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IgE Cross-linking Critically Impairs Human Monocyte Function by Blocking Phagocytosis

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Abstract

Background—IgE cross-linking triggers many cellular processes that drive allergic disease. While the role of IgE in mediating allergic responses is best described on basophils and mast cells, expression of the high-affinity IgE receptor on other innate immune cells, including monocytes, suggests that it may impact the function of these cells in allergic environments.

Objectives—To determine the effect of IgE cross-linking on the function of human monocytes.

Methods—Monocytes purified from healthy donor blood samples were cultured for 4–96 hr with media alone, a cross-linking anti-IgE antibody, or control IgG. Surface CD14 and CD64 expression and secreted cytokine concentrations were determined. Monocyte function was determined by assessing: 1) phagocytosis of E. coli or apoptotic HEp2 cells and 2) killing of intracellular E. coli. Select experiments were performed on monocytes obtained from participants with elevated versus normal serum IgE concentrations.

Results—IgE cross-linking on monocytes increased CD14 expression and induced secretion of TNF-á, IL-6, and autoregulatory IL-10. These effects were greatest in individuals with elevated serum IgE concentrations. In contrast, IgE cross-linking reduced CD64 expression and significantly impaired phagocytic function without disrupting the capacity of monocytes to kill bacteria.

Conclusion—IgE cross-linking drives monocyte pro-inflammatory processes and autoregulatory IL-10 in a serum IgE-dependent manner. In contrast, monocyte phagocytic function is critically impaired by IgE cross-linking. Our findings suggest that IgE cross-linking on monocytes may contribute to allergic disease by both enhancing detrimental inflammatory responses and concomitantly crippling phagocytosis, a primary mechanism utilized by these cells to resolve inflammation.

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Keywords

Monocyte; IgE; FcεRI; IgE cross-linking; Allergy; Pro-inflammatory; Autoregulatory; Phagocytosis; Apoptotic debris

Introduction

Despite the significant healthcare burden of atopic disease in the United States[1–3], the mechanisms underlying pathogenesis are incompletely understood. IgE plays a critical role in mediating atopic disease: significant correlations between serum IgE concentration and disease have been demonstrated in allergic asthma and atopic dermatitis[4, 5]. Indeed, serum IgE concentration represents a diagnostic criterion for these conditions[6, 7]. Therapies that reduce serum IgE concentration, such as omalizumab, result in clinical improvement in patients with severe atopic disease[8, 9].

IgE exerts its effect on atopic disease via the high-affinity IgE receptor, FcεRI[10]. Upon cross-linking of allergen-specific IgE by a multivalent allergen the receptor is activated, resulting in intracellular signaling and cell-type specific effects[10]. Surface FcεRI expression on several immune cells, including basophils and dendritic cells, is increased in individuals with atopic disease and correlates with serum IgE concentration[11, 12].

FcεRI mediates IgE-dependent pathways in several cell types, and its role is best characterized in basophils and mast cells. In these cells, IgE cross-linking induces release of inflammatory mediators including histamine, prostaglandins, and cytokines[13, 14]. FcεRI plays an important role on myeloid and plasmacytoid dendritic cells (mDC and pDC, respectively) as well[12]; IgE cross-linking on these cells induces pro-inflammatory cytokine secretion[15, 16]. In pDCs, IgE- and toll-like receptor(TLR)9-mediated pathways have been shown to oppose one other[16]; allergic stimulation via this pathway also interferes with *in vitro* pDC antiviral responses[17].

FcεRI is also expressed on monocytes and is increased in individuals with atopic diseases[11, 18, 19]. Present in high numbers at mucosal surfaces and in the skin both during steady state and inflammatory conditions, such as allergen exposure, monocytes and their progeny are poised to influence allergic responses[20–25]. Monocytes play many important roles during inflammatory processes, including regulating immune responses through the release of cytokines[26], and resolving inflammation though phagocytosis of cellular debris[27–29]. Expression of specific surface molecules can also reflect functional properties of monocytes. CD14 contributes to TLR4 signaling and is thus important for immune responses to lipopolysaccharide (LPS)[30]. CD64, the high affinity IgG receptor, contributes to phagocytosis; its expression reflects monocyte phagocytic function[31, 32].

Despite the expression of FcεRI on monocytes from both atopic and non-atopic individuals[11, 18, 19] and the importance of these cells in inflammatory processes, the consequences of FcεRI activation on monocytes remain incompletely characterized. Stimulation of FcεRI has been shown to induce activation of NF-kB and secretion of TNFα, IL-6, and MCP-1 in human monocytes[33, 34]. In addition, FcεRI cross-linking of GM-CSF and IL-4 treated monocytes *in vitro* has been shown to promote IL-10 secretion and differentiation into macrophages[35].

We set out to define the impact of IgE cross-linking on the function of human monocytes and to determine whether serum IgE concentration impacts the magnitude of these responses. Monocytes, by virtue of their expression of FcεRI, inflammatory capacity, and

prevalence in mucosal tissues, have the potential to significantly influence allergic inflammation. Determining how IgE cross-linking impacts monocyte function will lead to a better understanding of the role of this important cell type in allergic processes and may reveal critical pathways that contribute to the pathogenesis of allergic disease.

Methods

Monocyte Purification

Leukocyte-enriched blood samples were obtained from a local blood bank and diluted 1:1 (vol/vol) with PBS (GIBCO, Grand Island, NY; supplemented with 2% heat-inactivated FCS and 2 mM EDTA). For select experiments, blood was drawn from human donors into tubes containing acid citrate dextrose. Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation with Ficoll-Paque (GE Healthcare, Uppsala, Sweden) and monocytes were purified using the EasySep Negative Selection Human Monocyte Enrichment Kit (Stemcell Technologies, Vancouver, Canada). Purity ranged from 85%–95%.

Monocyte culture

Isolated monocytes were cultured in complete RPMI 1640 media (GIBCO; supplemented with 10% heat-inactivated FCS, 1% penicillin-streptomycin, 1% Na pyruvate, 1% glutamate, 1% HEPES buffer solution, 1% non-essential amino acids, and 100 mM β-mercaptoethanol) at a concentration of 1×10^6 monocytes/ml. Rabbit anti-human-IgE (αIgE) or rabbit IgG (IgG) (1 or 10 μg/ml; Bethyl Laboratories, Montgomery, TX) was added to monocyte cultures as indicated. For select experiments, F(ab)'$_2$ fragments derived from αIgE and IgG antibodies (GenScript, Piscataway, NJ) were added at 10 μg/ml. For cytokine neutralization experiments, mouse anti-human-IL-10, -IL-6, -TNFα, -IL-10Rα, -IL-6R, -TNFRI or IgG1 or IgG2b isotype controls (R&D Systems, Minneapolis, MN) were added to monocyte cultures at 10 μg/ml (anti-TNFα, IgG1) or 5 μg/ml (others). Time points reflect distinct cultures for indicated times, with no removal or replacement of media or antibodies.

Flow cytometry

The following fluorochrome-conjugated anti-human antibodies were used: CD14-V450, CD64-FITC, CD64-PE, FcεRI-PE (BD Biosciences, San Diego, CA). Cells were rinsed with PBS and stored in Streck Cell Preservative (Streck, Omaha, NE) at 4° C prior to staining. Preserved samples we re washed, resuspended in 100 μl PBS and incubated with 2.5 μl of each antibody for 30 min at 4° C. Cells were then washed and resuspended in 1% paraformaldehyde. Samples were subsequently acquired on a BD LSR II flow cytometer (BD Biosciences, San Diego, CA) and analyzed with FlowJo software (Tree Star, Ashland, OR). Mean fluorescence intensity for $CD14^+$ cells was determined and subsequently converted to mean equivalent standard fluorescence (MESF), using Ultra Rainbow Calibration Particles (Spherotech, Lake Forest, IL) and FlowJo.

Cytokine analysis

Supernatants were harvested and stored at −80° Cels ius (C) until use. Concentrations of TNFα and IL-10 in monocyte culture supernatants were measured by ELISA using Legend Max Human ELISA kits (BioLegend, San Diego, CA). IL-6 concentration was determined using READY-SET-GO! Human IL-6 ELISA kit (eBioscience, San Diego, CA).

Phagocytosis assays

BODIPY FL-conjugated E. coli BioParticles (Molecular Probes, Eugene, OR) were opsonized with E. coli Opsonizing Reagent (Molecular Probes) according to manufacturer

instructions, added to monocyte cultures at 10 bacteria/monocyte and incubated at 37° C for 2 hr. For microscopy, mo nocytes were washed and mounted onto slides with a Cytospin 4 Centrifuge (Thermo Scientific, Waltham, MA). Slides were fixed with methanol and coverslipped with Vectashield Mounting Medium with DAPI (Vector Laboratories, Burlingame, CA). Images were acquired on a Deltavision Deconvolution Microscope (Applied Precision, Issaquah, WA) for 50 cells per sample. Internalized particles were counted in ImageJ using a macro written by D Pyle. For flow cytometry, monocytes were washed, stained for CD14, and acquired on the LSR II.

For selected experiments, HEp2 cells were grown in complete DMEM media (GIBCO; supplemented as above). Cells were incubated with 5 μM Carboxyfluorescein Succinimidyl Ester (CFSE) (Molecular Probes) for 10 minutes, washed extensively with media, and incubated for 24 hr with 1 μg/ml Actinomycin D (Sigma, St. Louis, MO) to induce apoptosis. Apoptotic cells were washed, opsonized with 100 μg/ml whole human IgG (Bethyl Laboratories, Montgomery, TX) and added to monocyte cultures at a 1:1 ratio. After 4 hr, cells were washed and stained for CD14 for flow cytometry analysis. In some experiments, $CD14^+$ monocytes were sorted on a BD FACSAria flow cytometer (BD Biosciences, San Diego, CA) based on CFSE fluorescence and imaged as above.

Bacterial killing assays

E. coli (DH5α strain, a kind gift from David Farrar) were grown in LB media (Sigma) and added to monocyte cultures at 10 CFU/monocyte for 45 min. Gentamicin (Amresco, Solon, OH) was added (100 μg/ml) and monocytes were harvested immediately (0 hr) or after 16 hr. Monocytes were washed extensively, counted, and lysed in sterile deionized H_2O. Lysates were plated on LB agar (Sigma) overnight and colonies were counted. CFU/Cell was determined for each harvest and % of bacteria killed was calculated as $\%Killed = (CFU/Cell_{0hr} - CFU/Cell_{16hr}) \div CFU/Cell_{0hr} \times 100$. This calculation reflects bacterial killing regardless of the amount of phagocytosis.

Patient recruitment

Individuals with a history of serum IgE concentration >100 U/ml were recruited for select experiments. Individuals with lower IgE were recruited as controls. All participants had positive skin test to ≥1 indoor allergen. Skin tests were performed[36] and serum IgE levels were determined[17] as previously described. This study was approved by the University of Texas Southwestern institutional review board. Written informed consent and assent were obtained.

Data analysis and Statistics

Data are presented as means ± SEM. For all data sets with N>8, Grubb's test for outliers was applied with $\alpha=0.0001$ and outliers were removed from analysis. Where indicated, data were normalized to the media condition for each experiment. For three or more conditions, one-way repeated measures ANOVA and pairwise Tukey's post hoc comparisons were performed for each time point. For comparison of two conditions, paired or unpaired t tests were performed with Holm-Sidak correction where appropriate. For experiments comparing high vs. low IgE individuals, Pearson correlations between experimental results and log of serum IgE concentration were performed. $p<.05$ was considered significant. All statistical analyses were performed with GraphPad Prism versions 5 and 6.

Results

The majority of the assays reported hereafter were performed on monocytes from healthy human blood donors. To establish potential clinical impact, we performed certain assays on

monocytes obtained from participants with either low (<100 U/ml) or high (>100 U/ml) serum IgE levels. Participant information is reported in Table I. The majority of participants in both groups had a history of asthma and all tested positive for at least one environmental allergen by skin test. There was no statistically significant difference in age or demographic characteristics between groups. As we have reported previously for pDCs from individuals with elevated IgE levels[17], monocyte expression of FcεRI was significantly elevated in the high IgE group. Data from these experiments were compared for correlation with serum IgE concentration; these analyses are summarized in Table EI in the Online Repository.

IgE cross-linking alters monocyte surface marker expression

To determine the effect of IgE-mediated stimulation on functionally relevant monocyte surface markers, we analyzed the impact of IgE cross-linking on the surface expression of CD14 and CD64.

IgE cross-linking resulted in significant up regulation of CD14 expression at both time points measured (Fig. 1 A). In contrast, surface expression of CD64 was significantly diminished by IgE cross-linking (Fig. 1 B). An F(ab)'$_2$ fragment of the IgE cross-linking antibody induced similar up regulation of CD14, indicating that the effects of the whole antibody were not mediated by Fcγ receptors (Online Repository Fig. E1 A).

Additionally, CD14 up regulation after IgE cross-linking was greater in individuals with elevated serum IgE (Fig. 1 C) at both time points. Moreover, the expression of CD14 after IgE cross-linking was positively correlated with serum IgE levels (Fig. 1 C) at 96 hr.

IgE cross-linking induces secretion of pro-inflammatory cytokines and autoregulatory IL-10

To determine the temporal patterns and interactions of cytokines induced by IgE cross-linking, we analyzed three cytokines commonly secreted by monocytes: TNFα, IL-6, and IL-10.

TNFα secretion was significantly increased by IgE cross-linking at all time points measured (Fig. 2 A, left). Interestingly, the concentration of TNFα induced by IgE cross-linking was greatest after 4 hr and diminished significantly by 24 hr. This reduction could reflect degradation of TNFα between 4 and 24 hr. IgE cross-linking induced robust IL-6 secretion at 4 and 24 hr; in contrast to TNFα, IL-6 levels were maintained at 48 hr (Fig. 2 A, middle). The F(ab)'$_2$ fragment of the IgE cross-linking antibody induced similar secretion of IL-6 after 48 hr (Online Repository Fig. E1 B), confirming that effects of αIgE on monocytes are not Fcγ-mediated.

IgE cross-linking also induced significant IL-10 secretion (Fig. 2 A, right). Notably, the greatest IL-10 concentrations were observed after 24 hr of IgE cross-linking and corresponded with lower TNFα concentrations. To evaluate a potential regulatory role of IL-10 on TNFα production, we used neutralizing antibodies against both IL-10 and its receptor in the presence of IgE cross-linking. The neutralizing antibodies were chosen such that they did not interfere with cytokine detection by ELISA. IL-10 blockade prevented the reduction in IgE-mediated TNFα secretion over time (Fig. 2 B, left), suggesting an autoregulatory role for IL-10. Interestingly, IL-10 blockade also led to a dramatic increase in IgE-mediated secretion of IL-6, as well as IL-10 itself (Fig. 2 B, middle and right). However, neutralization of TNFα and IL-6 did not affect IL-10 levels (Online Repository Fig. E2), suggesting that the induction of IL-10 by IgE cross-linking is not mediated by TNFα or IL-6.

Interestingly, this autoregulatory IL-10 secretion was increased in monocytes from individuals with elevated serum IgE (Fig. 2 C, left); in fact, IL-10 secretion after IgE cross-linking significantly correlated with serum IgE concentration (Fig. 2 C, right). While the increased autoregulatory IL-10 response in individuals with elevated serum IgE might predict reduced pro-inflammatory cytokine secretion, this was not the case. TNFα secretion upon IgE cross-linking was actually increased and IL-6 secretion was 4-fold higher in participants with elevated IgE (Fig. 2 D).

We next examined the effects of αIgE concentration on IgE-mediated surface marker expression and cytokine secretion. IgE cross-linking induced concentration dependent up regulation of CD14, down regulation of CD64, and secretion of IL-6, and IL-10, while TNFα secretion was similar at both concentrations (Online Repository Fig. E3). Subsequent experiments were performed using 10 μg/ml, as this concentration of anti-IgE induced the maximum effect.

IgE cross-linking impairs monocyte phagocytosis

Given the inflammatory nature of IgE-mediated monocyte cytokine secretion, we next explored the impact of IgE cross-linking on a critical monocyte function: phagocytosis.

Utilizing microscopy to quantitate internalized bacteria, we determined that IgE cross-linking significantly impairs monocyte phagocytosis. Monocytes exposed to IgE cross-linking internalized fewer killed, opsonized bacteria compared to monocytes cultured in control conditions (Fig. 3 A). Quantitation of internalized bacteria revealed a significant reduction in phagocytosis after IgE cross-linking (Fig. 3 B). To extend these findings to multiple time points and assess the role of specific cytokines in phagocytosis, we utilized a higher throughput flow cytometry assay to similarly measure phagocytosis; this revealed a significant reduction in monocyte phagocytosis at both 48 and 96 hr after IgE cross-linking (Fig. 3 C). Interestingly, the impairment of phagocytosis induced by IgE cross-linking was not altered by neutralization of TNFα, IL-6, or IL-10 (Fig. 3 D, Online Repository Fig. E4), suggesting that this effect of IgE cross-linking is independent of IgE-mediated cytokine secretion and not subject to autoregulation by IL-10.

To assess the extent of the functional impairment resulting from IgE cross-linking, we next investigated whether bacterial killing was altered in monocytes that had already engulfed bacteria. By comparing the number of live, internalized bacteria immediately after phagocytosis and after a 16 hr period, we were able to determine the ability of monocytes to kill internalized bacteria even when different numbers of bacteria were initially engulfed. Confirming our findings with killed, opsonized bacteria (Fig. 3 A–C), monocytes exposed to 48 hr of IgE cross-linking internalized fewer live, unopsonized bacteria than monocytes in control conditions (Fig. 3 E, left). Surprisingly, IgE cross-linking did not affect the killing of internalized bacteria after a further 16 hr incubation (Fig. 3 E, right), suggesting that the IgE-mediated functional deficit on monocytes is specific to phagocytosis.

One important function of monocytes and their progeny is the clearing of apoptotic debris after infection or inflammation[27–29]. We next determined the effect of IgE cross-linking on phagocytosis of apoptotic cells. After exposure of monocytes to CFSE-labeled apoptotic cells, monocyte CFSE fluorescence was diminished in the IgE cross-linking condition, indicating diminished phagocytosis of apoptotic cells (Fig. 4 A). Interestingly, two distinct populations of monocytes were observed: $CFSE^{low}$which contained small apoptotic debris (Fig. 4 B,C), and $CFSE^{high}$which contained large apoptotic cell remnants (Fig. 4 B,D). IgE cross-linking significantly reduced monocyte phagocytosis of small debris ($CFSE^{low}$ monocytes; Fig. 4 E) as well as the percentage of monocytes that phagocytosed large

apoptotic cells ($CFSE^{high}$ monocytes; Fig. 4 F). In combination, these two measures reflect diminished phagocytosis of apoptotic cells by monocytes exposed to IgE cross-linking.

Unlike the pro-inflammatory effects of IgE cross-linking, the impairment of monocyte phagocytosis was not dependent on serum IgE concentration. Monocytes from participants with elevated IgE levels showed similar levels of phagocytosis after 48 and 96 hr of IgE cross-linking (Fig. 5 A). Moreover, there was no significant correlation between serum IgE and phagocytosis after IgE cross-linking (Fig. 5 B), again suggesting that IgE-mediated inhibition of phagocytosis is independent of the pro-inflammatory effects of IgE cross-linking.

The effects of IgE cross-linking on monocytes are not mediated by contaminating basophils

Because basophils express high levels of FcεRI and secrete immunomodulatory mediators upon IgE cross-linking[14], we examined possible basophil contamination by determining the percentages of $CD14^-$ $HLA\text{-}DR^-$ $Fc\varepsilon RI^+$ cells in each experiment. While most purified monocyte preparations contained <1% $CD14^-$ $HLA\text{-}DR^-$ $Fc\varepsilon RI^+$ cells, some contained more (1–4.8%). To rule out potential basophil contribution to the above results, we compared the magnitude of IgE-mediated effects to the % $CD14^-$ $HLA\text{-}DR^-$ $Fc\varepsilon RI^+$ cells in each experiment and no relationships were observed (data not shown).

Discussion

In this report, we demonstrate for the first time that IgE cross-linking impairs the function of human monocytes. Despite the inflammatory phenotype induced by IgE cross-linking, the phagocytic function of these cells is concomitantly crippled (Fig. 6). In addition, this study is the first to demonstrate the impact of serum IgE concentration, a biomarker of allergic disease, on the magnitude of IgE-mediated monocyte responses.

IgE-mediated induction of CD14 represents one potential mechanism by which monocytes may contribute to allergic inflammation. Our finding that IgE-mediated CD14 expression correlates with serum IgE concentration may explain the clinical observation that allergen exposure up regulates CD14 on monocytes from sensitized individuals[37]. Since CD14 is essential for LPS responses, one potential consequence of increased CD14 expression is enhancement of this response. This is relevant to allergic disease considering that individuals with allergic asthma have increased bronchial reactivity to inhaled LPS[38], which itself contributes to airway inflammation in mouse models of allergic asthma[39]. Our results suggest a potential link between increased CD14 expression and allergic airway disease and a role for IgE in this process.

Another prominent finding in our study was the rapid and robust secretion of the inflammatory cytokines TNFα and IL-6. Several studies have implicated TNFα in the pathogenesis of allergic disease, where it has been shown to impact airway inflammation[40–42]. Additionally, IL-6 sputum concentrations correlate inversely with respiratory function in asthma patients[43]. The results of our study suggest that IgE cross-linking on monocytes could thus contribute to allergic disease via the induction of TNFα and IL-6 secretion.

The gradual rise in IL-10 secretion was in marked contrast to the kinetics of TNFα induced by IgE cross-linking. The ability of IL-10 neutralization to reverse the TNFα decline and dramatically augment secretion of IL-6, and even IL-10 itself, suggests that IL-10 acts in an autocrine fashion to limit IgE-mediated cytokine secretion and possibly induce degradation of existing TNFα. In fact, IL-10 is proposed to play a suppressive role in allergic asthma[44].

Since IL-10 has been shown to suppress T cell and monocyte/macrophage responses to pathogens[45, 46], excess IL-10 could potentially disrupt these immune responses. Given the importance of pathogen-associated exacerbations of allergic diseases[47], this potential effect of IgE-mediated monocyte IL-10 secretion represents an exciting direction for future studies.

We report for the first time that IgE cross-linking specifically disrupts monocyte phagocytosis without affecting bacterial killing. The apparent discrepancy between IgE-mediated impairment of phagocytosis and induction of a pro-inflammatory program – including TNFα, a cytokine known to promote phagocytosis[48] – suggests potential activation of divergent pathways by IgE cross-linking. One possible mechanism is impairment of TNFα responsiveness after IgE cross-linking, as increased TNFα concentration after IL-10 neutralization did not rescue the IgE-mediated repression of phagocytosis. However, TNFα unresponsiveness cannot completely account for IgE-mediated effects on monocyte function, as bacterial killing, another TNFα-responsive process[48], remained intact. CD64 is also involved in phagocytosis and its expression is known to reflect monocyte phagocytic ability[31, 32]. The down regulation of CD64 induced by IgE cross-linking represents another potential mechanism contributing to impaired phagocytosis.

Another key regulator of phagocytosis is SH2-domain-containing inositol 5’ phosphatase 1 (SHIP), which inhibits macrophage phagocytosis through its action on membrane phospholipids[49]. Interestingly, SHIP has also been reported to augment TLR-induced pro-inflammatory cytokine secretion[50] and to mediate formation of reactive oxygen intermediates, which promote killing after phagocytosis[51]. In addition to its roles in macrophages, SHIP is a negative regulator of allergic signaling in basophils; upon activation by IgE cross-linking it limits degranulation[52]. Activation of SHIP by IgE cross-linking in monocytes could potentially explain our observation of impaired phagocytosis despite secretion of pro-inflammatory cytokines and intact bacterial killing.

The relevance of IgE-mediated disruption of phagocytosis to allergic disease is evidenced by studies demonstrating that alveolar macrophage phagocytosis of bacteria and apoptotic cells is impaired in individuals with severe allergic asthma[53, 54]. Indeed, reduced macrophage phagocytosis has been correlated with increased sputum eosinophils and reduced respiratory function in individuals with allergic asthma[32]. Furthermore, macrophage ingestion of apoptotic granulocytes has been shown to reflect the resolution of asthma symptoms[55], underscoring the importance of this process in allergic disease. IgE-mediated impairment of monocyte and macrophage phagocytosis *in vivo* could thus lead to reduced clearance of apoptotic debris and delayed resolution of inflammation.

In summary, we demonstrate that IgE cross-linking drives select functions in human monocytes including the secretion of both pro-inflammatory and autoregulatory cytokines, which is enhanced in monocytes from individuals with elevated serum IgE levels. In contrast, the ability of monocytes to engulf bacteria or cellular debris is significantly impaired by IgE cross-linking. This suggests that while allergic stimulation of monocytes promotes some aspects of inflammation, it concomitantly impairs the ability of these cells to resolve inflammation via phagocytosis. For individuals with elevated serum IgE concentration, the inflammation induced by allergic stimulation may be more pronounced considering their enhanced secretion of TNFα and IL-6. Yet, their ability to resolve such inflammation is blocked by IgE-mediated signaling. The ability of IL-10 to modulate IgE-mediated pro-inflammatory cytokine secretion without impacting the impairment of phagocytosis suggests that divergent pathways are induced by IgE cross-linking (Fig. 6). Further, the discordant regulation of cytokine secretion and phagocytosis may perpetuate

inflammation that occurs during infection in the context of allergic stimulation. Future studies designed to delineate how IgE-mediated signaling leads to these disparate functional effects will further elucidate the consequences of IgE cross-linking on human monocytes and provide a foundation for understanding the role of this important cell type in IgE-mediated allergic disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

αIgE	cross-linking anti-IgE antibody
IgG	control rabbit IgG antibody
mDC	myeloid dendritic cell
pDC	plasmacytoid dendritic cell
FcεRI	high-affinity IgE receptor
MESF	mean equivalent standard fluorescence
LPS	lipopolysaccharide
PBMC	peripheral blood mononuclear cells
TLR	toll-like receptor
SHIP	SH2-domain-containing inositol 5’ phosphatase 1

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Key Messages

- IgE cross-linking on monocytes increases expression of CD14 and the inflammatory cytokines TNFα and IL-6; these effects are enhanced in individuals with elevated serum IgE concentration.
- IgE cross-linking induces IL-10 secretion in a serum IgE dependent manner, which acts in an autocrine fashion to limit TNFα and IL-6 secretion.
- In contrast to the inflammatory phenotype, IgE cross-linking specifically impairs a critical monocyte function – phagocytosis; this effect is not subject to regulation by IL-10.

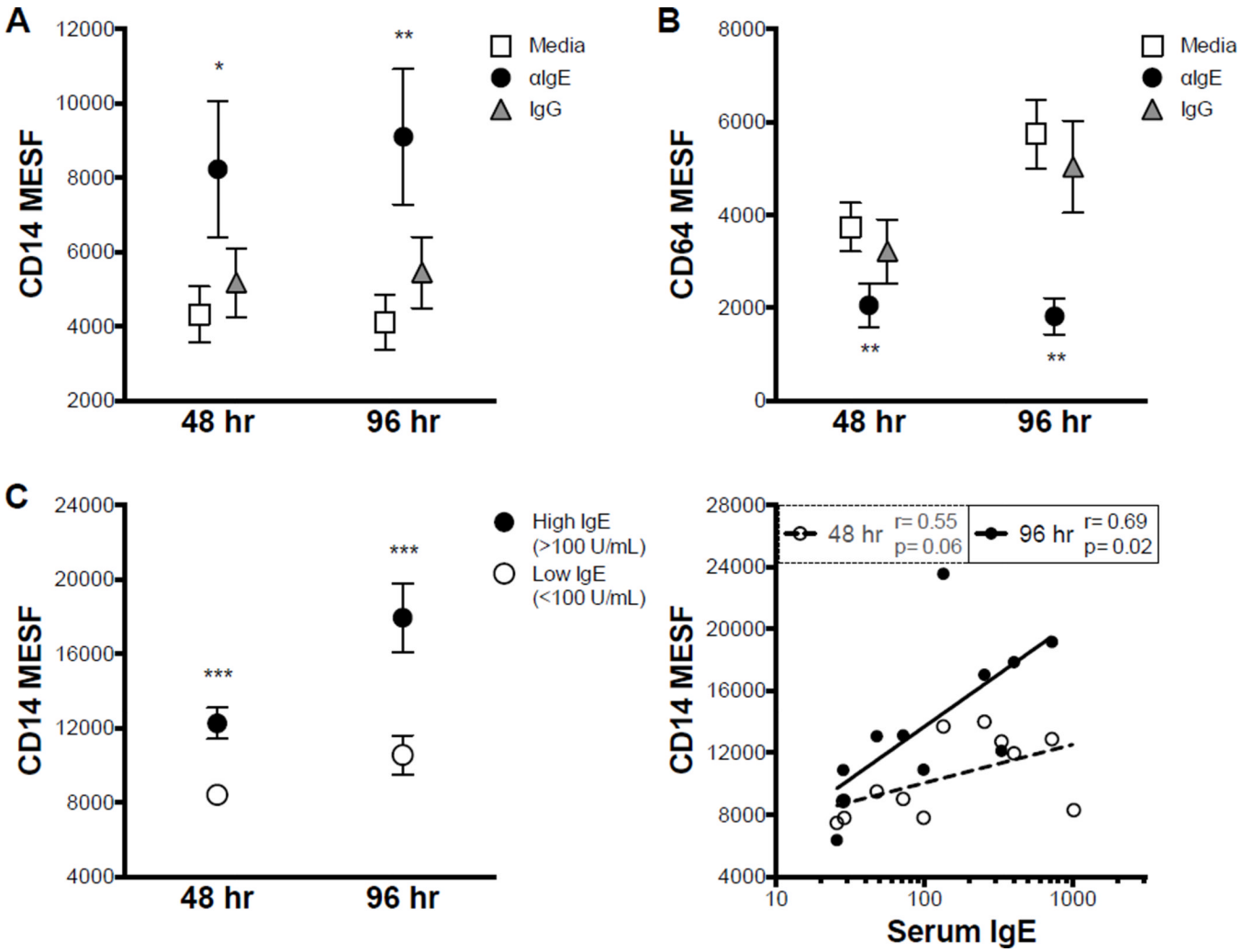

Figure 1. IgE cross-linking up regulates surface CD14 and down regulates CD64

A,B. Monocyte CD14 (A) and CD64 (B) expression after 48 or 96 hr culture in indicated conditions (N≥9). **C.** CD14 expression after IgE cross-linking on monocytes from individuals with low or high serum IgE (left); Pearson correlation between CD14 expression and serum IgE (right; N≥11). * p<.05, ** p<.01, *** p<.001 for αIgE vs. Media and IgG within time points (A,B) or High IgE vs. Low IgE (C).

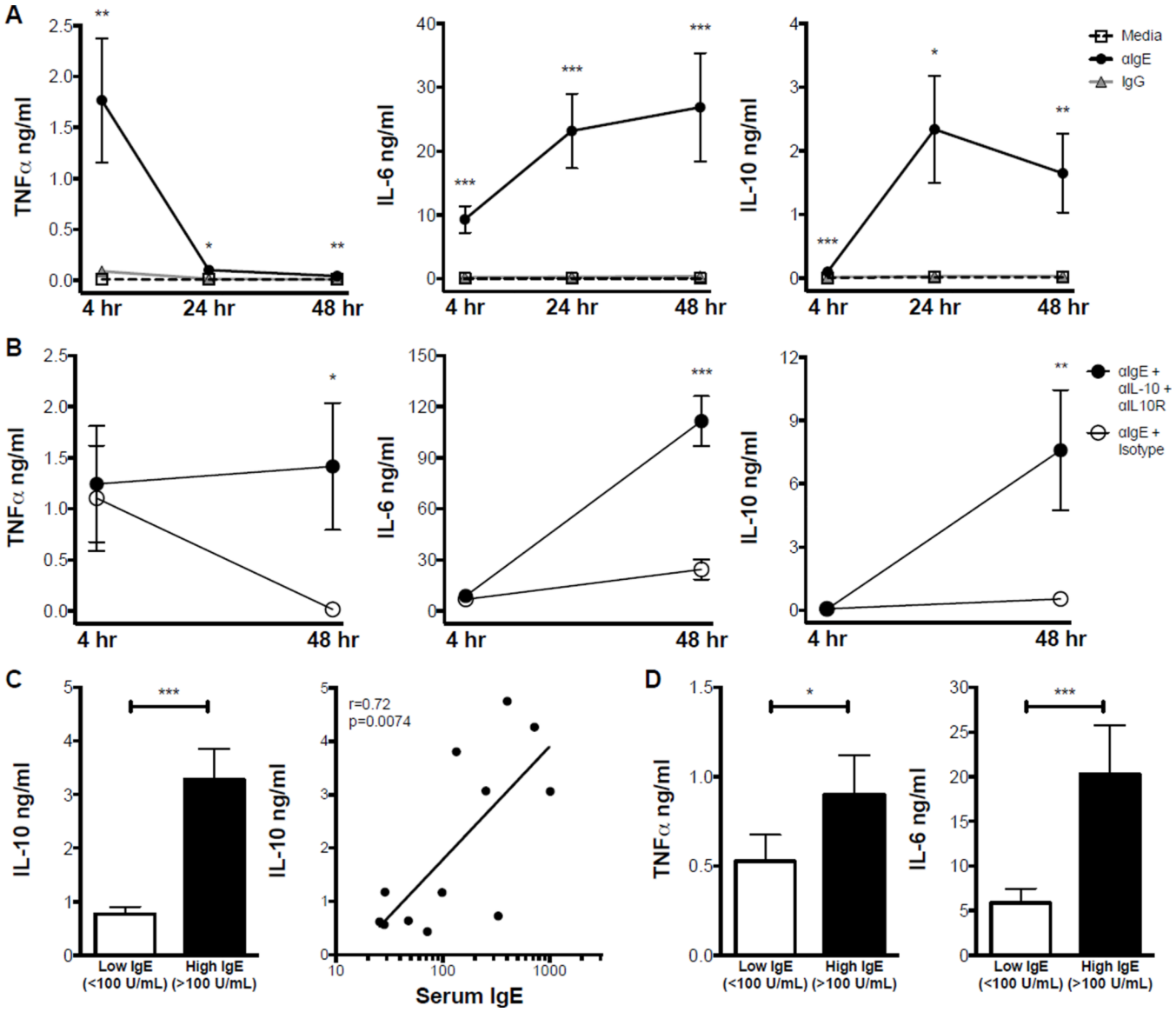

Figure 2. IgE cross-linking induces monocyte secretion of TNFá, IL-6, and autoregulatory IL-10

A. TNFα (left), IL-6 (middle), and IL-10 (right) concentrations in indicated monocyte culture conditions after 4, 24, or 48 hr (N≥9). **B.** Similar data for monocytes cultured with αIgE in the presence of IL-10 and IL-10R neutralizing antibodies or isotype control (N=3). **C.** IL-10 concentration after 48 hr of IgE cross-linking on monocytes from individuals with low (<100 U/ml) or high (>100 U/ml) serum IgE (left) and corresponding Pearson correlation (right; N=12). **D.** Concentration of TNFα after 4 hr (left); IL-6 after 48 hr (right) of IgE cross-linking on monocytes from individuals with low vs. high serum IgE (N≥10). * p<.05, ** p<.01, *** p<.001 for αIgE vs. Media and IgG (A), Blocking Antibodies vs. Isotype (B), or High IgE vs. Low IgE (C,D).

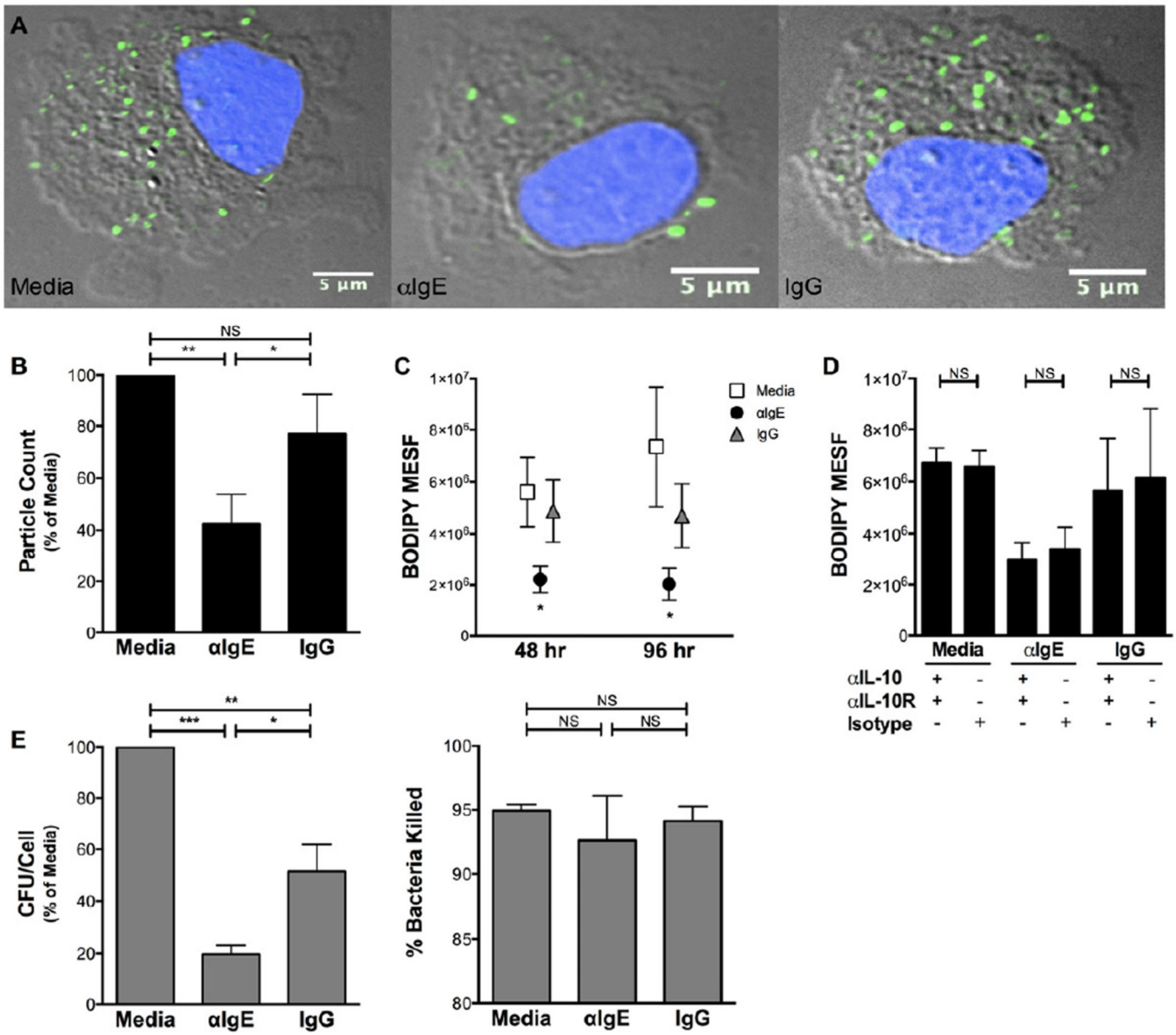

Figure 3. IgE cross-linking impairs monocyte phagocytic function

A,B. Photomicrographs (A, grey=DIC, blue=DAPI, green=BODIPY-FL) and particle count per cell (B) of BODIPY-labeled bacteria phagocytosed by monocytes after 96 hr culture in indicated conditions (N=5). **C.** Fluorescence of internalized bacteria determined by flow cytometry for indicated conditions (N≥9). **D.** Effect of IL-10 neutralization on phagocytosis in indicated conditions (N=3). **E.** Internalization (CFU/Cell, left) of live bacteria after 48 hr culture; percent of internalized bacteria killed (right) after additional 16 hr incubation (N=4). * p<.05, ** p<.01, *** p<.001, NS p>.05 for αIgE vs. Media and IgG (C) or indicated comparisons (B,D,E).

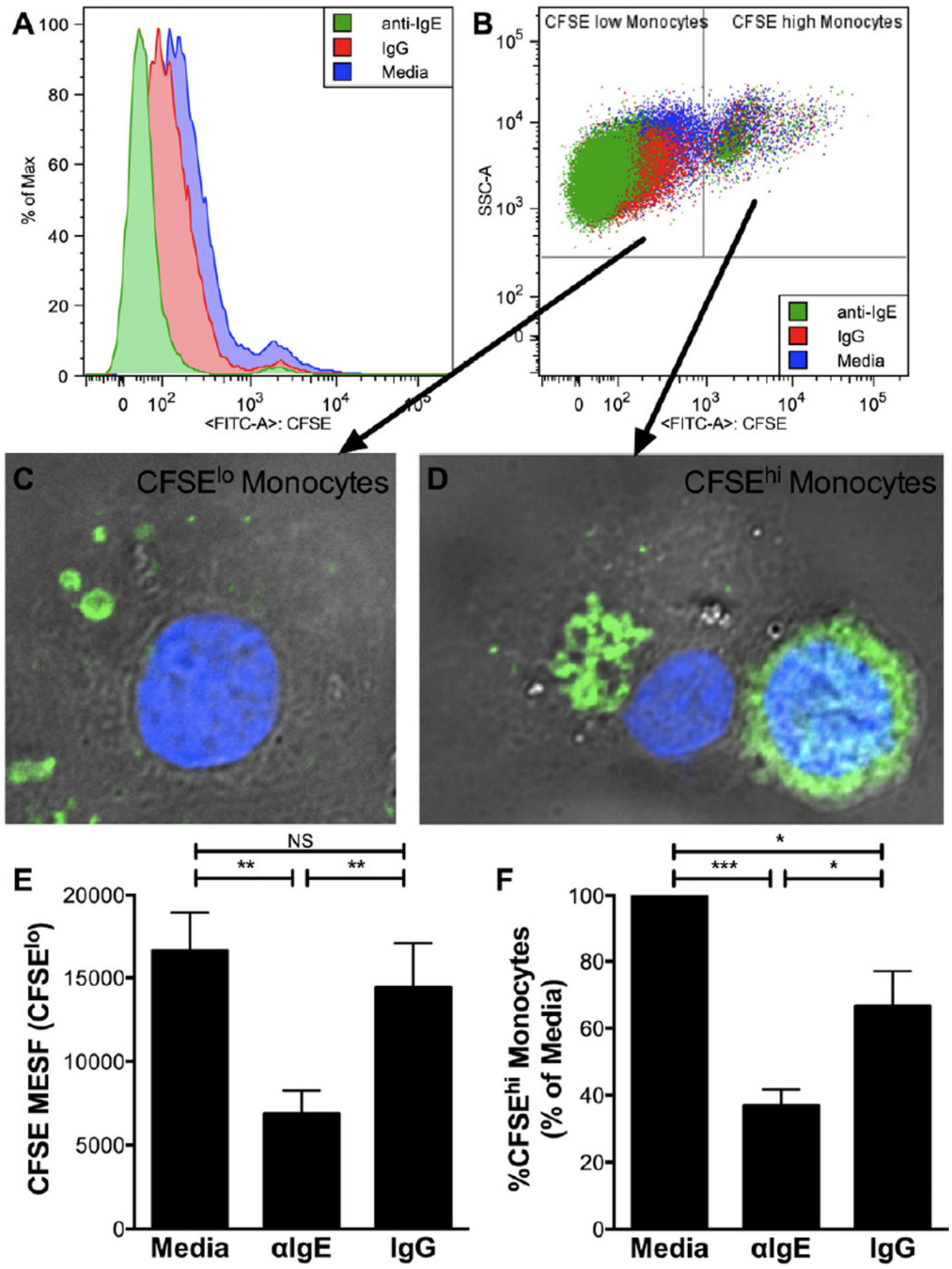

Figure 4. IgE cross-linking inhibits phagocytosis of apoptotic cells

A,B. CFSE fluorescence in monocytes (gated on $CD14^{+}$) after phagocytosing CFSE-labeled apoptotic cells (A); gating strategy for $CFSE^{low}$ and $CFSE^{high}$ monocytes(B). **C,D.** Photomicrographs (grey=DIC, blue=DAPI, green=CFSE) of sorted $CFSE^{low}$ monocytes(C) and $CFSE^{high}$ monocytes(D). **E,F.** CFSE fluorescence of $CFSE^{low}$ monocytes (E) and percent $CFSE^{high}$ of total monocytes (F) for indicated culture conditions (N=4). * p<.05, ** p<.01, *** p<.001, NS p>.05 for indicated comparisons (E,F).

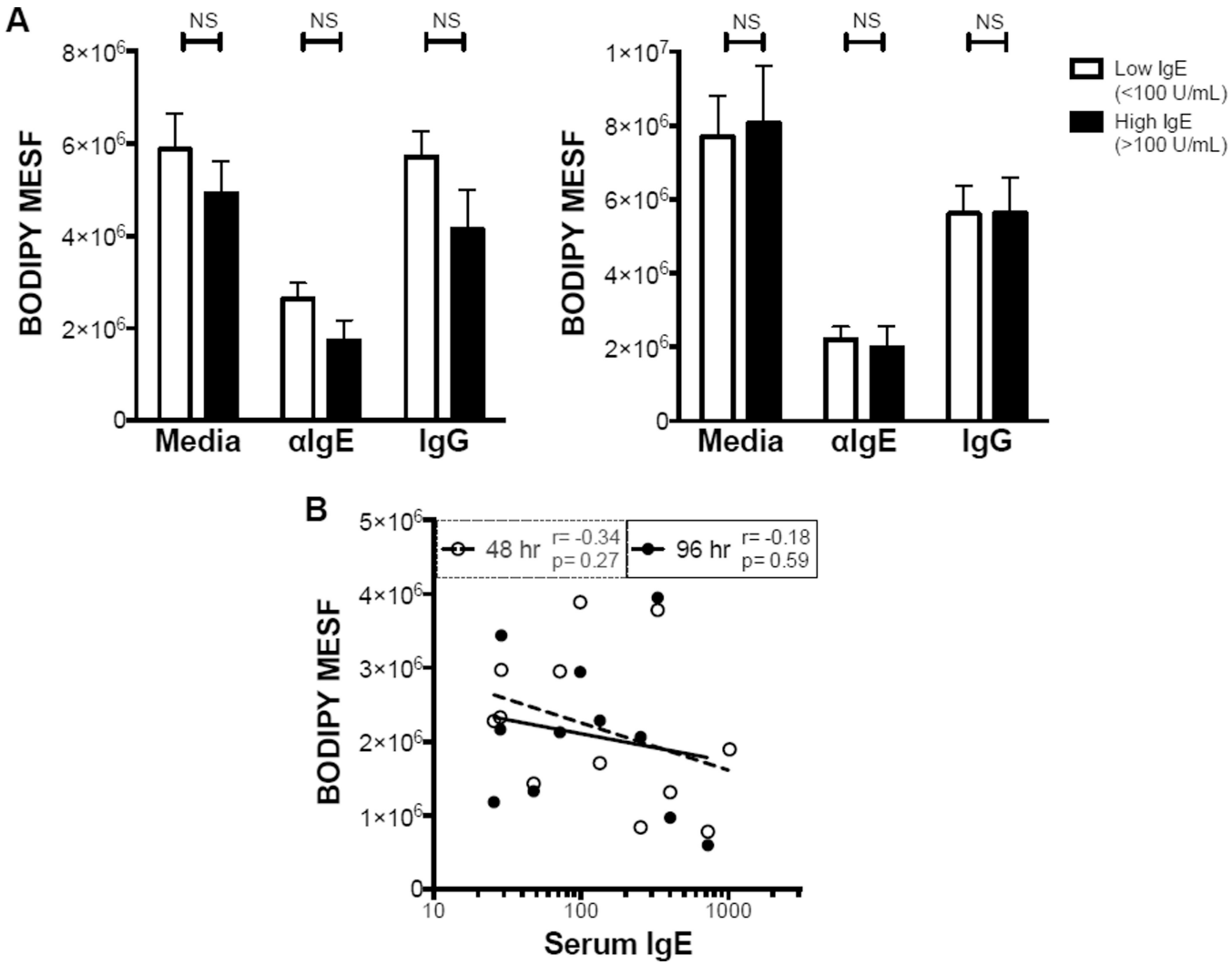

Figure 5. Inhibition of phagocytosis by IgE cross-linking is independent of serum IgE concentration

A,B. Fluorescence of internalized bacteria in monocytes from individuals with low (<100 U/ml) or high (>100 U/ml) serum IgE, cultured for 48 (A, left) or 96 (A, right) hr in indicated conditions; corresponding Pearson correlation (B) for αIgE condition (N=12). NS p>.05 for indicated comparisons.

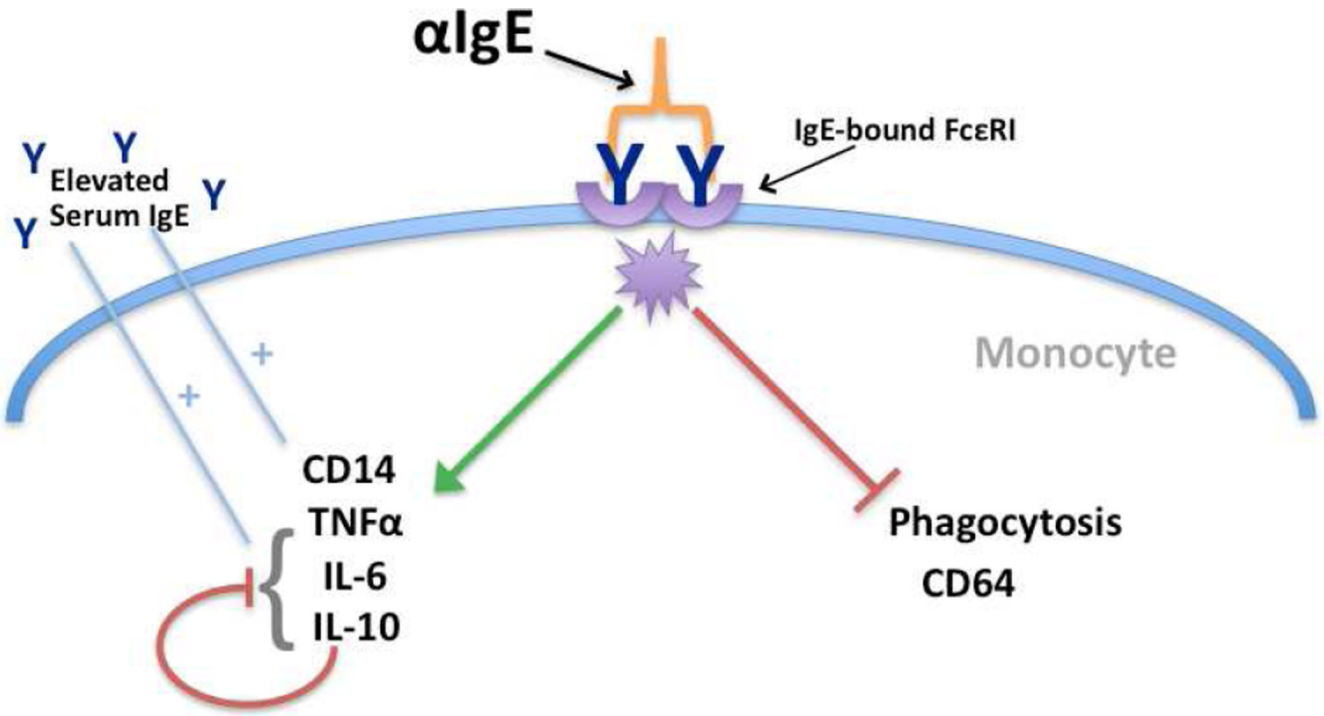

Figure 6. Proposed model of IgE cross-linking on human monocytes

IgE cross-linking drives (green arrow) increased CD14 expression and secretion of both pro-inflammatory cytokines (TNFα and IL-6) and autoregulatory IL-10. These effects are enhanced in individuals with elevated serum IgE (blue lines). In contrast, phagocytosis and CD64 expression are suppressed by IgE cross-linking (red line). The IgE-mediated impairment of phagocytosis is independent of regulation by IL-10, suggesting that divergent pathways are activated by IgE cross-linking on monocytes.

Table I

Participant Information

	Low IgE	High IgE	P value
Number	6	6	n/a
Age	23.0 (18–27)	21.2 (14–44)	0.71
Gender	3 Male, 3 Female	4 Male, 2 Female	1.00
Ethnicity	2 Black, 2 White, 2 Hispanic	5 Black, 1 White	0.16
Atopy			
-Asthma	4	6	0.45
-Allergic Rhinitis	3	6	0.18
-Atopic Dermatitis	0	1	1.00
-Skin Test (≥1 Positive)	6	6	n/a
-Skin Test (# of Positive)	3.5 (1–7)	5.8 (3–9)	0.11
Serum IgE (U/mL)	50.2 (25–99)	477.0 (134–1017)	**0.01**
Monocyte FcεRI (MESF)	2951 (1592–4582)	5171 (2011–8065)	**0.04**

Demographic information, atopic status, serum IgE concentration, and monocyte FcεRI expression are presented for enrolled participants. P values for categorical values were calculated by Chi-square or Fisher's Exact Test. Mean (range) and t test are shown for quantitative values.