Aged mice have increased inflammatory monocyte concentration and altered expression of cell-surface functional receptors

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The expression of monocyte cell-surface receptors represents one index of immune dysfunction, which is common with aging. Although mouse models of aging are prevalent, monocyte subset assessment is rare. Our purpose was to compare cell receptor expression on classic ($CD115^{+}/Gr\text{-}1^{high}$) and non-classic ($CD115^{+}/Gr\text{-}1^{low}$) monocytes from 80- or 20-week-old CD-1 mice. Three-colour flow cytometry was used to determine the concentration of monocyte subsets and their respective cell-surface expression of TLR2, TLR4, CD80, CD86, MHC II and CD54. These receptors were selected because they have been previously associated with altered monocyte function. Data were analysed with independent *t*-tests; significance was set at $P<0.05$. Old mice had a greater concentration of both classic (258%, $P=0.003$) and non-classic (70%, $P=0.026$) monocytes. The classic : non-classic monocyte ratio doubled in old as compared with that in young mice ($P=0.006$), indicating a pro-inflammatory shift. TLR4 (↓27%, $P=0.001$) and CD80 (↓37%, $P=0.004$) were decreased on classic monocytes from old as compared with those from young mice. TLR2 (↑24%, $P=0.002$) and MHCII (↓21%, $P=0.026$) were altered on non-classic monocytes from old as compared with those from young mice. The increased classic : non-classic monocyte ratio combined with changes in the cell-surface receptor expression on both monocyte subsets is indicative of immune dysfunction, which may increase age-associated disease risk.

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1. Introduction

Increased biological aging is associated with immune dysfunction, which has been implicated in the pathophysiology of cardiovascular disease (CVD) and type 2 diabetes mellitus (DM2) (Saurwein-Teissl, *et al.* 2000; Esch and Stefano 2002). Complications associated with chronic disease onset increase morbidity and mortality rates in older adult populations (Cesari *et al.* 2003; Licastro *et al.* 2005). In humans, phenotyping of peripheral blood monocytes has improved the understanding of age-related immune dysfunction and its role in chronic disease (Frankenberger *et al.* 1996; Sadeghi *et al.* 1999; Afiune Neto *et al.* 2006). Aging tends to favour pro-inflammatory cytokine production, which can be accounted for by shifts that favour the pro-inflammatory/non-classic ($CD14^{dim}/CD16^{bright}$) monocyte subset (Sadeghi *et al.* 1999). As in humans, mouse monocytes exist in at least two subpopulations. These populations are categorized by universal expression of the macrophage – colony stimulating factor (M-CSF) receptor, CD115, and differential expression of GR-1 (Ly-6C epitope) (Sunderkötter *et al.* 2004). $CD115^{+}/GR\text{-}1^{high}$ monocytes are considered the classic subset while the $CD115^{+}/GR\text{-}1^{low}$ monocytes are considered the non-classic subset; classic monocytes are recent immigrants from the bone marrow and are preferentially recruited to sites of inflammation or infection (Sunderkötter *et al.* 2004; Swirski *et al.* 2007). Conversely, when little to no inflammation is present, classic monocytes mature into the non-classic phenotype, which have lost the ability to migrate into inflamed tissue (Sunderkötter *et al.* 2004). Thus, the classic subset is comprised of 'inflammatory' monocytes, whereas the non-classic subset consists of 'resident' monocytes that are surmised to circulate in the blood and enter only healthy tissue to replenish resident macrophages (Gordon and Taylor 2005).

In addition to shifts in monocyte subset concentration, the expression of various cell-surface receptors is considered

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predictive of changes in monocyte functional capacity (van Duin *et al.* 2007). In our laboratory, we have previously linked monocyte TLR2 and TLR4 cell-surface expression to inflammatory capacity (McFarlin *et al.* 2004, 2006). While monocyte function receptors have been explored in human models, few studies have comprehensively examined them in mice. Mice are an ideal model for studying age-associated effects vis-à-vis humans because of their short life span and greater ability to delimit key outcome variables. We hypothesized that, similar to those in humans, monocytes from old mice would have a shift toward an increased proportion of inflammatory monocytes with altered functional receptors. The purpose of our study was to compare peripheral blood monocyte subset concentration and cell-surface receptor expression in young *versus* old mice.

2. Methods

2.1 *Animals*

All procedures were approved by the University of Houston Institutional Committee for the Care and Use of Animals. Outbred, CD-1 male mice (Charles River Labs; Wilmington, MA) were used for the present study and grouped according to age: young (N=18; 20 weeks old) or old (N=18; 80 weeks old). This particular strain of mice was selected, because the inter-mouse variability is consistent with human populations due to their outbred origin. Body weight was determined using a digital scale and within normal growth curves for CD-1 mice in our facility.

2.2 *Non-lethal venous blood collection*

Following an overnight fast (>8 h), approximately 80 μL of blood was collected from the saphenous vein using a non-lethal technique (Esposito *et al.* 2010). All blood samples were collected during the last 2 h of the light cycle. Briefly, each mouse was humanely restrained in a modified 50 mL centrifuge tube, exposing one of the hind limbs. Hair was removed using electric clippers and a thin layer of petroleum jelly was applied to the skin. The saphenous vein was punctured using a sterile 5 mm lancet and blood was collected into a microvette tube treated with lithium heparin (Sarstedt; Newton, NC). Approximately 1 μL of blood was analysed for glucose using a commercial glucose analyser (Advocate Medical Services Inc.; Tampa, FL).

2.3 *Set-up of flow cytometry experiments*

Prior to analysis of study samples, we set up our flow cytometry analysis protocols based on a series of methods we have described previously (Breslin *et al.* 2011). During the set-up of the protocols used in the present study, we found several instances where the isotype control antibodies stained in a similar position but at a lower level than the positive peaks (CD80, CD86 and MHCII). In order to investigate this further, we completed some additional preliminary tests. First, we stained with an unlabelled antibody specific to the target marker, then followed this with staining with a labelled antibody against the target marker. This staining procedure identified the amount of non-specific binding associated with a given antibody in a more specific manner than a generic isotype control. When using this procedure, we found that the non-specific staining was approximately one log decade less than what was observed with the isotype control. A series of back-gating tests demonstrated that non-specific staining was <11% for all monocyte subsets when CD80, CD86 or MHCII were considered. Our preliminary testing suggests that care should be taken when using isotype control antibodies as the sole negative test because it might not always reflect true non-specific antibody binding. These controls along with isotype staining for TLR2 and TLR4 were used to place the negative populations in the first log decade of the acquisition histograms.

2.4 *Flow cytometry labelling*

All flow cytometry antibodies and reagents were purchased from e-Bioscience (San Diego, CA) unless otherwise noted. Aliquots of heparin-treated whole blood (10 μL) were transferred to 1.2 mL library tubes and treated with FC (CD16/32) blocking cocktail for 10 min. FC-blocked cells were washed with phosphate buffered saline (PBS; Sigma-Aldrich; St. Louis, MO) and re-suspended in staining buffer with three-colour cell surface antibody cocktail: CD115-Biotin, Gr-1-PECy5 and a PE-labelled antibody against TLR2, TLR4, CD80, CD86 or MHC II. The particular clone of the antibody against Gr-1 (RB6-8 C5) that was utilized is known to bind to the Ly6C epitope on monocytes (Daley *et al.* 2008); thus, Gr-1 is synonymous with Ly6C for the purposes of the current study. Anti-mouse monoclonal antibodies were diluted 1:50 with staining buffer. Following a 30 min incubation, FITC-labelled streptavidin was added to each tube and samples incubated for 30 min in the dark. Red blood cells were lysed with a commercial RBC lysis solution validated for mouse blood. The lysing action was stopped with 500 μL of PBS added prior to centrifugation (2000g, 10 min). Cells were suspended in 75 μL of staining buffer and 75 μL of 1% paraformaldehyde (Electron Microscopy Sciences; Hatfield, PA). Single-colour samples were used for compensation controls. All samples were run uncompensated on the Millipore-Guava EasyCyte Mini flow cytometer (Hayward, CA). Absolute cell counting (i.e. concentration) was possible because the flow cytometer uses a precise volumetric syringe to deliver cell preps into the flow cell.

2.5 *Flow cytometry gating and acquisition*

FCS data files were exported to FCS Express for compensation and analysis of our markers (v.3.0; DeNovo Software; Los Angeles, CA). A primary two-parameter dot plot (CD115 vs SSC) was used to quantify the percentage of monocyte events. A secondary dot plot (CD115 vs Gr-1) was used to distinguish classic and non-classic subsets. The classic-to-non-classic ratio was calculated by dividing classic concentration by non-classic concentration. Two, one-parameter histograms (count vs PE-labelled marker) were gated on classic, or non-classic, monocytes to determine the geometric mean fluorescent intensity (gMFI) of PE-labelled cells-surface receptors.

2.6 *Statistical analysis*

SPSS v.17 (Chicago, IL) was used for statistical analysis. Prior to formal statistical testing, data were individual checked for normality and constant error variance using the explore function in SPSS. Outliers (>3 SD from the mean) were discarded prior to analysis. Separate independent *t*-tests were used to assess all dependent variables. Significance was set at a $P<0.05$.

3. Results

3.1 *Total monocyte and subset concentrations*

The mean concentration of $CD115^+$ monocytes was 120% greater in old than in young mice ($t=3.05$, $P=0.006$; figure 1). Similarly, mean classic (258%, $t=5.60$, $P=0.001$; figure 1) and non-classic (70%, $t=2.34$, $P=0.026$; figure 1) concentrations were greater in old than in young mice. Regarding the average proportions of monocyte subsets, 22% of monocytes were classic and 77% were non-classic in young mice. In old mice, 37% were classic while 62% were non-classic (figure 2). The classic-to-non-classic monocyte ratio was significantly greater in old (0.56) than in young mice (0.26) ($t=3.111$, $P=0.006$), which may be indicative of a pro-inflammatory shift within blood monocytes.

3.2 *Antigen detection and inflammatory response: toll-like receptors*

gMFI values for each cell-surface receptor can be found in table 1. Non-classic TLR2 expression was 24% greater in old than in young mice ($t=3.33$, $P=0.002$), whereas classic TLR4 expression was 27% lower than that of young mice ($t=3.04$, $P=0.005$). A representative dot plots for non-classic TLR2 is presented in figure 3A. This relative distribution of TLR4 was similar to that of TLR2. We did not find any evidence of additional secondary populations other than the two subsets of monocytes. The receptors appeared to be normally distributed within a given receptor.

3.3 *Receptors involved in T-lymphocyte priming and antigen presentation*

Similar to TLR4, classic CD80 expression in old mice was 37% lower than in young mice ($t=3.32$, $P=0.004$). MCH II

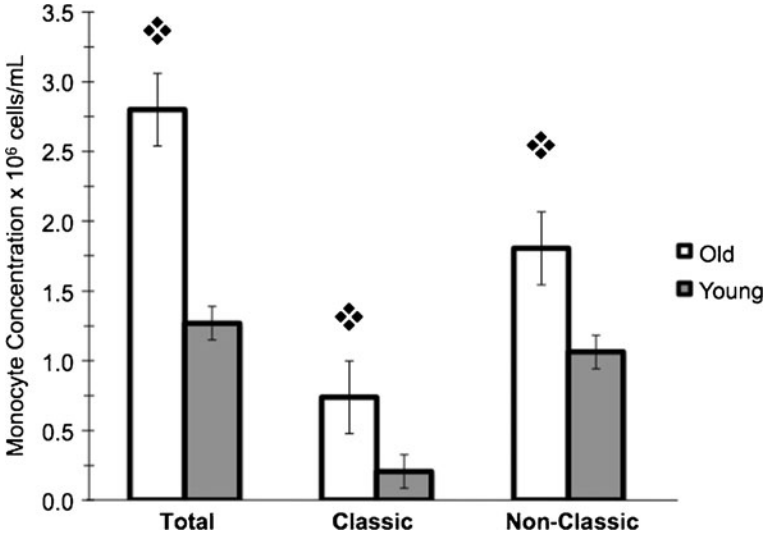

Figure 1. Total monocyte and subset concentration. Blood monocytes from young (20 weeks; $N=18$) and old (80 weeks; $N=18$) were compared for total and subset concentrations and cell-surface receptor expression using flow cytometry. Expression was assessed in both classic ($CD115^+/Gr\text{-}1^{high}$) and non-classic ($CD115^+/Gr\text{-}1^{low}$) monocyte subsets. This figure demonstrates group differences in cell concentration within total, classic and non-classic monocyte populations. ❖ $P<0.05$ between young and old mice within a monocyte population.

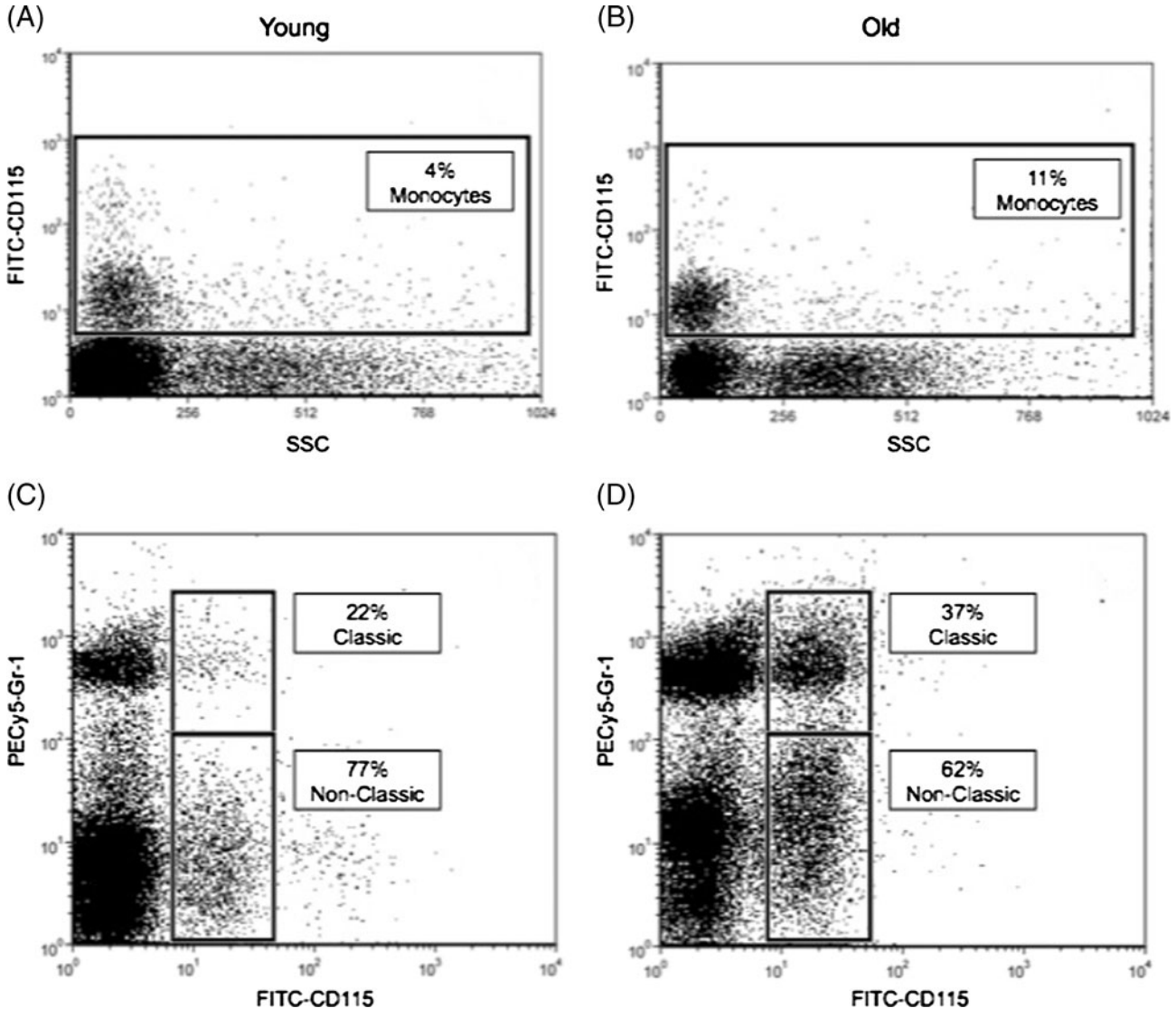

Figure 2. Flow cytometric determination of monocyte subsets. Blood monocytes from young (20 weeks; N=18) and old (80 weeks; N=18) were compared for total and subset concentrations and cell-surface receptor expression using flow cytometry. Expression was assessed in both classic ($CD115^+/Gr\text{-}1^{high}$) and non-classic ($CD115^+/Gr\text{-}1^{low}$) monocyte subsets. Panels (**A**) and (**B**) illustrate the identification and proportion of $CD115^+$ monocytes in young and old mice, respectively; panels (**C**) and (**D**) illustrate the differentiation of $CD115^+$ monocytes based upon high and low expression of Gr-1 in young and old mice, respectively.

expression on non-classic monocytes in old mice was 21% lower than in young mice (t=3.34, P=0.026). No significant age difference was found for CD86 on either monocyte subset. A representative dot plot for classic CD80 cell-surface expression is presented in figure 3B. The relative distribution of MHCII was similar to that of CD80. We did not find any evidence of additional secondary populations other than the two subsets of monocytes. The receptors appeared to be normally distributed within a given receptor.

4. Discussion

The key findings of the present study were that old mice had a significantly greater monocyte concentration, which was accounted for by an increase in both the classic (inflammatory) and non-classic subsets. The classic-to-non-classic monocyte ratio doubled in old mice, which is indicative of a shift toward accumulation of pro-inflammatory monocytes. There were also changes in the cell-surface receptor expression on both subsets, which is consistent with a

Table 1. Geometric mean fluorescent intensity (gMFI) of cell-surface receptors on classic ($CD115^+/Gr\text{-}1^{high}$) and non-classic ($CD115^+/Gr\text{-}1^{low}$) monocyte subsets in young (20 weeks; $N=18$) and old (80 weeks; $N=18$) CD-1 mice

	Classic			Non-Classic		
Cell-surface receptor	Young	Old	Difference	Young	Old	Difference
TLR2	18.10±0.72	18.69±0.59	3.3%	12.74±0.36	15.75±0.88*	23.6%
TLR4	26.78±1.66	19.49±1.62*	27.2%	11.74±0.33	14.00±1.14	19.2%
CD80	23.13±2.44	14.61±0.95*	36.8%	9.79±0.35	10.03±0.34	2.5%
CD86	30.46±3.34	28.10±5.36	7.8%	11.47±0.37	10.93±0.34	4.6%
MHC II	34.86±2.91	30.92±1.78	11.3%	33.83±2.20	26.73±2.03*	21.0%

Cell-surface receptor expression was determined using three-colour flow cytometry. Values represent Group Mean for gMFI±SEM. Percentage differences in gMFI between young and old mice are indicated within each monocyte subset. *$P<0.05$ between young and old mice within a monocyte subset.

pattern of reduced monocyte functional capacity. Collectively, these findings reflect that, in aged mice, peripheral blood monocyte subsets display phenotypical, functional receptor expression and proportional differences that have previously been associated with immune dysfunction and increased disease risk in aged humans and mice (Renshaw *et al.* 2002; Boehmer *et al.* 2004; Stewart *et al.* 2005; McFarlin *et al.* 2006; Esposito *et al.* 2010).

In the current study, old mice had a significantly greater total ($CD115^+$), non-classic ($CD115^+/Gr\text{-}1^{Low}$) and classic ($CD115^+/Gr\text{-}1^{High}$) monocyte concentrations compared to young mice. Regardless of age, the largest portion of monocytes was accounted for by the non-classic subset; however, old mice had a shift toward a greater portion of classic monocytes than young mice. Our observed elevation in classic, inflammatory monocytes was consistent with what has been reported in humans, in that advanced age was associated with an increase in pro-inflammatory ($CD14^{dim}/CD16^{bright}$) monocytes (Sadeghi *et al.* 1999). Because classic monocytes in mice are preferentially recruited to atherosclerotic plaques (Tacke *et al.* 2007), it is likely that increases we observed may be associated with elevated cardiovascular disease risk in old than in young mice.

We also found that classic monocytes from old mice had lower TLR4 and CD80 cell-surface expression than young mice. Our findings are consistent in regard to TLR4 and CD80 with what others have reported in mouse macrophages and human monocytes (Renshaw *et al.* 2002; Boehmer *et al.* 2004). Since classic monocytes are considered to be naïve M1 (or classically activated) macrophages recruited to inflamed tissue (Swirski *et al.* 2007), it is logical that reduced classic TLR4 expression may mirror age-associated declines in monocyte functional capacity (Curat *et al.* 2004). The observed effect is also consistent with what we have reported following a period of diet-induced weight gain in CD-1 mice (Esposito *et al.* 2010); however, it is the opposite of what we have observed in overweight and physically inactive humans (McFarlin *et al.* 2004, 2006). Based on our previous findings and that of others, it appears that monocyte TLR4 expression may exhibit a different response between mice and men, while the net reduction in monocyte functional capacity is similar. Further research is needed to understand functional differences between mouse and human monocytes with respect to TLR4 expression. Our findings with respect to classic monocyte CD80 expression are consistent with what others have reported. Fleischer *et al.* (1996) reported that monocyte CD80, but not CD86, was an important co-stimulatory factor for *in vitro* T-lymphocyte response to tuberculin virus and other infectious agents (Boucher *et al.* 1998; van Duin *et al.* 2007). Thus, our observed changes with respect to CD80 expression are consistent with previous reports and indicative of diminished monocyte co-stimulatory activity, which diminishes the immune response. The alterations in classic monocytes in the present study have implications for the observed changes in non-classic monocytes, since classic monocytes mature into either peripheral tissue macrophages or blood non-classic monocytes (Sunderkötter *et al.* 2004). Thus, it is plausible that the diminished TLR4 and CD80 on classic monocytes was a precursor to observed differences in TLR2 and MHC II on non-classic monocytes. Collective changes in both monocytes subsets are likely reflective of diminished blood and peripheral tissue immune response.

Similar to classic monocytes, the cell-surface phenotype of non-classic monocytes in the present study was indicative of diminished functional capacity. Specifically, we found an increase in TLR2 and a decrease in MHC II cell-surface expression on non-classic monocytes from old as compared to young mice. The direction of change for TLR2 was opposite to that of TLR4 on classic monocytes; however, the TLR2 response was consistent with what we have observed in physically inactive humans (Stewart *et al.* 2005). One possible explanation for observed differences is that classic TLR4 expression increased as an attempt to

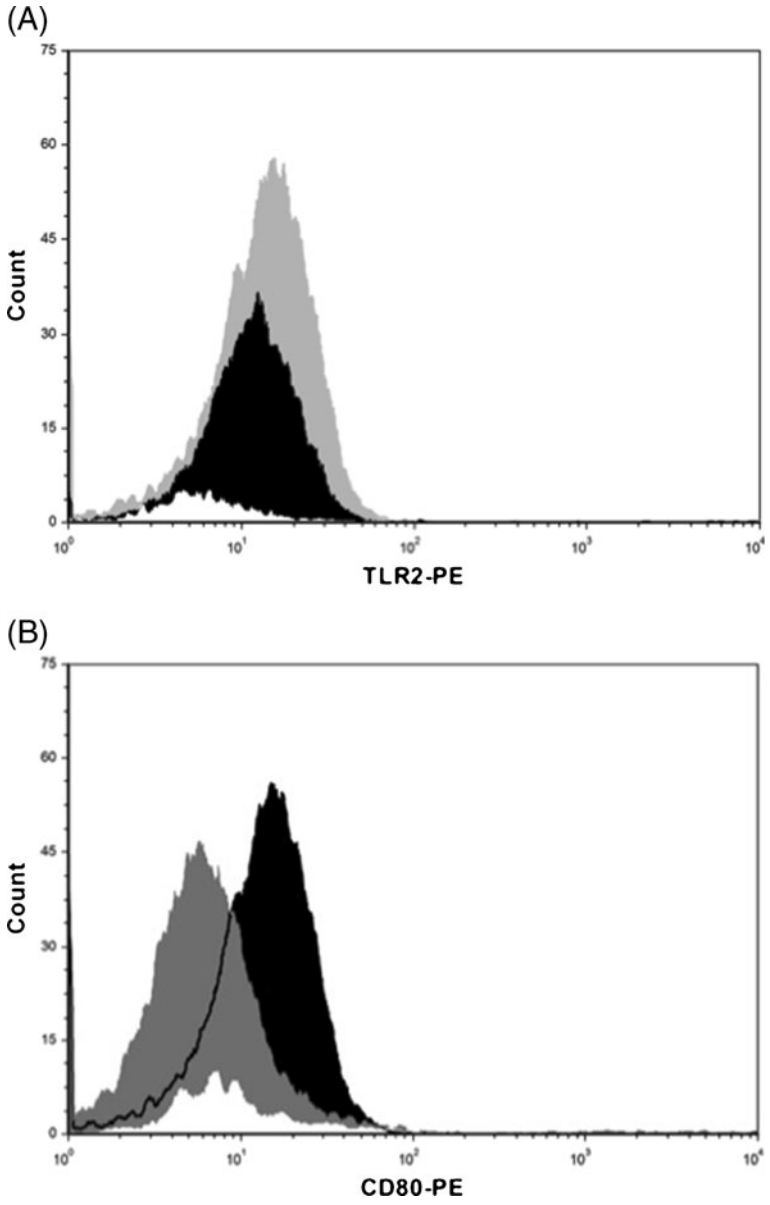

Figure 3. Representative dot plots monocyte cell-surface receptors. Panel (**A**) represents non-classic TLR2 expression. Blood monocytes from young (20 weeks; *N*=18; black lines) and old (80 weeks; *N*=18; grey lines) were compared for total and subset concentrations and cell-surface receptor expression using flow cytometry. The white line represents the isotype control antibodies that corresponded with each cell-surface receptor. The observed isotyping staining is consistent with that of TLR4, CD54, and CD25. Panel (**B**) represents classic CD80 expression. Blood monocytes from young (20 weeks; *N*=18; black lines) and old (80 weeks; *N*=18; grey lines) were compared for total and subset concentrations and cell-surface receptor expression using flow cytometry. The white line represents the 'inhibition' control that corresponded with each cell-surface receptor. The observed isotyping staining is consistent with that of CD86 and MHCII.

compensate for diminished non-classic TLR2 expression. The most likely factor contributing to decreased TLR2 is IFN-γ. It has been previously reported IFN-γ is elevated with advanced age (Ernst *et al.* 1993) and that prolonged *in vitro* stimulation with IFN-γ results in decreased TLR2 cell-surface expression (Matsuguchi *et al.* 2000). MHC II plays a key role in monocyte antigen-presenting capacity (de Waal Malefyt *et al.* 1991); thus, the functional capacity of non-classic monocytes was diminished in our older mice.

In summary, we found that aged mice had a greater monocyte concentration that was primarily accounted for by a shift toward increased classic or 'inflammatory' monocytes.

In addition to a change in total and subset monocyte concentration, the observed changes in cell-surface TLR2, MHC II and CD80 are consistent of age-associated declines in monocyte functional capacity. We also observed changes in TLR4 expression, which were consistent with what we have previously reported in mice, but different from what we have reported in humans. Further research is needed to determine how the observed changes in monocyte cell-surface phenotype contribute lifelong morbidity and premature mortality.

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References

Afiune Neto A, Mansur AP, Avakian SD, Gomes EP and Ramires JA 2006 Monocytosis is an independent risk marker for coronary artery disease *Arq. Bras. Cardiol.* **86** 240–244

Boehmer ED, Goral J, Faunce DE and Kovacs EJ 2004 Age-dependent decrease in Toll-like receptor 4-mediated proinflammatory cytokine production and mitogen-activated protein kinase expression. *J. Leukoc. Biol.* **75** 342–349

Boucher N, Dufeu-Duchesne T, Vicaut E, Farge D, Effros RB and Schächter F 1998 CD28 expression in T cell aging and human longevity. *Exp. Gerontol.* **33** 267–282

Breslin WL, Strohacker K, Carpenter KC, Haviland DL, McFarlin BK 2011 Mouse blood monocytes: standardizing their identification and analysis using CD115. *J. Immunol. Methods*. doi: 10.1016/j.jim.2011.03.005

Cesari M, Penninx BW, Newman AB, Kritchevsky SB, Nicklas BJ, Sutton-Tyrrell K, Tracy RP, Rubin SM, Harris TB and Pahor M 2003 Inflammatory markers and cardiovascular disease (The Health, Aging and Body Composition [Health ABC] Study). *Am. J. Cardiol.* **92** 522–528

Curat CA, Miranville A, Sengenès C, Diehl M, Tonus C, Busse R and Bouloumié A 2004 From blood monocytes to adipose tissue-resident macrophages: induction of diapedesis by human mature adipocytes. *Diabetes* **53** 1285–1292

Daley JM, Thomay AA, Connolly MD, Reichner JS and Albina JE 2008 Use of Ly6G-specific monoclonal antibody to deplete neutrophils in mice. *J. Leukoc. Biol.* **83** 64–70

de Waal Malefyt R, Haanen J, Spits H, Roncarolo MG, te Velde A, Figdor C, Johnson K, Kastelein R, Yssel H and de Vries JE 1991 Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. *J. Exp. Med.* **174** 915–924

Ernst DN, Weigle WO, Noonan DJ, McQuitty DN and Hobbs MV 1993 The age-associated increase in IFN-gamma synthesis by mouse CD8+ T cells correlates with shifts in the frequencies of cell subsets defined by membrane CD44, CD45RB, 3G11, and MEL-14 expression. *J. Immunol.* **151** 575–587

Esch T and Stefano G 2002 Proinflammation: a common denominator or initiator of different pathophysiological disease processes. *Med. Sci. Monit.* **8** HY1-9

Esposito LM, Simpson RJ, Strohacker K, Carpenter KC and McFarlin BK 2010 Defining a longitudinal survival model to examine forced treadmill running as a countermeasure for diet-induced weight gain. *Lab. Anim.* **44** 305–311

Fleischer J, Soeth E, Reiling N, Grage-Griebenow E, Flad HD and Ernst M 1996 Differential expression and function of CD80 (B7-1) and CD86 (B7-2) on human peripheral blood monocytes. *Immunology* **89** 592–598

Frankenberger M, Sternsdorf T, Pechumer H, Pforte A and Ziegler-Heitbrock HW 1996 Differential cytokine expression in human blood monocyte subpopulations: a polymerase chain reaction analysis. *Blood* **87** 373–377

Gordon S and Taylor PR 2005 Monocyte and macrophage heterogeneity. *Nat. Rev. Immunol.* **5** 953–964

Licastro F, Candore G, Lio D, Porcellini E, Colonna-Romano G, Franceschi C and Caruso C 2005 Innate immunity and inflammation in ageing: a key for understanding age-related diseases. *Immun. Ageing* **2** 8

Matsuguchi T, Musikacharoen T, Ogawa T and Yoshikai Y 2000 Gene expressions of Toll-like receptor 2, but not Toll-like receptor 4, is induced by LPS and inflammatory cytokines in mouse macrophages. *J. Immunol.* **165** 5767–5772

McFarlin BK, Flynn MG, Campbell WW, Craig BA, Robinson JP, Stewart LK, Timmerman KL and Coen PM 2006 Physical activity status, but not age, influences inflammatory biomarkers and toll-like receptor 4. *J. Gerontol. A Biol. Sci. Med. Sci.* **61** 388–393

McFarlin BK, Flynn MG, Campbell WW, Stewart LK and Timmerman KL 2004 TLR4 is lower in resistance-trained older women and related to inflammatory cytokines. *Med. Sci. Sports Exerc.* **36** 1876–1883

Renshaw M, Rockwell J, Engleman C, Gewirtz A, Katz J and Sambhara S 2002 Cutting edge: impaired Toll-like receptor expression and function in aging. *J. Immunol.* **169** 4697–4701

Sadeghi HM, Schnelle JF, Thomas JK, Nishanian P and Fahey JL 1999 Phenotypic and functional characteristics of circulating monocytes of elderly persons. *Exp. Gerontol.* **34** 959–970

Saurwein-Teissl M, Blasko I, Zisterer K, Neuman B, Lang B and Grubeck-Loebenstein B 2000 An imbalance between pro- and anti-inflammatory cytokines, a characteristic feature of old age. *Cytokine* **12** 1160–1161

Stewart LK, Flynn MG, Campbell WW, Craig BA, Robinson JP, McFarlin BK, Timmerman KL, Coen PM, Felker J and Talbert E 2005 Influence of exercise training and age on CD14+ cell-surface expression of toll-like receptor 2 and 4. *Brain Behav. Immun.* **19** 389–397

Sunderkötter C, Nikolic T, Dillon MJ, van Rooijen N, Stehling M, Drevets DA and Leenen PJM 2004 Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. *J. Immunol.* **172** 4410–4417

Swirski FK, Libby P, Aikawa E, Alcaide P, Luscinskas FW, Weissleder R and Pittet MJ 2007 Ly-6Chi monocytes dominate

hypercholesterolemia-associated monocytosis and give rise to macrophages in atheromata. *J. Clin. Invest.* **117** 195–205

Tacke F, Alvarez D, Kaplan TJ, Jakubzick C, Spanbroek R, Llodra J, Garin A, Liu J, *et al.* 2007 Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. *J. Clin. Invest.* **117** 185–194

van Duin D, Allore HG, Mohanty S, Ginter S, Newman FK, Belshe RB, Medzhitov R and Shaw AC 2007 Prevaccine determination of the expression of costimulatory B7 molecules in activated monocytes predicts influenza vaccine responses in young and older adults. *J. Infect. Dis.* **195** 1590–1597

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