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CD2 Identifies a Monocyte Subpopulation with IgE Dependent, High Level Expression of FcεRI

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Abstract

Background—FcεRI expression by monocytes can affect monocyte function via multiple mechanisms, thereby potentially influencing the generation of allergic inflammation. Previous studies on the in vivo regulation of monocyte FcεRI expression by ambient IgE have yielded conflicting results.

Objective—We hypothesized that monocyte FcεRI expression is limited to a specific monocyte subset and that within that subset FcεRI surface expression is correlated to serum IgE.

Methods—Study 1: Blood was obtained from nonallergic subjects (n = 14) and subjects with allergic asthma (n = 18), hypereosinophilic syndrome (n = 2), hyper-IgE syndrome (n = 6), and helminth infection (n = 4). Study 2: Blood was obtained from allergic subjects in a clinical trial of omalizumab before and during study drug treatment. Monocyte surface FcεRI expression was measured using flow cytometry.

Results—FcεRI expression was significantly greater in the $CD2^{high}$ vs. $CD2^{low}$ monocyte subsets (31% vs. 1.9% median $FcεRI^{+}$, respectively). In asthmatic and non-atopic healthy control subjects, $CD2^{low}$ monocytes expressed little or undetectable FcεRI. In study 1, FcεRI expression was highly correlated to serum IgE in the $CD2^{high}$, but not in the $CD2^{low}$ monocyte subpopulation (R values of 0.67 and 0.41, respectively). In study 2, omalizumab but not placebo, caused a significant and sustained drop in FcεRI expression within the $CD2^{high}$ monocyte subset.

Conclusions—CD2 defines a monocyte subset with high FcεRI expression, the magnitude of which is highly correlated to serum IgE. As such, this new description of $CD2^{high}$ monocytes as FcεRI bearing cells suggests that they may be potential targets of anti-IgE immunomodulatory therapies.

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Keywords

Monocyte; FcεRI; IgE; antigen presenting cell; CD2; allergy; omalizumab; allergy; asthma; flow cytometry

Abbreviations

APC: Antigen presenting cell; DC: Dendritic cell; FcεRI: High affinity IgE receptor; MEPE: Molecules of equivalent phycoerythrin

Introduction

The high-affinity IgE receptor (FcεRI) and allergen specific IgE play an essential role in mast cell and basophil activation, leading to immediate-type hypersensitivity. Antigen presenting cells (APCs), such as monocytes and dendritic cells (DCs), also express FcεRI[1–6]. FcεRI expression by APCs can increase the efficiency of allergen presentation to T cells up to 1000-fold, through an antigen focusing mechanism in which FcεRI-bound IgE on APCs selectively captures allergen[7]. In this manner, FcεRIα expression by APCs may augment the activation and differentiation of allergen-specific Th2 cells, thereby increasing allergic inflammation [8]. Additionally, FcεRI cross-linking on monocytes causes NF-κB activation[9], prevention of apoptosis[10], and induction of IL-10 expression, which then inhibits monocyte differentiation into DC[11]. Lastly, monocytes are the precursors of both DCs and macrophages, and thus FcεRI expression by monocytes may have the potential to influence the generation of allergic inflammation through multiple mechanisms.

IL-4 and IL-13 increase monocyte FcεRIα expression in vitro[12] and may play a major role in the increased expression found on monocytes obtained from atopic patients. In vitro studies of FcεRI transfected cell lines have shown that IgE binding to FcεRIα stabilizes the complex resulting in greater FcεRI surface expression[13]. This phenomenon is borne out in human disease in which there is a strong correlation between serum IgE and FcεRIα surface expression by basophils and DCs[14]. This suggests that the increased monocyte FcεRI expression found in allergic disease is at least in part a result of the increased serum IgE that is characteristic of these disorders[3,15,16]. However, a study that carefully examined monocyte FcεRIα expression in non-allergic patients with highly elevated serum IgE concentrations found no correlation between monocyte FcεRI expression and serum IgE[17]. This latter study suggests that monocyte surface FcεRIα expression is independent of ambient IgE concentrations and thus regulated in a fundamentally different manner from that of other FcεRI expressing cells.

Monocytes are defined by their characteristic morphology and cell surface phenotype. Recently it has been appreciated that monocytes, although morphologically uniform, are composed of subsets with different capacities for lineage differentiation, tissue homing and contribution to tissue inflammation[18,19]. These subsets have largely been phenotypically defined using cell surface markers including CD2, CD14, CD16, CD64 and chemokine receptor expression. In specific, $CD16^+$, $CX3CR1^+$ monocytes differentiate into tissue homing resident macrophages found in uninflammed spleen, liver and lungs. In contrast, $CD16^-$, $CCR2^+$ monocytes are the precursors of macrophage and DC populations found in inflammatory sites[18,20]. Recently, it has been reported that the $CD16^+$, $CD64^-$ monocyte subpopulation is increased in the blood of patients with atopic dermatitis[21].

However, previous studies of monocyte FcεRI expression have examined the total monocyte population without subset analysis and were technically limited by the use of unconjugated low signal:noise FcεRIα mAbs, that in some cases recognized an FcεRI epitope that competed

with IgE binding, and required acid stripping to remove bound IgE. The conflicting results in the literature thus led us to carefully reexamine the issue of monocyte FcεRI expression using multiparameter flow cytometry and a directly labeled high signal:noise non-competitive anti-FcεRIα mAb[22] to examine FcεRI expression. We used two complementary clinical approaches to examine the relationship between serum IgE and monocyte FcεRI : in study 1, we investigated patient populations with a 1000-fold range of serum IgE concentrations and in study 2, we employed a clinical trial of omalizumab, in which the serum IgE concentration was reduced 10–30 fold. In this work, we localize FcεRIα expression to a subpopulation of $CD2^{high}$ human monocytes, and using the above clinical approaches, we demonstrate a high correlation between serum IgE and FcεRI expression within the $CD2^{high}$ monocyte subpopulation. These findings explain the conflicting results from previous studies and demonstrate that surface FcεRI expression by monocytes is, in fact, regulated in a similar manner to other hematopoietic cells, such as basophils, mast cells and DC.

Methods

Antibodies

Anti-FcεRIα (clone AER-37, originally described as CRA1 [22]) PE, APC, biotin was obtained form eBiosciences, San Diego, CA. This mAb does not compete with IgE for FcεRI binding and thus is an accurate means to assess surface FcεRIα expression. Anti-IgE phycoerythrin (PE) (clone G7-26); CD123/anti–IL-3R phycoerythrin (PE), PE/cyanin-5 (PE/Cy5), biotin; mouse IgG1 unlabeled, biotin, PE; mouse IgG2b unlabeled, PE, PE/Cy5; mouse IgG2a PE; streptavidin-PE; CD64 (clone 10.1) biotin, PE; HLA-DR PE, PE/Cy5; CCR3 (clone 1C6) PE; CCR4 (clone 1G1) PE, CCR5 (clone 2D7/CCR5) PE; CCR6 (clone 11A9) PE were obtained form BD-PharMingen Corporation, San Diego, CA. Anti-CD14 (clone RM052) FITC, APC; CD2 unlabeled (clone T11) (Immunotech, Marseille, France); CCR2 (clone 48607) PE; CCR7 (clone 150503) PE (R&D Systems Inc, Minneapolis, MN.); CD1c/BDCA-1 biotin, PE (clone AD5-8E7); BDCA-2 PE (clone AC144); BDCA-3 (clone AD5-14H12) PE (Militenyi Biotec, Auburn, CA); Goat-anti-Mouse IgG1 Alexa 647; streptavidin-APC (Molecular Probes, Eugene, OR); anti-CD16 (clone 3G8) FITC (BioSource, Camarillo, CA) were obtained commercially. The basophil granule-specific mAb 2D7[23] was a gift from Dr. Lawrence Schwartz (Virginia Commonwealth University, VA).

Reagents

Paraformaldehyde, dimethyl sulfoxide, histopaque-1077 and -1088 (Sigma Chemicals, St. Louis, MO) and Sphero Rainbow Calibration Particles (BD-PharMingen) were obtained commercially.

Study subjects and cells

Study 1—Eighteen allergic asthmatic subjects (Figs. 1–5) met American Thoracic Society criteria for the diagnosis of asthma[24], had either moderate or severe asthma as defined by the National Asthma Education Prevention Program, had 3 or more positive skin test responses (≥3 mm induration over glycerol negative control) out of a panel of 10 aeroallergens, and were not experiencing an exacerbation at the time of the study. Thirteen of these asthmatic subjects were using inhaled corticosteroid therapy; none were receiving systemic corticosteroid therapy. Fourteen healthy control subjects had no history of allergic disease or asthma and had negative skin tests (≤2 mm induration) to the panel of aeroallergens. The helminth infected patients had intestinal helminths (n=1), onchocerciasis (n=2) and loiasis (n=1). The hyper IgE recurrent infection (Job's syndrome) patients had a history of multiple characteristic infections and elevated IgE. The 2 hypereosinophilic syndrome patients met established criteria[25].

Study 2—The omalizumab clinical study (Fig. 6) was a randomized, double blind, placebo-controlled clinical trial of 24 subjects (16 omalizumab and 8 placebo) with seasonal allergic rhinitis, as reported[5,26]. Subjects with a history of ragweed-induced seasonal allergic rhinitis, a positive ragweed skin test, and a serum IgE concentration of less than 700 IU/mL were enrolled. Subjects were randomly assigned to receive either omalizumab (0.016 $mg \cdot kg^{-1} \cdot IU^{-1} \cdot mL^{-1}$ of IgE) or placebo on days 0 and 28. At the completion of the trial, the data were analyzed in a blinded fashion. Monocyte FcεRIα expression was determined by flow cytometry on day 0 (baseline) and on days 7, 14, 28, and 42.

The clinical protocols for studies 1 and 2 were approved by the National Institute of Allergy and Infectious Diseases and the Creighton University Institutional Review Boards, respectively. All subjects gave informed consent.

PBMC were isolated from EDTA anticoagulated blood by means of density gradient separation with either Histopaque-1077 (study 1, Figs 1–5) or Histopaque-1088 (study 2, Fig 6), fixed in 4% paraformaldehyde for 5 minutes at 37°C, and cryopreserved in 10% dimethyl sulfoxide/PBS at −80°C, according to published methods[27]. Serum IgE determinations (Fig 4, Table I) were performed by the National Institutes of Health Clinical Center Department of Laboratory Medicine using a chemiluminescence immunoassay.

Antibody staining

Multiple combinations of antibodies were used in the experiments, employing a nonpermeabilizing adaptation of described procedures[4,27]. The following is an example of the staining for CD2, CD14, FcεRIα and BDCA-1 that was used in figures 3–6. Cryopreserved fixed cells were thawed, washed once in PBS with 0.1% BSA (PBS/BSA), and then blocked in PBS/BSA/5% nonfat dry milk (PBS/BSA/milk) for 1 hour on ice. Cells were incubated with CD14 FITC, FcεRIα or IgG2a PE, BDCA-1 biotin, and CD2 or IgG1 control in PBS/BSA/milk for 30 minutes at 4°C, washed twice in PBS/BSA, and incubated with Goat-anti-Mouse IgG1 Alexa 647 and streptavidin conjugated PE/Cy5.5 in PBS/BSA for 30 minutes, washed, and analyzed by flow cytometry. The CD2 mAb was an IgG1 isotype, the CD14, FcεRI and BDCA-1 mAbs were Ig2a and were thus not recognized by the Goat-anti-Mouse IgG1 secondary antibody in pilot experiments (data not shown).

Flow cytometry

Data were acquired with a 2-laser, 4-parameter FACSCalibur flow cytometer (Becton-Dickinson Biosciences) and analyzed on Cellquest (Becton-Dickinson Biosciences) or FlowJo software (Tree Star, San Carlos, CA). Typically, 200,000 total events were acquired to obtain adequate numbers of $FcεRIα^+$ monocytes. FcεRIα expression was quantitated as molecules of equivalent PE (MEPE) using Sphero Rainbow Calibration particles, as per the manufacturer's instructions.

The $CD14^+$, $CD2^{high}$ and $CD14^+$, $CD2^{low}$ subsets were identified by first gating on $CD14^+$ cells, then back gating on cells of the corresponding scatter, which yielded two distinct populations of cells differentially expressing CD2 and FcεRI (Fig. 2E, F). The $CD14^+$, $CD2^{high}$ population, although enriched for FcεRI+ cells, contained a minority population of $CD2^{bright}$, $FcεRI^-$ cells (bold arrows in Fig. 2E, F), which in additional experiments were found to be largely CD3+ T cells (mean 84% $CD3^+$, n=6 donors, 3 allergic asthma, 3 non-atopic control). Because these cells were not monocytes, in the additional experiments performed in figures 4–6, this population was excluded from the analysis of $CD2^{high}$ monocytes.

Statistical analysis

The Mann-Whitney U test was used to compare FcεRIα expression between different subject groups (Figs. 2 and 5). The Spearman rank test was used to evaluate correlative data in figure 4. Paired data in figure 6 were analyzed using the Wilcoxon signed rank test. A *P* value of less than 0.05 was considered significant. Statistical calculations and linear regression analysis were performed with Prism software (GraphPad Software, San Diego, CA).

Results

To explore monocyte heterogeneity, we first examined FcεRIα expression in monocytes as a function of CD14. As shown in figure 1A, the majority of monocytes stained negative for FcεRIα (gate I) and a minority subpopulation (gate II) extended as a characteristic curved $FcεRI^{+}$ tail. A third population of $CD14^{-}$, $FcεRI^{bright}$ cells was noted (gate III), which upon further analysis consisted of basophils and DCs (data not shown). In subsequent experiments in Figs. 1–3, we define $FcεRI^{-}$ and $FcεRI^{+}$ monocytes as the populations defined by gates I and II, respectively. These results demonstrate that monocytes consist of two populations: a majority or "mainstream" population with little or no FcεRI expression and a second population consisting of a tail of FcεRI+ cells extending 1–2 logs above the mainstream cluster.

Because basophils and DCs express FcεRIα, we next sought to demonstrate that the $FcεRI^{+}$ monocyte population was not composed of either of basophils or DCs. Both the $FcεRI^{+}$ and $FcεRI^{-}$ monocyte populations stained negatively for the basophil granule protein 2D7 (Fig. 1B) as well as for the BDCA-2 and BDCA-3 dendritic cell markers (Fig. 1D, E)[28]. Most of the $FcεRI^{+}$ monocytes were negative for the CD1c/BDCA-1 marker, however there was a shoulder of CD1c intermediate cells noted (Fig. 1C). These results demonstrate that the $FcεRI^{+}$ monocyte subpopulation is not contaminated by substantial numbers of other known FcεRI bearing cell populations.

We next sought to determine if any previously reported monocyte subset markers could be used to better define the $FcεRI^{+}$ monocyte subset. The best accepted monocyte subsets consist of reciprocal $CD16^{-}$, $CD64^{+}$ and $CD16^{+}$, $CD64^{-}$ subpopulations [18], both of which were found in the mainstream $FcεRI^{-}$ monocyte population (Fig. 2A). In contrast, the $FcεRI^{+}$ monocyte subpopulation consisted almost entirely of cells expressing the $CD16^{-}$, $CD64^{+}$ phenotype (mean 92% of $FcεRI^{+}$ monocytes were CD16−, CD64+). However, because most monocytes are CD16−, CD64+, these markers did not further discriminate the $FcεRI^{+}$ monocyte subpopulation. We next examined HLA-DR and CD123, which identify different DC subsets and maturation stages[29] and found they were expressed at 2.7 and 2.0 greater levels, respectively, in the $FcεRI^{+}$ vs. $FcεRI^{-}$ monocyte subsets (Fig. 2C and D), but did not sufficiently discriminate the $FcεRI^{+}$ monocyte population (data not shown). We further examined CCR2, CCR3, CCR4, CCR5, CCR6 and CCR7 and found none of these chemokine receptors were differentially expressed in the $FcεRI^{+}$ vs. $FcεRI^{-}$ monocyte populations (data not shown).

CD2 is expressed at high levels by a subset of monocytes, which are capable of rapid differentiation into functional dendritic cells and may represent DC precursors[30–32]. We thus examined CD2 expression and found that FcεRI was highly expressed on the $CD2^{high}$ monocyte subset (Figs. 2E and F). FcεRI staining by $CD2^{high}$ monocytes was verified by demonstrating that FcεRI+ cells stained concordantly for IgE (Fig. 2G), whereas no staining was found in $CD2^{low}$ monocytes (Fig. 2H).

We next analyzed the relative expression of FcεRI in the $CD2^{high}$ and $CD2^{low}$ monocyte subsets to determine if CD2 could be used to better discriminate the $FcεRI^{+}$ subset. The $CD2^{high}$ monocyte subset contained a significantly greater fraction of $FcεRI^{+}$ cells than were

present in the $CD2^{low}$ monocyte subset (39% vs. 1.9%, median) and this relationship was found in monocytes from both allergic asthmatic and non-atopic healthy control subjects (Fig. 3A). $CD2^{high}$ monocytes from allergic asthmatic donors contained greater numbers of $Fc\varepsilon RI^{+}$ cells relative to healthy non-atopic donors, and approached statistical significance ($p = 0.065$). Conversely, the $Fc\varepsilon RI^{+}$ monocyte subset contained a significantly greater fraction of $CD2^{high}$ cells, relative to the $Fc\varepsilon RI^{-}$ subset (Fig. 3B). In sum, these data demonstrate that the $CD2^{high}$ monocyte subset is highly enriched for FcεRI expressing cells and suggests that CD2 may be used as a marker to identify FcεRI expressing monocytes.

Previous studies have shown that monocytes are capable of expressing FcεRI, but have yielded conflicting results regarding the relationship between monocyte FcεRI expression and serum IgE [16,17]. We hypothesized that this discrepancy between studies could be due to the inability to accurately measure the small fraction of $Fc\varepsilon RI^{+}$ monocytes within the much larger pool of mainstream $Fc\varepsilon RI^{-}$ monocytes. To address this, we next examined the relationship between serum IgE and FcεRI expression within the $CD2^{high}$ and $CD2^{low}$ monocyte subsets.

We first used a similar study design to Saini et al[17], and studied subjects with serum IgE concentrations encompassing a 10,000-fold range. Five patient groups were studied: healthy non-atopic controls, allergic asthma, helminth infection, hyper-IgE (Job's) syndrome and hypereosinophilic syndrome (Table I). All 4 disease cohorts had elevated serum IgE levels, but the latter three did not have allergic disease. Within the $CD2^{high}$ monocyte subset, FcεRI expression correlated well with serum IgE ($r= 0.67$, Fig. 4A). In contrast, a lower correlation was found in the $CD2^{low}$ subset ($r= 0.41$, Fig. 4B). A subgroup analysis using only the allergic asthmatic and non-atopic control subjects yielded a significant correlation for the $CD2^{high}$ ($r= 0.39$, $p= 0.028$), but not the $CD2^{low}$ ($r= 0.30$, $p= 0.99$) monocyte subsets. We additionally examined the percentage of $CD2^{high}$, $Fc\varepsilon RI^{+}$ monocytes (Fig. 4C), but did not find a significant correlation ($r= 0.26$, $p=0.09$). Repeat analyses of the $CD2^{high}$ monocyte FcεRI MEPE from 6 asthmatic and 6 non-atopic control subjects acquired at separate time points over a year apart demonstrated a high degree of correlation ($r= 0.71$), demonstrating that this is a stable characteristic. These data demonstrate that FcεRI expression and serum IgE are highly correlated in the $CD2^{high}$ but not the $CD2^{low}$ monocyte subset.

We then examined surface FcεRIα expression as a function of disease status. In the $CD2^{high}$ monocyte subset, FcεRI was expressed at significantly higher levels in all 3 disease categories relative to the non-atopic subjects (Fig. 5). In contrast, no such relationship to disease status was found for $CD2^{low}$ monocytes when comparing allergic asthma to non-atopic healthy control subjects. Interestingly, $CD2^{low}$ monocytes from both the helminth infected and hyper-IgE syndrome subjects had higher FcεRI expression than those from the non-atopic control subjects, although this finding was less significant than that for the $CD2^{high}$ monocytes.

We next hypothesized that the correlation between monocyte FcεRI and serum IgE found in figures 4 and 5 would be reiterated when serum IgE was therapeutically decreased 10–30-fold during a clinical trial of omalizumab[26]. Omalizumab caused a significant drop in surface FcεRI expression in the $CD2^{high}$ monocyte subpopulation that was noted at all study time points (Fig. 6A). In contrast, the change in FcεRI expression within the $CD2^{low}$ population was not significant at any of the first 3 times points measured (days 7, 14 and 28), but did reach statistical significance at the single day 42 time point (Fig. 6C). The drop in FcεRI expression during omalizumab treatment was 22% and 11% for the $CD2^{high}$ and $CD2^{low}$ monocyte subsets, respectively. These data demonstrate that therapeutic reduction in serum IgE causes a reduction in FcεRIα surface expression in the $CD2^{high}$ monocyte subset.

Discussion

Monocytes express low levels of FcεRI, however the relative magnitude of this expression and its relationship to serum IgE concentration has been disputed. In this report, we demonstrate for the first time that FcεRIα surface expression is limited to the $CD2^{high}$ monocyte subset, and that FcεRI expression by this monocyte subset is highly correlated to serum IgE concentration. These results demonstrate that FcεRI surface expression by this subset of $CD2^{high}$ monocytes responds to ambient IgE levels similarly to that shown previously in other FcεRI bearing cells.

In basophils,[17] dendritic cells,[5] and mast cells[33] *in vivo*, as well as in *in vitro* studies of transfected cell lines[13] and monocyte derived DC[34], surface FcεRIα expression is highly dependent on ambient IgE concentration. These studies have led to the general understanding that surface FcεRI is stabilized by IgE occupancy of the receptor, resulting in greater levels of surface FcεRI expression. Sirha et al [16] found a high correlation between monocyte FcεRI and serum IgE. However, a carefully performed study of monocytes found no correlation between serum IgE and monocyte FcεRI, suggesting that the regulation of monocyte surface FcεRI expression was singularly different from that of other cell lineages previously studied [17]. Our results in Figs. 1–3, demonstrating that FcεRI surface expression is largely limited to a subset of $CD2^{high}$ monocytes, help explain the seeming contradiction of these previous reports. We found that surface FcεRI expression by the majority $CD2^{low}$ monocyte subset was generally below the level of detection of our high signal:noise assay, and in that subset, FcεRI expression was not responsive to ambient IgE levels. In contrast, as shown in Figs. 4–6, the $CD2^{high}$ monocyte subset expresses substantial amounts of surface FcεRI, the expression of which is highly responsive to ambient IgE concentrations. These data support the concept that surface FcεRI expression regulation by $CD2^{high}$ monocytes is regulated in a similar manner to other cell lineages studied.

We used two complementary clinical approaches, both of which demonstrate the IgE dependence of monocyte FcεRI surface expression. First, in a similar manner to Saini et al [17], we utilized a cohort of subjects with a 4 log range of serum IgE concentrations (Figs. 4 and 5). Second, we utilized a clinical trial of omalizumab to decrease serum IgE (Fig. 6). The magnitude of reduction in FcεRI expression induced by omalizumab was less than that seen for basophils and DCs analyzed from the same clinical trial [5,26]. Further work is needed to determine if this finding is a technical artifact or represents actual differences in the biology of FcεRI surface expression.

To more clearly define the $Fc\varepsilon RI^{+}$ monocyte population, we examined a number of different cell surface markers. In these experiments, CD2 was uniquely capable of identifying the $Fc\varepsilon RI^{+}$ monocyte subpopulation. However, despite our identification of the $CD2^{high}$ monocyte as being the major FcεRI bearing monocyte population, some FcεRI expressing monocytes were found in the $CD2^{low}$ population. This spillover may explain the higher levels of FcεRI in $CD2^{low}$ monocytes from subjects with hyper-IgE syndrome or helminth infections. This finding may also explain the drop in FcεRI expression in $CD2^{low}$ monocytes in the omalizumab treated group on study day 42. Future advances in understanding FcεRI expression by monocyte subsets will require additional markers to more precisely identify this subpopulation.

$CD2^{high}$ monocytes rapidly acquire DC activity in vitro and may represent the immediate precursors to DC [30–32]. Given the capacity for $CD2^{high}$ monocytes to acquire DC activity, it may be argued that $CD2^{high}$ monocytes are simply DC themselves and thus, express FcεRI in a similar manner to DC. However, FcεRI+ monocytes did not express the DC specific BDCA-2 and BDCA-3 markers, supporting the conclusion that these $Fc\varepsilon RI^{+}$ monocytes are not simply $CD14^{+}$ DC. A fraction of $Fc\varepsilon RI^{+}$ monocytes expressed intermediate levels of CD1c/

BDCA-1, which although lower expression than that found on DCs, could indicate that these cells are in transition from the monocyte to DC lineages. Further investigation is needed to better define the lineage relationship between the FcεRI^{+} monocyte subset, the mainstream FcεRI^{-} monocyte population, and DCs; as well as the role of the CD2^{high} monocyte subset in allergic disease pathogenesis and as a target for immunomodulatory therapies. These results confirm and extend our previous findings on DCs[5] and suggest that surface FcεRI is upregulated prior to monocyte differentiation into DCs.

FcεRI expression by APCs increases the efficiency of antigen presentation of allergen to T cells via an antigen focusing mechanism by as much as 1000-fold[8]. Given that we have shown that the CD2^{high}, FcεRI^{+} monocyte is the dominant FcεRI expressing monocyte subset, it is possible that these cells are responsible for IgE mediated antigen focusing by monocytes. Alternatively, crosslinking of FcεRI on antigen presenting cells may induce expression of proinflammatory mediators and chemokines[8].

In conclusion, we have demonstrated high level FcεRI expression by CD2^{high} monocytes, and furthermore, that this FcεRI expression is limited to the CD2^{high} monocyte subset, and is highly correlated to serum IgE. These findings help explain the conflicting results of previous studies on the relationship of monocyte FcεRI to serum IgE[3,16,17], and suggest that CD2^{high} monocytes may be a potential target of anti-IgE immunomodulatory therapy [35].

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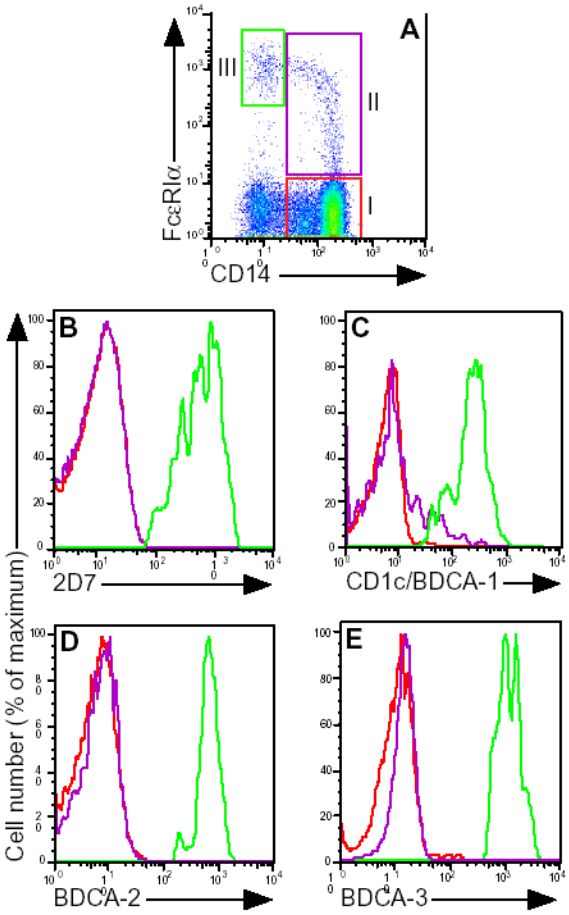

Figure 1. Identification of CD14$^+$, FcεRIα$^+$ cells as monocytes

A, after gating on typical monocyte scatter, a CD14 vs. FcεRI dot plot was generated. Gates for FcεRI$^-$ (I) and FcεRI$^+$ monocytes (II) and basophils/DC (III) are shown. Expression of **B,** 2D7; **C,** CD1c/BDCA-1; **D,** BDCA-2; and **E,** BDCA-3, after gating on the FcεRI$^-$ (red histograms) or FcεRI$^+$ (purple histograms) monocyte, basophil (green histogram in **B**) or DC (green histograms in **C–E**) populations. Results shown are representative of 3 non-atopic control and 3 allergic asthmatic subjects.

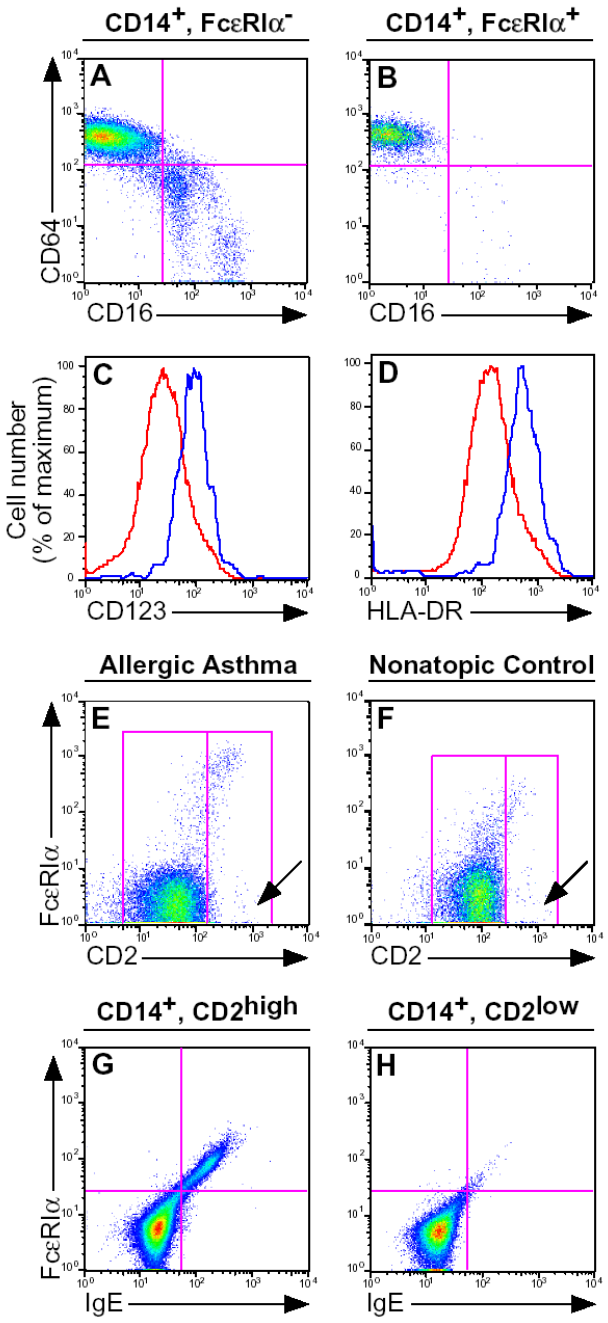

Figure 2. FcεRIα expression by monocyte subpopulations

After gating on FcεRI^{-} **(A)** or FcεRI^{+} **(B)** monocyte populations, a CD16 vs. CD64 dot plot was generated. FcεRI^{-} (red histogram) or FcεRI^{+} monocytes (blue histogram) expression of CD123 **(C)** and HLA-DR **(D)**. After gating on CD14+ monocytes from allergic asthmatic **(E)** and non-allergic **(F)** donors, a CD2 vs. FcεRI dot plot was generated. After gating on **(G)** CD14^{+}, CD2^{+} or **(H)** CD14^{+}, CD2^{-} monocytes from an allergic asthmatic donor, an FcεRI vs. IgE dot plot was generated. Results shown are representative of 6 **(A, B)**, 7 **(C, D)**, 18 asthmatic and 14 non-atopic control **(E, F)** and 6 **(G, H)** subjects, respectively.

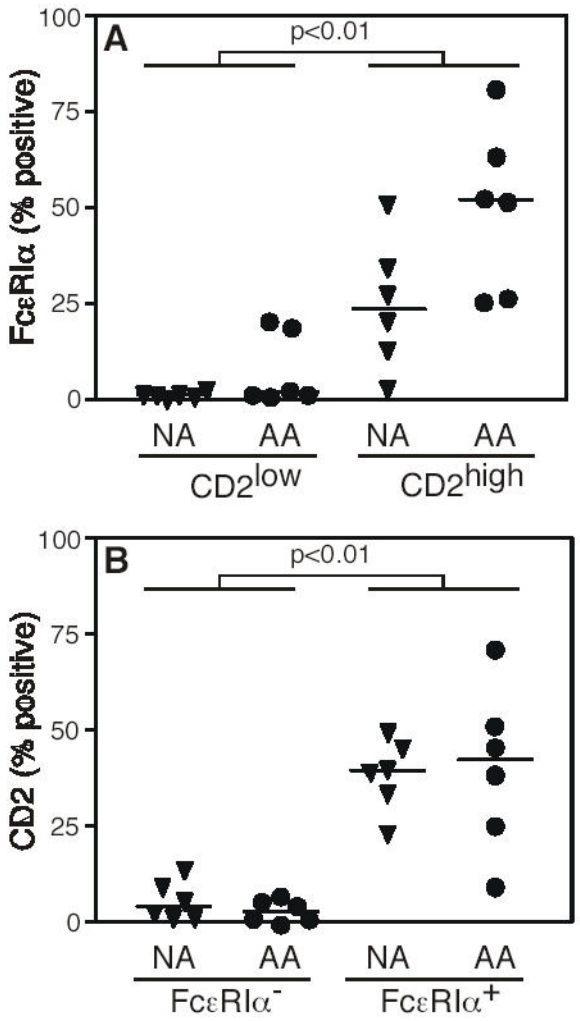

Figure 3. CD2^{high} monocytes express high levels of surface FcεRIα

A, CD2^{low} and CD2^{high} monocytes from 6 allergic asthmatic (AA) and 6 healthy non-atopic healthy control (NA) subjects were analyzed for FcεRI expression. **B,** FcεRI^{-} and FcεRI^{+} monocytes from allergic asthmatic and healthy non-atopic control subjects were analyzed for CD2 expression. Each symbol represents a unique subject. Horizontal bars denote median values. Statistical significance was determined using the Mann-Whitney U test.

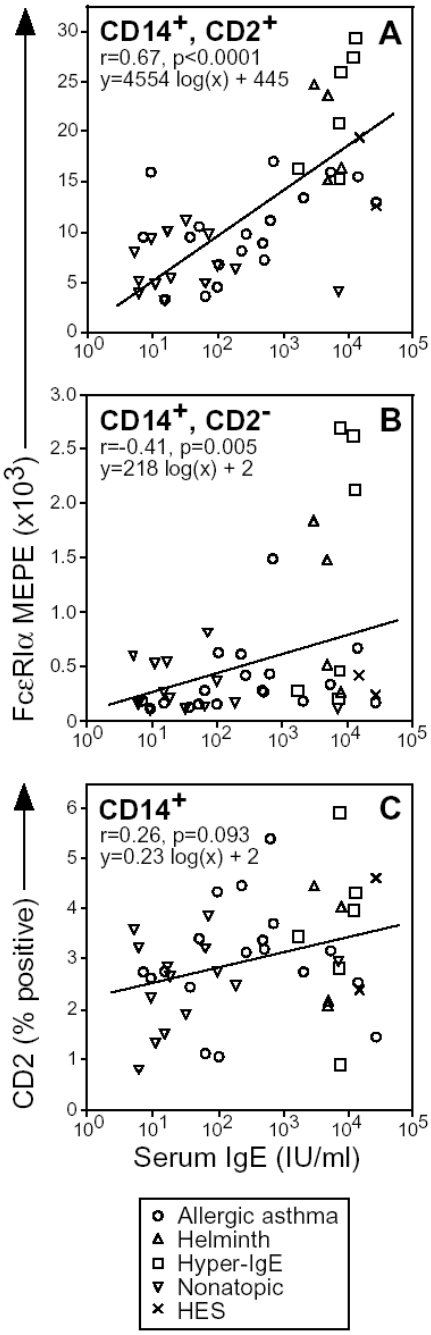

Figure 4. CD2^{high} monocyte FcεRIα expression correlates with serum IgE

Surface FcεRIα expression by CD2^{high} **(A)** and CD2^{low} **(B)** monocytes was plotted vs. serum IgE concentration. In (C) the percentage of monocytes staining CD2^{high}, FcεRI^{+} was plotted vs. serum IgE. Subject information is detailed in table I. Each symbol represents a unique subject. Correlation of FcεRI and serum IgE was determined using the Spearman rank correlation test. The line fitting these results was determined using linear regression analysis.

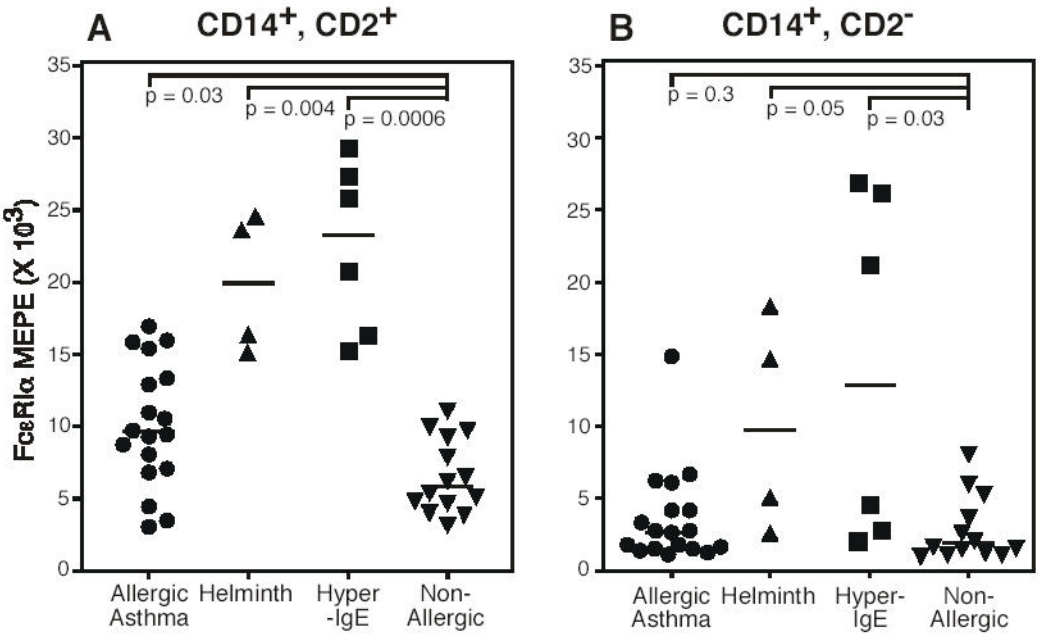

Figure 5. Monocyte FcεRIα expression as a function of disease category

Surface FcεRIα expression by $CD2^{high}$ **(A)** and $CD2^{low}$ **(B)** monocytes was determined and plotted against diseases category. Subject information is detailed in table I. Each symbol represents a unique subject. Horizontal bars denote median values. Statistical significance was determined using the Mann-Whitney U test.

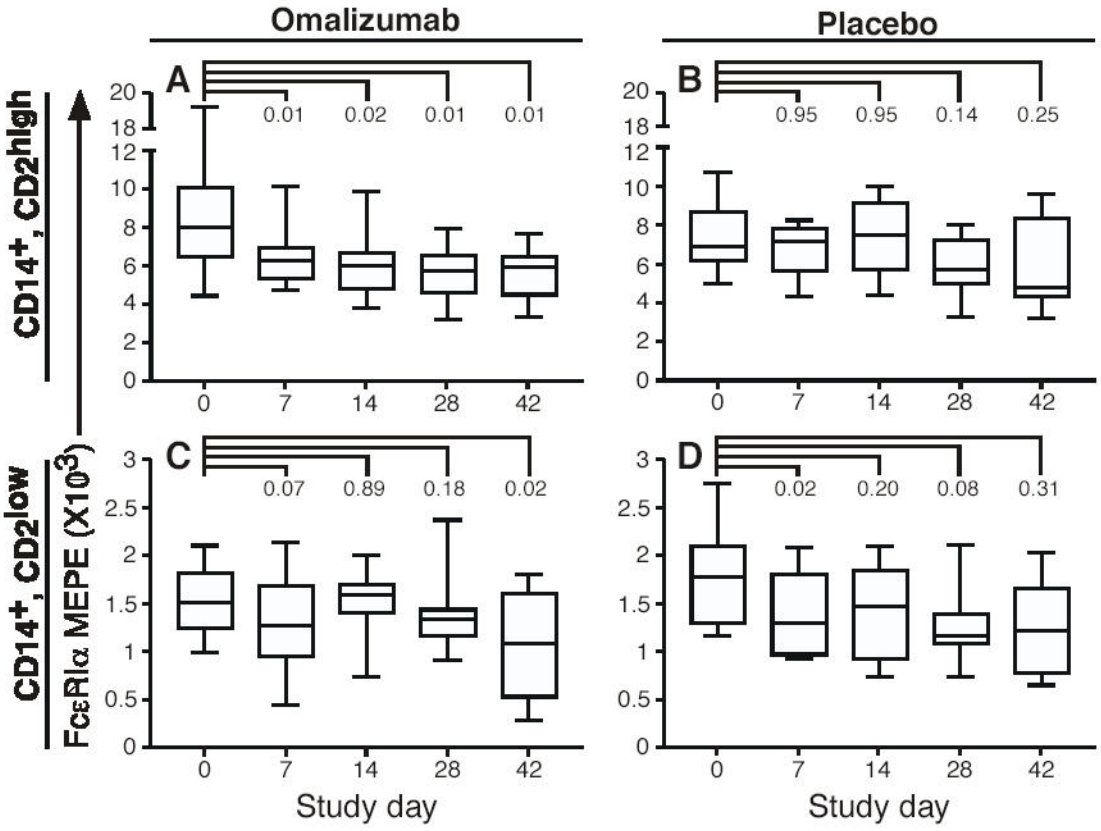

Figure 6. Omalizumab downregulates FcεRIα expression selectively in CD2^{high} monocytes

A–D, Box-and-whisker plots showing FcεRIα expression in CD2^{high} (top) and CD2^{low} monocytes plotted for each study visit. The *horizontal middle line* represents the median, the *top* and *bottom lines* represent the quartile values, and the T *bars* represent the maximum and minimal values. Statistical significance was determined by using the Wilcoxon signed-rank test.

Table I

Subject characteristics

Diagnosis	Number of subjects	Age (years)	Sex (M/F)	Serum IgE (IU/ml)
Allergic Asthma	18	36	10/8	361
Non-atopic Healthy Control	14	46	9/5	16
Hyper-IgE/Job's	6	26	2/4	7188
Helminth	4	58	1/3	4780
Hypereosinophilic	2	29	1/1	20,212

Values for age and serum IgE are median values for each subject group.