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A mouse model of yellow fluorescent protein (YFP) expression in hematopoietic cells to assess leukocyte–endothelial interactions in the microcirculation

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Abstract

In this study, we describe the use of intravital microscopy in a transgenic mouse model expressing yellow fluorescent protein (YFP) under the control of a monocyte specific promoter c-fms (CD115) to track and quantify specific leukocyte subsets. Flow cytometry on peripheral and bone marrow leukocytes revealed that YFP was predominantly expressed by CD11a⁺, CD11b⁺, and CD14⁺ monocytes. In the bone marrow, 67±4% of Ly6C^{high} F4/80⁺ cells were YFP^{high} while 55±1% of Ly6C^{low} F4/80⁺ cells were YFP^{low} supporting the use of c-fms^{YFP} expression as a marker of monocyte lineage. 70±7% of CD11b⁺ F4/80⁺ Ly6C⁺ (“triple positive”) cells expressed YFP. To assess leukocyte–endothelial interactions in YFP⁺ cells in c-fms^{YFP} mice, we evaluated leukocyte adhesion, rolling and local shear stress responses in the cremasteric endothelium 4 h following administration of TNFα. TNFα resulted in a five-fold increase in adhesion of YFP⁺ cells to the endothelium and provided superior discriminative ability in assessing rolling and adhesion events when compared with bright field microscopy. Additionally, when compared with Rhodamine-6G labeled leukocytes or GFP⁺ cells in mice transplanted with green fluorescent protein (GFP) positive bone marrow, the level of detail observed in the c-fms^{YFP} was greater, with both GFP⁺ and YFP⁺ cells demonstrating superior signal to noise compared to bright field microscopy. A weak positive linear correlation between wall shear stress and YFP⁺ cell adhesion ($r^2=0.20$, $p<0.05$) was seen in the cremasteric microcirculation. Taken together, these data demonstrate the use of c-fms^{YFP} mice in identifying distinct monocyte subsets and highlight the potential of this model for real-time monocyte–endothelial interactions using intravital microscopy.

Keywords

Yellow fluorescent protein (YFP); Leukocyte; Intravital microscopy; Adhesion; Monocyte

Introduction

Monocyte margination to the endothelium and subsequent adhesion and migration are essential steps in inflammatory disorders of the vasculature (Ross, 1999; Granger and Kubes, 1994). It is now appreciated that monocytes are not a homogenous population of cells, demonstrating a complex differentiation from progenitor cell populations in the bone marrow to eventual cell subsets that differ vastly in physiology and function.

There has been considerable interest in identifying monocyte subsets using unique surface markers or combinations of markers, with the premise that specific monocyte sub-types may be involved in physiological defense and inflammation (Geissmann et al., 2008, 2003; Tsou et al., 2007; Tacke and Randolph, 2006). An early approach used the Gr1 antigen to differentiate monocyte populations from neutrophils. Subsequent studies have shown that Gr1 is composed of two antigens, Ly6C and Ly6G. The Ly6G component is neutrophil specific while Ly6C is monocyte specific, with the degree of expression of Ly6C reflecting differentiation (Ly6C^{high}, Ly6C^{int} and Ly6C^{low}, etc.). Sunderkotter et al. (2004) have reported that bone marrow monocytes (representing an earlier developmental stage) express high levels of Ly6C, while blood monocytes express lower levels of Ly6C. Ly6C^{high} monocytes released from bone marrow have been shown to be significantly increased in the circulation following monocyte depletion, infections and home to areas of inflammation, presumably through adhesion and migration across the endothelium (Swirski et al., 2007; Sunderkotter et al., 2004). An alternate approach used 7/4, a marker expressed on both neutrophils and monocytes (Tsou et al., 2007). Using a combination of Ly6G and 7/4, Tsou et al. (2007) identified a homogenous population of monocytes that is 7/4⁺, Ly6G⁻ which increased in response to high fat feeding.

In this report, we describe the utility of a novel transgenic mouse model, c-fms^{YFP+} that expresses yellow fluorescent protein (YFP) under the control of a monocyte specific promoter c-fms (CD115, Csf-1R) allowing for targeted expression of this marker in cells of monocyte lineage. c-fms (CD115, Csf-1R) is a well characterized monocyte marker encoding the macrophage colony-stimulating factor receptor (Auffray et al., 2009). The goal of this study was to examine the expression of YFP in various monocyte subsets and to further demonstrate the utility of this mouse model in delineating real-time monocyte–endothelial interactions using intravital microscopy.

Material and methods

Animal models

c-fms^{YFP+} transgenic mice were generated at the Transgenic Animal Service of Queensland, Brisbane, Queensland, Australia (www.tasq.uq.edu.au) by injection of the transgenes into pronuclei of (C57BL/6×CBA)F₁ (BCBF1) fertilized eggs (Sasmono et al., 2003). The Committee on Use and Care of Animals from the Ohio State University (OSU) approved all experimental procedures. c-fms^{YFP+} mice of the FVB/N strain were bred and genotyped at OSU and housed in cages individually. The mice were studied at the age of 8–16 weeks. C57BL/6 mice were acquired from the Jackson Laboratory (Bar Harbor, ME, USA) at the age of 4–5 weeks and were studied at the age of 8–12 weeks.

Bone marrow transplant of GFP⁺ cells

C57BL/6 mice at the age of 8 months underwent irradiation (1100 cGy×2 doses, 4 h apart, Shepherd irradiator) and were transplanted with marrow derived from transgenic mice expressing GFP. Bone marrow was collected out of femur and tibia of the donor mouse and injected to the recipient mouse intravenously. GFP⁺ mice were studied at the age of 9 months.

In a subset of c-fms^{YFP+} mice, TNF α (1 μ g/kg, Sigma Chemical Co., St. Louis, MO, USA) was administered intraperitoneally (i.p.) 4 h before the experimental procedures.

Flow cytometry

Whole blood was collected to assess the expression of YFP in the peripheral leukocytes in c-fms^{YFP+} mice while bone marrow was collected by flushing the femur and tibia with 1 $\times$ PBS. Blood (1 ml) was treated with red blood cell lysis buffer (155 mM NH₄Cl, 10 mM KHCO₃, and 0.1 mM EDTA) for 5 min (two times) prior to further steps. Leukocytes were isolated from bone marrow with Histopaque-1077 (Sigma). Isolated leukocytes from both blood and bone marrow were then resuspended in flow buffer (1 $\times$ PBS containing 5% bovine serum albumin (BSA) and 0.02% NaN₃) and washed twice. This was followed by incubation with anti-CD14-APC (eBioscience, San Diego, USA), anti-Ly6G-PE-Cy7 (eBioscience), anti-Ly6C-APC (eBioscience) anti-CD3-APC (Pharmingen, San Jose, USA), anti-CD45.R-PE (Pharmingen), anti-F4/80-APC (Biolegend, San Diego, USA), anti-CD11a-PE (Biolegend), anti-CD11b-PE (Biolegend) and anti-7/4-PE (AbD Serotec, Raleigh, USA). Cells were subsequently analyzed by flow cytometry (BD FACSCantoTM flow cytometer, Becton Dickinson, San Jose, USA) and the data were analyzed on BD FACSDiva software (Becton Dickinson).

Intravital microscopy and analyses

Under anesthesia, the testicular cremaster muscle was exposed using a dissecting microscopy (2 $\times$; Nikon SMZ 645, Japan). The cremaster muscle bathed in Ringers Lactate at 37 °C and leukocyte–endothelial interaction was assessed in 15–25 using a Nikon Eclipse FN1 microscope (Nikon, Japan) with a 40 $\times$ /0.80 W water immersed objective at a 2.0 mm working distance. In some experiments, Rhodamine-6G (0.4 mg/kg) (Biochemika, Steinheim, Germany) was injected into the tail vein (Batz et al., 1995) to label leukocytes. In all experiments video images were captured and digitalized to 12-bit TIF images using Metamorph software (version 7.1.2.0, Metamorph, Downingtown, USA). Flowing leukocytes (labeled or unlabeled) or YFP⁺ cells were counted per minute for different vessel diameters and vessel segments. All labeled leukocytes or YFP⁺ cells, per 100 μ m of vessel length, that were immobile for at least 30 s were interpreted as adherent cells (Lim et al., 2002). Signal intensity (SI) levels in the cells (in AU) and calculated signal-to-noise ratio (SNR) and contrast-to-noise ratio (CNR) were calculated using custom software (Metamorph). Mean velocity of leukocytes in venules (V_{mean}) was calculated by the velocity divided by 1.6 (Russell et al., 2003). The wall-shear rate (WSR) was calculated by $8 \times (V_{\text{mean}}/\text{diameter})$ (Bienvenu and Granger, 1993). Calculations to determine the number of rolling and adherent cells according to vessel diameter were performed using Optimap (Version 1.4.1.0).

Statistical analysis

All data are expressed as means $\pm$ standard deviation (SD). Statistical tests were performed using one-way ANOVA followed by Mann–Whitney test or unpaired *t*-test using GraphPad Prism (version 4.0). Statistical significance was set at $p < 0.05$.

Results

Table 1 depicts the leukocyte surface markers that were employed to study leukocyte subsets in peripheral blood (left columns) and in the bone marrow (right columns). Furthermore, the relative expression of YFP among cells detected with various surface markers are presented as YFP⁺ cells of leukocyte subset. In the peripheral blood, YFP was predominantly expressed by CD11a⁺ and CD11b⁺ monocytes (92.2 $\pm$ 7.1% and 88.8 $\pm$ 4.3%, respectively) and by CD14⁺ and F4/80⁺ monocytes (90.4 $\pm$ 4% and 64.4 $\pm$ 0.9%, respectively). CD3⁺

lymphocytes had the lowest degree of co-expression of YFP with only $3.6 \pm 0.1\%$ of $CD3^+$ cells expressing YFP⁺. Peripheral blood cells expressing F4/80, Ly6G and 7/4 all expressed YFP to some degree as outlined in Table 1. Similar trends were observed in the bone marrow, with YFP expression seen in $76.1 \pm 1.8\%$, $88.2 \pm 5.2\%$, $92.3 \pm 5.4\%$ and $61.4 \pm 1.3\%$ of cells expressing CD11a, CD11b, and CD14 and F4/80, respectively. $CD3^+$ lymphocytes were the cell type to co-express YFP the least.

Distinct $Ly6C^{high}$ ($8.3 \pm 0.6\%$) and $Ly6C^{low}$ ($16.9 \pm 0.9\%$) populations within $F4/80^+$ cells isolated from the bone marrow were identified (Fig. 1a). We characterized these cells further, and showed that $Ly6C^{high} F4/80^+$ cells tended to be YFP^{high} ($66.6 \pm 3.8\%$) and $Ly6C^{low} F4/80^+$ cells tended to be YFP^{low} ($54.6 \pm 1.2\%$). $Ly6C^{high} F4/80^+$ monocytes have been shown to infiltrate sites of inflammation, while $Ly6C^{low} F4/80^+$ cells have been shown to represent a population that differentiates to “resident” macrophages. Based on these experiments, YFP status corresponds to Ly6C expression in monocyte populations. Fig. 1b depicts YFP expression in cells that express a combination of F4/80 and Ly6C while Fig. 1c depicts YFP in relationship to CD11b and Ly6C expression. $66.2 \pm 4.8\%$ of $F4/80^+ Ly6C^+$ cells expressed YFP while $66.5 \pm 7.8\%$ of $CD11b^+ Ly6C^+$ cells expressed YFP. Interestingly most $CD11b^+$ cells co-expressed YFP while a significant proportion of cells expressing F4/80 did not co-express YFP. Fig. 2 shows the YFP expression in a triple positive population of F4/80, CD11b and Ly6C. About $49.8 \pm 5.7\%$ of all bone marrow cells are F4/80 and CD11b positive. Of these cells, $97.3 \pm 2.1\%$ express Ly6C. $69.6 \pm 7.0\%$ of $F4/80^+ CD11b^+ Ly6C^+$ cells co-express YFP. Alternatively, $7/4^+$, $Ly6G^-$ status can be used to identify an inflammatory monocyte subset. In our experiments, $2.3 \pm 0.5\%$ of all peripheral blood leukocytes were $7/4^+ Ly6G^-$ monocytes and of this population $46.6 \pm 9.5\%$ expresses YFP. In contrast, only $0.4 \pm 0.1\%$ of all bone marrow derived leukocytes were $7/4^+ Ly6G^-$ and of this population, approximately $32.2 \pm 10.3\%$ also co-expressed YFP.

Experiments were performed to validate the utility of using the $c-fms^{YFP+}$ mouse model to evaluate leukocyte–endothelial interactions in the cremasteric muscle bed. This was then compared to conventional bright field microscopy and other approaches such as Rhodamine-6G labeling of leukocytes and evaluation of GFP^+ cells in a bone marrow transplant model (C57BL/6) serving as recipient of marrow from GFP mice (C57BL/6). YFP^+ cells were easily identified owing to higher signal to background noise ratio compared with non-specific labeling approaches including Rhodamine-6G. Although signal to noise ratio was comparable between GFP^+ and YFP^+ cells (Fig. 3), non-specific fluorescence of other hematopoietic cells such as platelets precluded accurate identification of leukocytes. Fig. 4 demonstrates the relative noise ratio for each of the detection techniques using the cremasteric muscle model.

Using *in vivo* detection of cells in the cremasteric muscle bed, we noted that there were notable differences in the number of adherent cells according to the size of the venule. Since the number of rolling and adherent cells varied according to the size of the venule imaged, we initially tried to establish the relationship between vessel diameter and the events of adhesion and rolling using the Least Square Method (LSQ), by the formula, $\text{adherent/rolling cells} = c_1 + c_2 \times \text{diameter}$ (in micrometers). The number of rolling and adherent cells was then inputted for a theoretical 20, 30 or 40 μm vessel, assuming a linear dependency between the vessel diameter and leukocyte adherence/rolling. Fig. 5 depicts the positive correlation between vessel diameter and the number of adherent cells, whereby the larger the vessel, the larger the number of adherent leukocytes ($r^2 = 0.1964$, $p < 0.0007$). Based on the regression equation $y = 0.05712x - 0.4283$, we then stratified the number of rolling and adherent cells according to standardized vessel diameters after calculating the constants. This is important to identify differences in adherence and rolling according to vessel diameter which might not be detected by summarizing adherence and rolling in vessels of varying sizes.

We then assessed rolling and adherent cells in the same c-fms^{YFP+} animal first with bright field microscopy and then by YFP fluorescence microscopy, 4 h following challenge with the pro-inflammatory cytokine TNF α (1 μ g/kg) (Fig. 6A and B). Fig. 7A and B depicts responses to TNF α at a dose of 1 μ g/kg administered intraperitoneally, on leukocyte adhesion and rolling compared to untreated mice. TNF α resulted in a five-fold increase in the number of adherent YFP⁺ cells in 40 μ m vessels suggesting preserved responses to inflammatory signals. Although there was an increase in leukocyte rolling in larger vessels as shown in Fig. 6A with TNF α , this was statistically not significant. There was no difference in rolling velocity between YFP⁺ cells and rolling cells measured by bright field microscopy (data not shown).

The correlation of wall shear rate (WSR) to adherent YFP⁺ cells is depicted in Fig. 8. In TNF α treated mice, the highest amount of adherent cells (13 $\pm$ 3 cells/100 μ m) was observed in vessels with a WSR of 50 s⁻¹. With an increase of the WSR correlating with a decrease of vessel size the number of adherent cells decreases to 2 $\pm$ 2 cells/100 μ m.

Discussion

We show in c-fms^{YFP+} mice that YFP is preferentially expressed by cells of a monocyte lineage, corresponding to a Ly6C⁺ CD11b⁺ F4/80⁺ population of monocytes. The degree of expression of YFP correlates with the degree of expression of Ly6C and may provide a novel approach to identify monocyte populations using live imaging approaches such as intravital microscopy. Importantly, we show that YFP is expressed by immature (F4/80⁻) and mature (F4/80⁺) monocytes demonstrating the usefulness of this model in identifying different monocyte subsets. YFP⁺ cells are easily identified during intravital microscopic evaluation and that once challenged the TNF α , leukocyte–endothelial interactions of inflammatory monocyte subsets can be better elucidated on account of the superior signal and specificity elicited by cell specific labeling.

Prior studies by Auffray et al. (2007) have described a CX₃CR1^{gfp/+} mouse model, which express green fluorescent protein (GFP) in monocytes and allows one to identify monocyte subsets. c-fms also referred to as CD115 was used as a pan-monocyte marker in these studies. We re-affirmed this in our study by demonstrating that YFP⁺ monocytes do not always express other classical markers of differentiated monocytes such as F4/80. Among murine blood cells, monocytes can be distinguished from granulocytes and lymphocytes by a high level of expression of c-fms (CD115). At least two main subsets of monocytes have been recognized in mice and humans both of which express CD115. Our intent in this paper was to evaluate the utility of using an approach of fluorescently tagging cells of monocyte lineage with YFP and evaluating the co-expression of this marker with other more traditional markers used to delineate monocytes and to test the utility of this mouse model in *in vivo* investigations assessing leukocyte–endothelial interactions. Recent studies involving inflammatory subsets of monocytes have revealed that there is remarkable heterogeneity in monocyte populations. Furthermore, distinct subsets of monocytes participate in inflammation and contribute to disorders such as atherosclerosis and insulin resistance (Drevets et al., 2004; Ouedraogo et al., 2007; Tacke et al., 2007). Tacke and Randolph (2006) distinguish between Ly6C^{low} and Ly6C^{high} monocytes with a lower and higher recruitment to inflammatory sites, respectively. A majority of cells (>80%) expressing CD11b also expressed YFP, with a greater fraction of CD11a/CD11b cells in the marrow expressing YFP compared with peripheral cells. The expression of YFP in neutrophils was limited (15% of Ly6G⁺ cells and 21% of 7/4 expressing cells) in the peripheral circulation. These data suggest that a YFP⁺ cell in the peripheral circulation is more likely a monocyte than a neutrophil. Our observation that YFP was minimally expressed in CD3⁺ cells also suggests that the expression is perhaps unique to non-T lymphocyte populations further

supporting that YFP status can be used to approximate the Ly6C status of monocyte populations. In previous studies comparing monocyte expression of surface markers, F4/80⁺ has been used as the benchmark for monocytes. However many studies have shown that a substantial percentage of immature monocytes may not express this marker. As the monocyte matures into a macrophage, F4/80 expression increases (Swirski et al., 2007). Our studies clearly demonstrate that YFP⁺ in our mouse model is likely a “panmonocyte” marker that identifies monocytes of various stages of maturity.

Intravital techniques using bright field microscopy have been used extensively for investigating the relationship between leukocyte adhesion and migration in the inflammatory process (Gavins and Chatterjee, 2004; Nishimura, 2008). Although these approaches are useful to assess leukocyte–endothelial interactions, they suffer from non-specificity, in that they do not allow one to define a leukocyte sub-population of interest, and are further limited by poor discriminative ability of cells. Approaches such as fluorescent tagging of cell subsets have been utilized but also suffer from limitations pertaining to non-specific labeling of cells (Abbitt et al., 2000) and background noise interference. Alternatively, transplantation of bone marrow from mice expressing green fluorescent protein (GFP) has been utilized to study bone marrow derived cells (Abedi et al., 2007). Although an attractive approach, all of the cells in the bone marrow are non-selectively labeled. Our investigations using intravital microscopy suggest that c-fms^{YFP+} mice are a suitable model to study leukocyte–endothelial interactions. The tissue specific transgenic expression of fluorescent proteins besides being more specific also amplifies the signal intensity in the targeted cell, allowing easy discrimination of these cells. The high signal intensity of YFP⁺ cells enables the detection of more rolling and adherent leukocytes and is clearly superior to bright field techniques that have no such discriminative ability. Our finding of an identical number of rolling and adherent cells with both bright field microscopy (a technique that should technically detect all cells) and fluorescent microscopy (detects only YFP⁺ cells, a small percentage of overall leukocytes) indicates that a percentage of cells are not being detected by bright field microscopy. Another important finding of the study is that transgenic over-expression of YFP does not impair responsiveness to pro-inflammatory signals such as TNF α . Taken together these findings affirm the applicability of these model systems to investigations evaluating the role of the monocyte system in inflammation.

Our studies confirm a strong relationship between wall shear rate and leukocyte adhesion as previously described by other investigators (Stein et al., 1999; Kim and Sarelius, 2004). Larger vessels exhibit a lower wall shear rate which leads to a higher leukocyte adherence. Most previous studies using intravital microscopy have pooled values from vessels across a range of diameters (20–40 μ m) and our studies would indicate that vessels around 40 μ m diameters are better suited for quantification of leukocyte rolling and adhesion events.

In conclusion we have characterized the expression of YFP in leukocyte subsets in a transgenic model of YFP expression and assessed its *in vivo* utility in vascular inflammation using intravital microscopy. This model may prove useful in inflammatory disorders characterized by exaggerated leukocyte–endothelial interactions.

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Fig. 1.

Flow cytometry analysis of mouse monocyte subsets in the bone marrow. (A) Dot plot depicting Ly6C high and low populations positive for F4/80 marker and histogram showing YFP positive monocytes as YFP low and high populations. (B) Dot plot depicting Ly6C high and low populations positive for F4/80 and histogram showing YFP positive monocytes as F4/80 low and high Ly6C⁺ cells. (C) Dot plot depicting Ly6C high and low populations positive for CD11b marker and histogram showing YFP positive monocytes as CD11b low and high Ly6C⁺ cells. All data are presented as mean±SD ($n=3$ independent experiments).

Fig. 2.

Flow cytometry analysis of monocytes expressing YFP in the bone marrow. $F4/80^+$, $CD11b^+$ cells were gated for Ly6C followed by YFP in bone marrow derived leukocytes. All data are presented as mean $\pm$ SD ($n=3-5$ independent experiments).

Fig. 3. Intravital microscopy allows for YFP⁺ cell detection in cremasteric venular preparations. Panels A and B depict venules under bright field microscopy (40× magnification) from C57BL/6 mice, after administration of Rhodamine-6G. Panels C and D depict a typical venule from GFP transplanted mice and from a c-fms^{YFP+} mice, respectively.

Fig. 4.

Comparison of signal intensity of three different fluorescent methods (Rhodamine-6G, c-fms^{YFP+}, BMT-GFP) reveals that YFP⁺ cells are easily identified. Three different fluorescent methods were used for assessment of *in vivo* detection of cells in the cremasteric muscle bed. YFP⁺ and GFP⁺ bone marrow cells had significantly higher signal-to-noise ratio (SNR) and contrast-to-noise ratio (CNR) when compared to Rhodamine-6G (* $p < 0.05$). Data are expressed as the mean $\pm$ SD ($n = 25$ independent measurements).

Fig. 5.

Correlation of adherent c-fms^{YFP+} cells to vessel diameter reveals that as the vessel diameter increases, the adherence of the leukocytes increases proportionally. Fig. 4 reveals that there is a direct proportional relationship between leukocyte adhesion and vessel diameter. An increase in the diameter allows more leukocytes to adhere within a 100 μm vessel segment ($n=38$, total observed vessel numbers).

Fig. 6.

Comparison of leukocyte adhesion and rolling in the cremasteric muscle bed by bright field and fluorescence microscopic methods resulted in similar efficiency in response to $\text{TNF}\alpha$ treatment. The different visualization methods show similar results in (A) leukocyte rolling using bright field microscopy (BFM) ($n=12$) and fluorescence microscopy (YFP) ($n=19$) and (B) leukocyte adhesion using bright field microscopy (BFM) ($n=12$) and fluorescence microscopy (YFP) ($n=19$). Data are expressed as the mean $\pm$ SD.

Fig. 7.

TNF α administration increased leukocyte adhesion and rolling in the cremasteric muscle bed. The effect of TNF α (1 $\mu\text{g/kg}$) intraperitoneal administration on (A) leukocyte rolling ($n=15$) and (B) leukocyte adhesion ($n=19$) as compared to non-treated controls revealed that TNF α administration increased both leukocyte rolling and adhesion of YFP $^{+}$ cells (c-fms $^{YFP+}$ TNF α data in Figs. 6B and 5B are the same while control groups differ). * $p<0.05$ with respect to the control. Data are expressed as the mean $\pm$ SD.

Fig. 8. TNF α administration increased leukocyte adherence at consistent wall shear rate (WSR). The effect of TNF α (1 μ g/kg) intraperitoneal administration on leukocyte adherence as compared to non-treated controls. Results revealed that TNF α administration increased leukocyte adhesion in vessels with a lower WSR ($n=15$). * $p<0.05$ with respect to the control. Data are expressed as the mean $\pm$ SD.

Table 1

Leukocyte surface markers and YFP expression in peripheral blood and bone marrow.

Monocyte/neutrophil/Lymphocyte lymphocyte marker					
Cell subsets	Surface markers	Peripheral blood % of leukocyte population	% of subset YFP⁺	Bone marrow % of leukocyte population	% of subset YFP⁺
Monocyte	F4/80	4.0±0.6	64.4±0.9	61.4±1.3	60.0±1.7
	CD11a	27.8±0.5	92.2±7.1	64.8±17.9	76.1±1.8
	CD11b	31.2±0.6	88.8±4.3	43±3	88.2±5.2
	CD14	28.9±3	90.4±4	16.4±0.8	92.3±5.4
Neutrophil	Ly6G	48.4±15.2	15.2±0.9	59.4±10.6	39±3.5
	7/4	48.2±15.1	20.7±1.7	55.3±11.1	39.7±3.7
Lymphocyte	CD3	22.4±1.1	3.6±0.1	7.6±2.3	5.7±1.6

This table outlines the expression markers used to detect the specific leukocyte subsets in the peripheral blood and bone marrow.