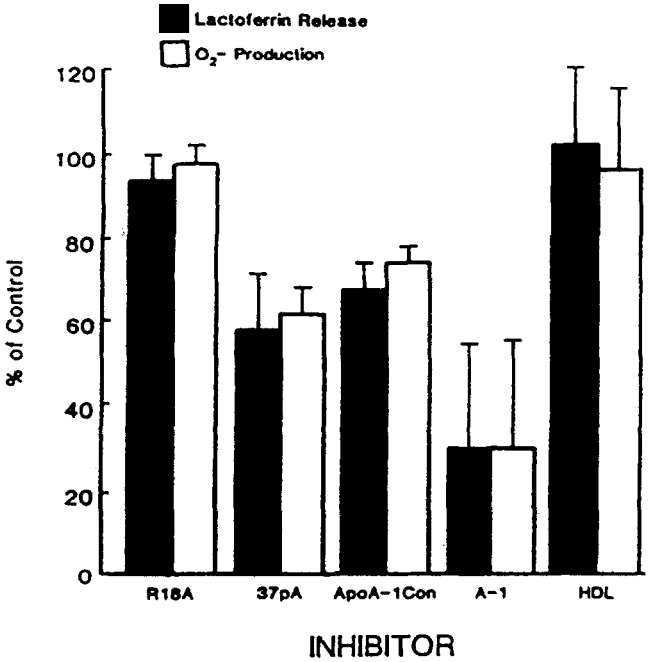

Fig. 1. The effect of HDL, apoA-I, and model amphipathic peptides on surface-bound IgG-mediated neutrophil activation: the effect of 10^{-6} M HDL, apoA-I, or peptide (n=8). Purified human neutrophils from normal donors were incubated for 15 min with either apoA-I or test peptides at 37°C. Cell suspensions were then added to microtiter wells that had been precoated with human IgG. After a 10-min incubation at 37°C, cell-free supernatants were assessed for superoxide production. Degranulation was measured after a 1-h incubation at 37°C by solid phase radioimmunoassay, of the cell-free supernatants, for lactoferrin (24). Each point represents the mean and one standard deviation of at least six observations. Degranulation and superoxide production were statistically different from controls ($P < 0.05$) for cells incubated with 37pA, apoA-I_{con}, and apoA-I.

Previous physicochemical studies of synthetic peptide analogs of apoA-I have shown that the lipid-associating regions are composed of amphipathic α-helixes. To further explore the importance of the amphipathic α-helical lipid-associating domains of apoA-I in mediating neutrophil activation, three synthetic peptide analogs with amphipathic α-helical structures and differing lipid affinities (23) were studied for their effects on neutrophil function. A helical wheel representation of their structures is shown in **Fig. 2.** Peptide 18A is a model peptide in which the charged residue positions were arranged to mimic the apolipoprotein class amphipathic helixes (29). This peptide has positively charged residues at the polar–nonpolar interface and negatively charged residues at the center of the polar face. Peptide 37pA is a proline-linked dimer of an 18mer amphipathic helix (18A-Pro-18A) that mimics apoA-I in some of its properties (21, 22). Reversal of the location of the positively and negatively charged residues in 18A, such that the basic amino acid residues are no longer at the polar–nonpolar interface, produces a peptide with relatively weak lipid affinity, designated R18A (21, 22). A third peptide, apoA-I_{con}, is a consensus sequence of the multiple lipid-associating amphipathic helical repeats of apoA-I and, therefore, is a close mimic of the native protein. This sequence was constructed by the selection of the most prevalent amino acid residues in each position of the different tandem repeating 22mer units of apoA-I. Most importantly, this analog has a glutamic acid residue at position 13 that is critical for LCAT activity (23). Because Glu-13 interrupts the helix hydrophobic face, this peptide has only intermediate affinity for lipid (23). ApoA-I has greater lipid affinity than apoA-I_{con} presumably because of cooperativity of its multiple amphipathic α-helical domains, most of which do not contain a Glu-13. Previous studies have shown that the rank order of affinity of the three amphipathic helix analog peptides for egg lecithin is 37pA > apoA-I_{con} > > R18A (23).

To determine whether these peptides associated with neutrophil lipid, lipids were extracted from whole neutrophils as described and fluorescein-entrapped liposomes were prepared. As demonstrated in **Fig. 3,** there was a marked difference in the ability of the synthetic peptides to release fluorescein from liposomes composed of neutrophil lipid.

It was next determined whether these synthetic peptides altered neutrophil function. Neutrophils were incubated for 15 min with the three peptides followed by incubation in wells coated with IgG. Degranulation, as measured by lactoferrin release, was diminished by preincubation with apoA-I_{con} and 37pA (**Fig. 4**). These two analogs were also able to decrease IgG-induced superoxide generation (Fig. 1). In contrast, R18A had no effect in either assay at the same concentration (Fig. 1). The effects of apoA-I_{con} and 37pA were dose-dependent (Fig. 4).

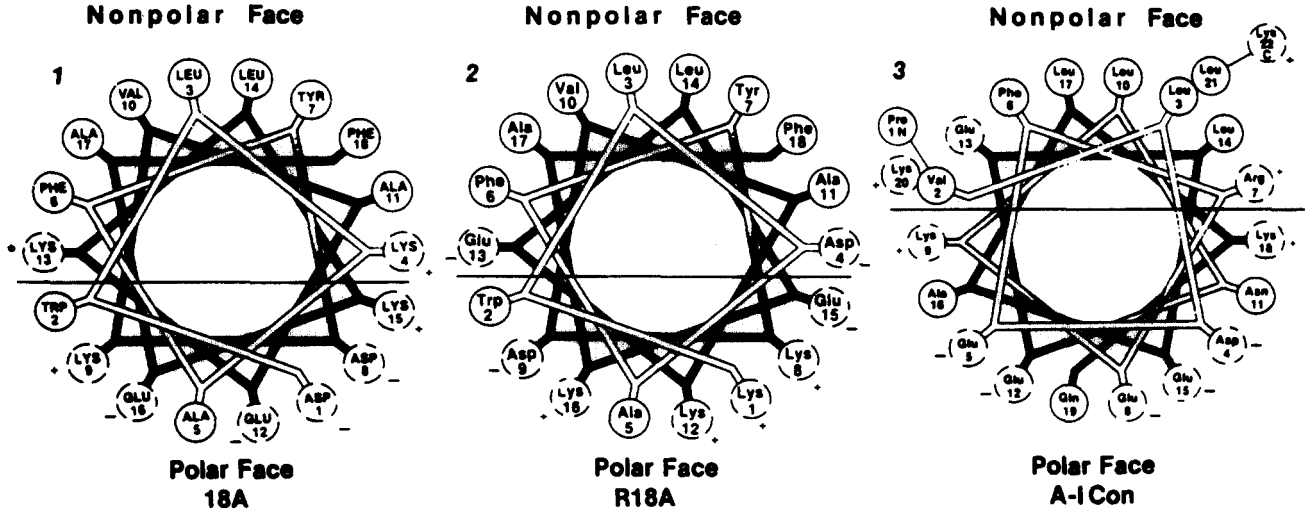

Fig. 2. Helical wheel representations of synthetic amphipathic peptide analogs. 1. Sequence of peptide 18A: Asp Trp Leu Lys Ala · Phe Tyr Asp Lys Val · Ala Glu Lys Leu Lys · Glu Ala Phe. Peptide 37pA is 18A-Pro-18A. 2. Sequence of peptide R18A: Lys Trp Leu Asp Ala · Phe Tyr Lys Asp Val · Ala Lys Glu Leu Glu · Lys Ala Phe. 3. Sequence of peptide apoA-I_{con}: Pro Val Leu Asp Glu · Phe Arg Glu Lys Leu · Asn Glu Glu Leu Glu · Glu Leu Lys Gln Lys · Leu Lys. *Dotted circles represent charged amino acids. Note the position of a glutamic acid residue interrupting the* nonpolar face of apoA-I_{con}. The figure also demonstrates the difference between 18A and R18A being the reversal of the position of the lysine residues which are positioned in 18A at polar–nonpolar interface and contribute to the hydrophobicity and higher lipid affinity of the hydrophobic face of 18A in comparison to R18A.

Maximal decrease of degranulation was seen at 10^{-6} M. Measurable decreases in degranulation were observed at concentrations as low as 5×10^{-8} M of apoA-I_{con}.

Preincubation of neutrophils with each of these peptides alone did not result in a detectable increased production of superoxide or release of lactoferrin when compared to control cells (data not shown). The ability of cells to exclude trypan blue and the release of lactate dehydrogenase were not altered by incubation with these peptides. Similar to the results reported above with apoA-I and HDL, chemotaxis through a nitrocellulose membrane in response to 10^{-8} M FMLP was not inhibited by the synthetic peptides (data not shown).

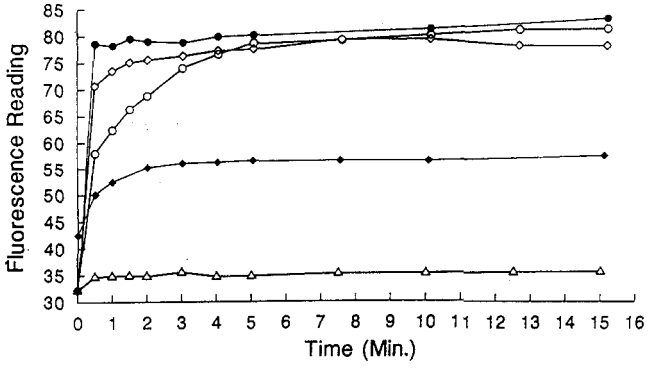

Fig. 3. The effect of apoA-I and peptide analogs of the amphipathic helix on dye leakage from neutrophil liposomes. Purified human neutrophils were extracted with chloroform–methanol 2:1 and the resultant lipids were sonicated in the presence of carboxyfluorescein dye to create dye-entrapped liposomes. The ability of peptides to interact with the liposomes was measured in terms of dye leakage, measured by fluorescence intensity at 525 nm. Symbols indicate the following compounds: solid circles, Triton X-100; open diamonds, 37pA; open circles, apoA-I; closed diamonds, apoA-I_{con}; and open triangles, R18A.

To determine whether the effects of these amphipathic peptides were restricted to F_c receptor-mediated activation alone, neutrophils were preincubated with peptides (10^{-6} M) and then stimulated with the soluble chemotactic peptide FMLP (10^{-8} M). ApoA-I inhibited FMLP-stimulated neutrophil degranulation (**Fig. 5**). Furthermore, inhibition of 30% and 36%, respectively, was noted by 37pA and apoA-I_{con}, but not by the peptide R18A. These results suggest that the inhibitory effects of apoA-I and peptide analogs were not due to peptide binding to and blocking of a specific receptor for neutrophil activation.

Because FMLP and portions of IgG are hydrophobic, it was important to determine whether the inhibitory effect of apoA-I was due to its direct binding of these agents rather than to a cell surface effect. ^{125}I-labeled apoA-I (100,000 cpm) was added to the standard microtiter plates that had been coated with human IgG and blocked with BSA or only coated with BSA. The wells were washed and bound radioactivity was determined. There was no excess radiolabeled apoA-I bound to the plates when compared to apoA-I bound to the plates not containing IgG. The degree to which apoA-I could associate with FMLP was measured by its ability to prevent FMLP leakage through a dialysis membrane (1000 mol wt cutoff). The quantity of FMLP remaining in the dialysis bag containing apoA-I was not different from the amount left in the bag not containing apoA-I. These data suggest that the inhibitory

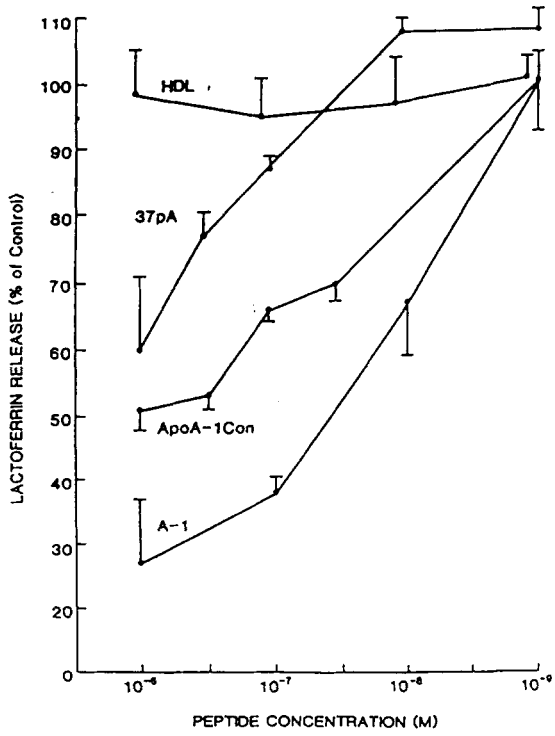

Fig. 4. Dose response of inhibition of apoA-I and model peptides. Purified human neutrophils from normal donors were incubated for 15 min with either apoA-I or test peptides at 37°C at the indicated concentrations. Cell suspensions were then added to microtiter wells that had been precoated with human IgG. Degranulation was measured after a 1-h incubation at 37°C by solid phase radioimmunoassay, of the cell-free supernatants, for lactoferrin. Each point represents the mean and standard deviation of at least six observations. Lactoferrin release was significantly less than control for concentrations as low as 10^{-8} M apoA-I, 5×10^{-8} M of apoA-I_{con}, and 5×10^{-7} M 37pA ($P < 0.05$).

effects of apoA-I are not due simply to its direct binding of the neutrophil activators, FMLP and IgG.

Since FMLP and surface-bound IgG both interact with specific membrane receptors, it was of interest to determine whether a nonreceptor-dependent neutrophil stimulus was also affected by apoA-I. Neutrophils were preincubated with or without 10^{-6} M apoA-I followed by activation with the phorbol ester PMA (500 μg/ml). Neither superoxide production nor degranulation in response to PMA was affected by apoA-I (data not shown).

Competitive binding studies were undertaken (30) to further define the interaction between apoA-I and 37pA with neutrophils. ^{125}I-labeled apoA-I was incubated with resting neutrophils and the ability of unlabeled apoA-I to displace cell-associated radioactivity was determined. A displacement curve and Scatchard analysis (**Fig. 6**) indicated that there was competitive binding to one class of binding site with K_d of approximately 1.7×10^{-7} M with approximately 190,000 binding sites per resting cell.

To determine the physiological relevance of these studies, serum was depleted of apoA-I by passage over an affinity column. As demonstrated in **Fig. 7**, removal of apoA-I from normal serum actually augmented superoxide production by neutrophils activated in the presence of surface-bound IgG by 55%.

DISCUSSION

Neutrophils actively participate in the inflammation and tissue destruction characteristic of the acute phase response (31–33). Molecules such as tumor necrosis factor, interferon-γ, and granulocyte/macrophage-colony stimulating factor (GMCSF) "prime" the neutrophil, thereby augmenting cellular responses to known activators (34–38). In contrast to many biological systems, little is known regarding factors that negatively regulate neutrophil function. However, neutrophils obtained from some inflammatory sites do not respond to well-characterized stimuli as well as cells isolated from noninflammatory sites (39–41). One explanation for such observations is that during the acute phase response there are factors to which the cells are exposed that decrease neutrophil function.

Our observations demonstrate that apoA-I significantly diminishes neutrophil degranulation and superoxide production in response to surface-bound IgG and FMLP. In contrast, preincubation with HDL purified from normal donors resulted in no decrease. The lipid-associating do-

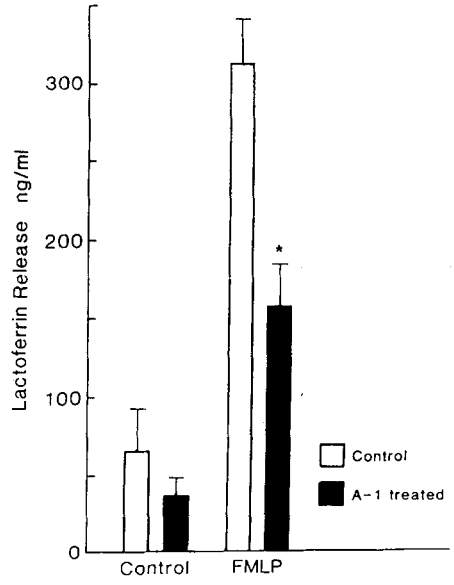

Fig. 5. Effect of apoA-I on FMLP-induced neutrophil degranulation. Normal human neutrophils were incubated with 10^{-7} M apoA-I for 15 min at 37°C and then incubated with 10^{-8} M FMLP. After 1 h incubation, cell-free supernatants were assayed for lactoferrin content by radioimmunoassay. Each bar represents the mean and standard deviation of six observations; *indicates a difference relative to control with a $P < 0.05$.

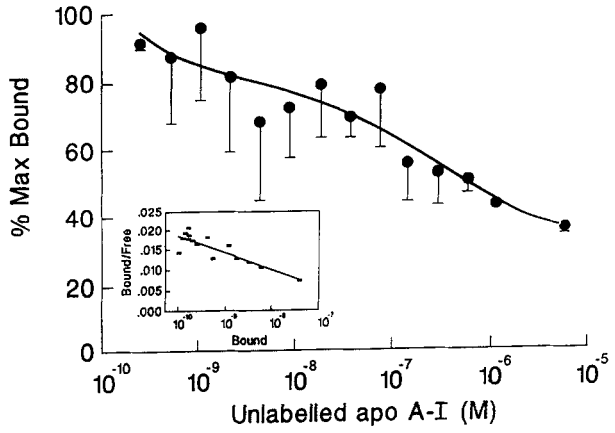

Fig. 6. Binding of ^{125}I-labeled apoA-I to resting human neutrophils. Human neutrophils were incubated with ^{125}I-labeled apoA-I and competing unlabeled apoA-I. Radioactivity bound to the neutrophil was quantitated. Each data point was run in triplicate. The displacement curve data are expressed as percent maximal binding of ^{125}I-labeled apoA-I and represent the mean ± 1 SD of three experiments. Insert: Scatchard analysis of a representative displacement experiment using the LIGAND program (27).

mains of apoA-I, which have amphipathic helical structural features, may be buried when apoA-I is in the presence in HDL. This suggested that this region might be responsible for the inhibitory effect on neutrophil function. This possibility was explored by testing three amphipathic helical peptides with differing lipid affinities for their ability to cause a similar inhibition of neutrophil activation. The finding that the two peptides with strong lipid affinity, but not the peptide with weak lipid affinity, inhibited cell activation supports this hypothesis. ApoA-I has been postulated to contain eight tandem 22mer amphipathic helical domains (23). Each tandem repeat is similar to the 22mer of peptide apoA-I_{con}. The fact that apoA-I at 10^{-8} M was as effective as 10^{-7} M of the consensus lipid-binding peptide analog apoA-I_{con} suggests a cooperative effect of the multiple amphipathic helical domains of apoA-I.

These observations suggest a further correlation between lipid affinity and decrease in neutrophil function. A similar correlation has been noted with the lipid affinities of these peptides and the release of lactogen from placenta (42). This correlation with neutrophils is not precise, however, because apoA-I_{con} was at least as effective as 37pA in spite of its significantly lesser lipid affinity. This observation may indicate that more complex factors than simply lipid interaction are responsible for the alterations in neutrophil function. The glutamic acid residue at position 13 of apoA-I_{con}, which interrupts the hydrophobic face of the helix and thus lowers lipid affinity, may be particularly important for effects on cell function. Although glutamic acid residues are found at the thirteenth position in only two out of eight 22mer repeating units of apoA-I, they have been postulated to be critical for LCAT activation (23).

An initial concern was that the noted decrease in the neutrophil response was due to cellular toxicity. However, this possibility was excluded by the observation that release of cytoplasmic lactate dehydrogenase and ability of the cells to exclude trypan blue was not affected by either apoA-I or the peptide analogs. In addition, chemotaxis was not decreased by these peptides or apoA-I. Furthermore, previous studies using human placenta have also demonstrated that these peptides and apoA-I at equivalent concentrations are not cellular toxins (42).

Our binding studies are compatible with a receptor-mediated mechanism of diminished neutrophil function. In addition, the correlation between the K_d of 1.7×10^{-7} M and the concentration eliciting 50% of the maximum decreased degranulation (10^{-8} M) is supportive of a receptor-mediated effect. ApoA-I has been reported to bind to the putative HDL receptor ($K_d = 2 \times 10^{-8}$ to 5×10^{-8}) (43–45). The presence of an HDL receptor has been described for macrophages and it is possible that we have detected an apoA-I receptor in neutrophils. In those systems where it has been studied, HDL and apoA-I appear to compete for the same receptor (43, 44). The lack of neutrophil inhibition by HDL itself raises the possibility that interaction with an apoA-I/HDL receptor may occur under the conditions of the binding study, but inhibition might be due to apoA-I interacting with other sites on activated cells, possibly through a nonreceptor-mediated mechanism. Interestingly, since HDL did not diminish the neutrophil functions studied, it would appear that with the HDL examined in our studies, there is not adequate exchange of apoA-I with the neutrophil

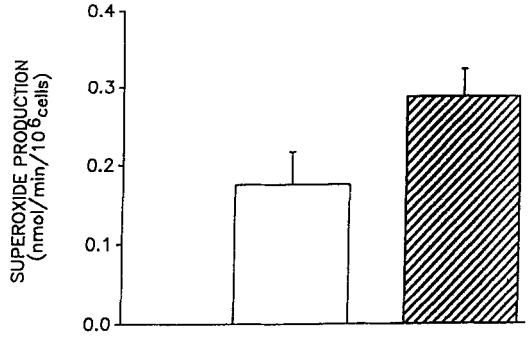

Fig. 7. The effect of depletion of serum apoA-I on IgG-induced neutrophil superoxide production. Heat-inactivated pooled human serum was depleted of apoA-I by filtering over a Sepharose column to which a polyclonal antibody with specificity for apoA-I was coupled. The ability of this serum to inhibit surface-bound IgG-induced neutrophil superoxide production was compared to that of control serum containing normal amounts of apoA-I. Data are expressed as the mean and one standard deviation from three experiments. Cells incubated with apoA-I depleted sera produced more superoxide radicals than control sera ($P < 0.05$).

membrane to cause a measurable difference in function. This would indicate that the equilibrium in our system would be in favor of apoA-I association with HDL.

Since apoA-I decreased neutrophil activation by surface-bound IgG and FMLP, but did not decrease PMA-induced activation, it suggests that apoA-I interferes with the initial steps in the signal transduction pathway.

The recent observation that at least 4% of plasma apoA-I (approximately 62 μg/ml) is not HDL-bound makes it easier to envision a cellular interaction via the normally lipid-occupied amphipathic helical domains (46). Indirect experimental support for an ability of apoA-I to bind cell lipids also exists, based on the demonstration that plasma apoA-I separate from HDL is most active in picking up cellular cholesterol as part of reverse cholesterol transport (47). The ability of the acute phase apolipoprotein serum amyloid A to displace apoA-I from HDL in vitro (6, 13) also suggests that free apoA-I might be available to exert its effect on nearby cells in vitro. Our observation that sera depleted of apoA-I augmented IgG-induced superoxide production when compared with normal serum suggests that levels of the exchangeable apoA-I/HDL could influence the magnitude of neutrophil responses in vivo.

In summary, we have demonstrated a property of apoA-I, diminution of IgG-mediated neutrophil degranulation and superoxide production. The importance of the lipid binding domains of apoA-I is suggested by the lack of inhibition seen when apoA-I is bound to HDL. This has been supported by the effects observed using lipid-associating synthetic peptide analogs of the amphipathic helix. This effect, however, does not appear to be a simple function of lipid affinity. The results of competitive binding studies of apoA-I are mostly in agreement with a ligand-receptor interaction.

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