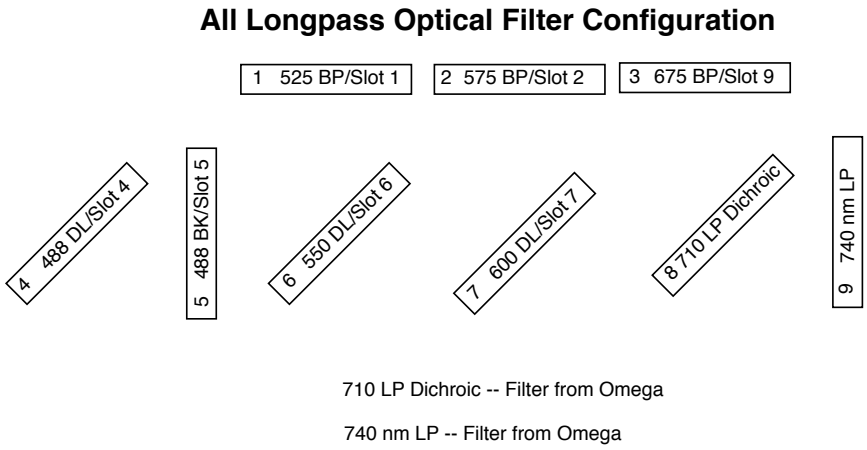
Antigen	Fluoro-chrome	Clone	Isotype	Supplier*
CD2	PC7	39C1.5	R IgG2a	BC
CD3e	FITC, PE, PC5, PC7	UCHT1	M IgG1 k	BC, eB
CD4	PC7	SFCI12T4D11	M IgG1	BC
CD4	PE	RPA-T4	M IgG1 k	BD
CD8a	PC5	RPA-T8	M IgG1 k	eB
CD8a	PC7	SFCI21Thy2D3	M IgG1 k	BC
CD14	PE, PC5	RM052	M IgG2a k	BC
CD15	FITC	HI98, 80H5	M IgM k	BC, BD
CD16	PC7	3G8	M IgG1 k	BC
CD19	PC7	J4.119	M IgG1 k	BC
CD20	PE	B9E9	M IgG2a k	BC
CD25	FITC	M-A251	M IgG1 k	BD
CD25	PE	BC96	M IgG1 k	eB
CD33	PC5	WM53	M IgG1 k	BD
CD33	PC5	D3HL60.251	M IgG1 k	BC
CD38	PC5	LS198	M IgG1 k	BC
CD45	PC7	J33	M IgG1 k	BC
CD56	PC5	N901	M IgG1 k	BC
CD57	FITC	NK-1	M IgM k	BD
CD80	FITC	2D10.4	M IgG1 k	eB
CD86	PE	IT2.2	M IgG2b k	eB
CD91	FITC, PE	A2MR-a2	M IgG1 k	BD
CD161	PE	191B8	M IgG2a	BC
CD163	PE	GHI61	M IgG1 k	BD
CD244	PC6	C1.7.1	M IgG1	BC
PD-1	FITC	J116	M IgG1 k	eB
B7-H1	PE	MIH1	M IgG1 k	eB
TLR1	PE	GD2.F4	M IgG1 k	eB
TLR2	FITC	TL2.1	M IgG2a k	eB
TLR4	PE	HTA125	M IgG2a k	eB
HLA-DQ	FITC	TU169	M IgG2a k	BD
HLA-DR	PC5	G46-6 (L243)	M IgG2a k	BD
PRF1	FITC	deltaG9	M IgG2b k	BD
Isotype	FITC	27-35	M IgG2b k	BD
TIA-1	PE	2G9	M IgG1 k	BC

* BC = Beckman-Coulter; BD = BDBiosciences; eB = eBioscience

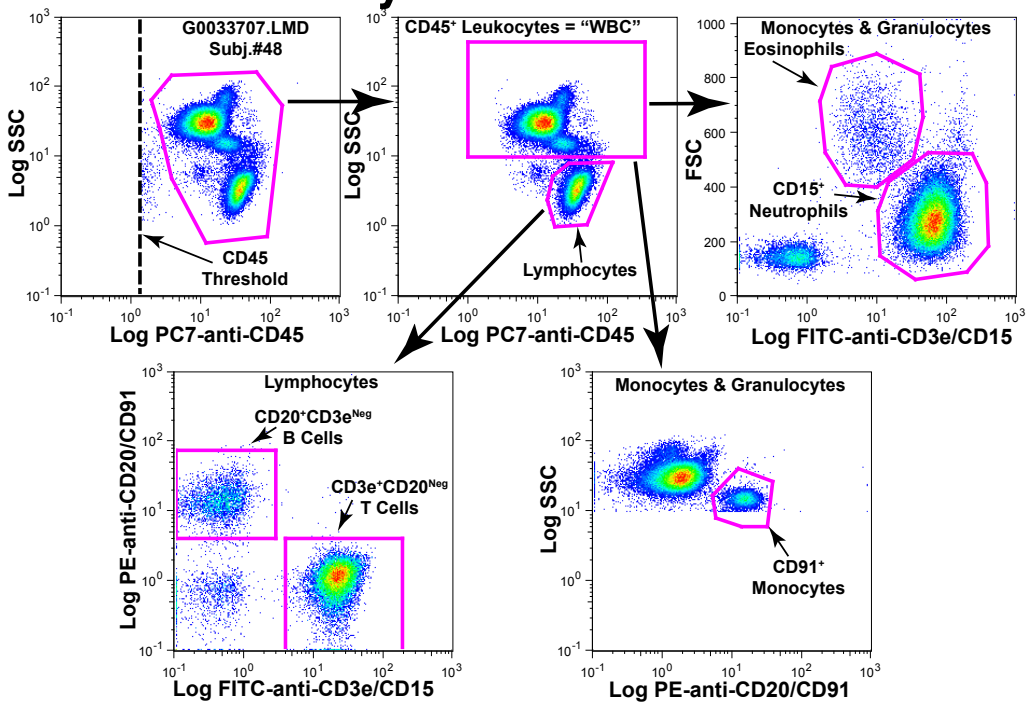
Supplemental Table ST1. Antibodies used in flow cytometric examinations

Antibodies listed above were used in the present studies. The exact combinations are described in the relevant legends.



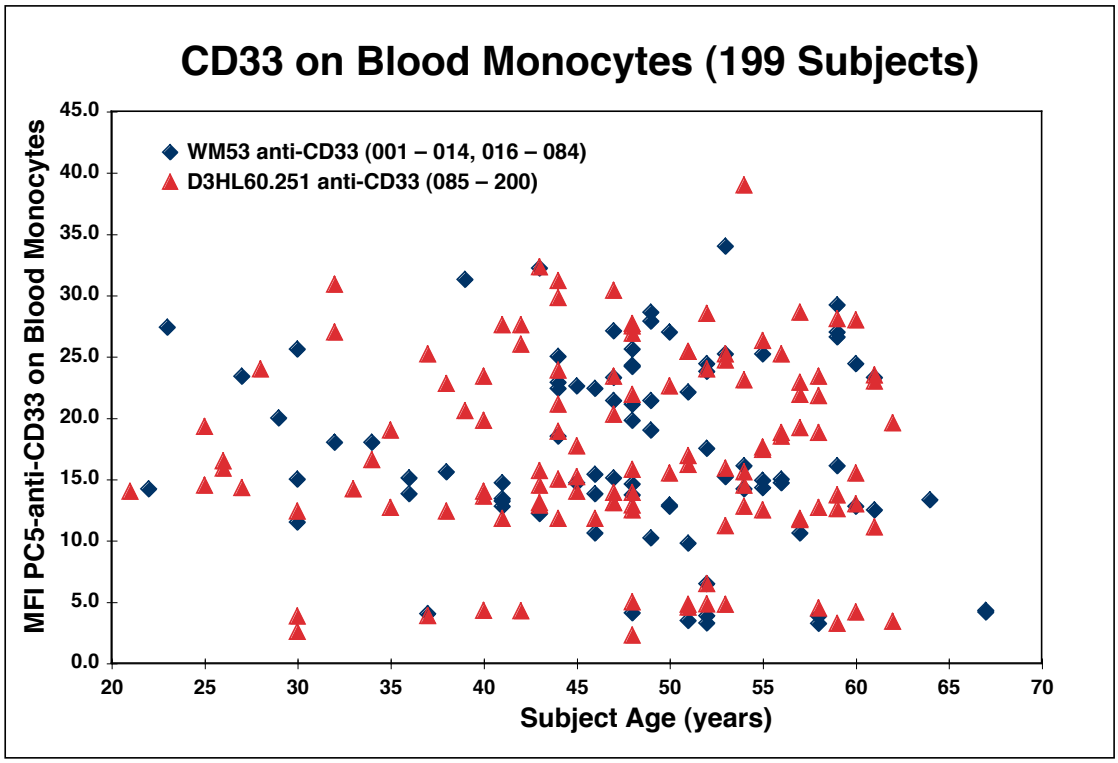
Supplemental Figure S1. Optical filter configurations for the XL/MCL cytometer

The diagram above depicts the modified arrangements of optical filters that were evaluated in order to measure fluorescein, PE, PC5 and PC7. The numbers correspond to the numbered filter slots in the XL cytometer. The standard optical filters in the Coulter Epics XL/MCL cytometer were configured to detect fluorescein, PE, PE-TxRed ("ECD") and PC5. The filters supplied for that original configuration as labeled as "Slot n" with "n" equal to the original position of that filter. Since there was considerable fluorescence spillover with the original arrangement, the optical filters were altered to detect fluorescein, PE, PC5 and PC7 as illustrated

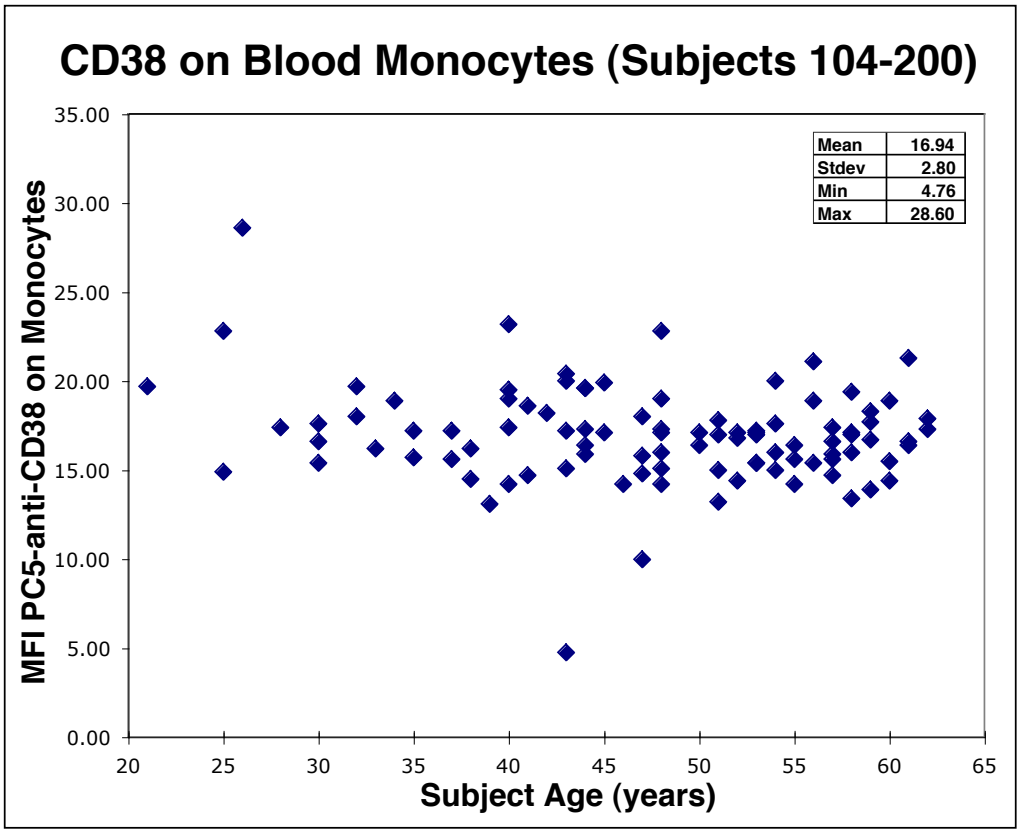


Supplemental Figure S2. Gating used to determine flow cytometric (FCM) differential leukocyte frequencies.

Aliquots of blood were labeled with FITC-anti-CD3e, FITC-anti-CD15, PE-anti-CD20, PE-anti-CD91, PC5-anti-CD56 and PC7-anti-CD45, treated with FACSLysing solution, washed and resuspended for analysis. Data files were collected with linear forward scatter (FSC, gain 2) and log amplification for side scatter (SSC) and all fluorescence parameters using a CD45 threshold (as indicated). All parameters were collected using the Beckman-Coulter standard pulse area signals. The SSC signals for granulocytes were nearly an order of magnitude greater than that for lymphocytes so log amplification was used in order to keep all $CD45^+$ blood leukocytes on scale. Antibody to CD56 was included since we initially planned to identify natural killer (NK) cells as $CD56^+$ lymphocytes that expressed neither CD3e nor CD20 (or CD19). However, we found that CD56 expression consistently underestimated the number of $CD3e^{Neg}$ lymphocytes that expressed perforin (PRF1) so we ultimately did not use CD56 expression to identify NK cells.

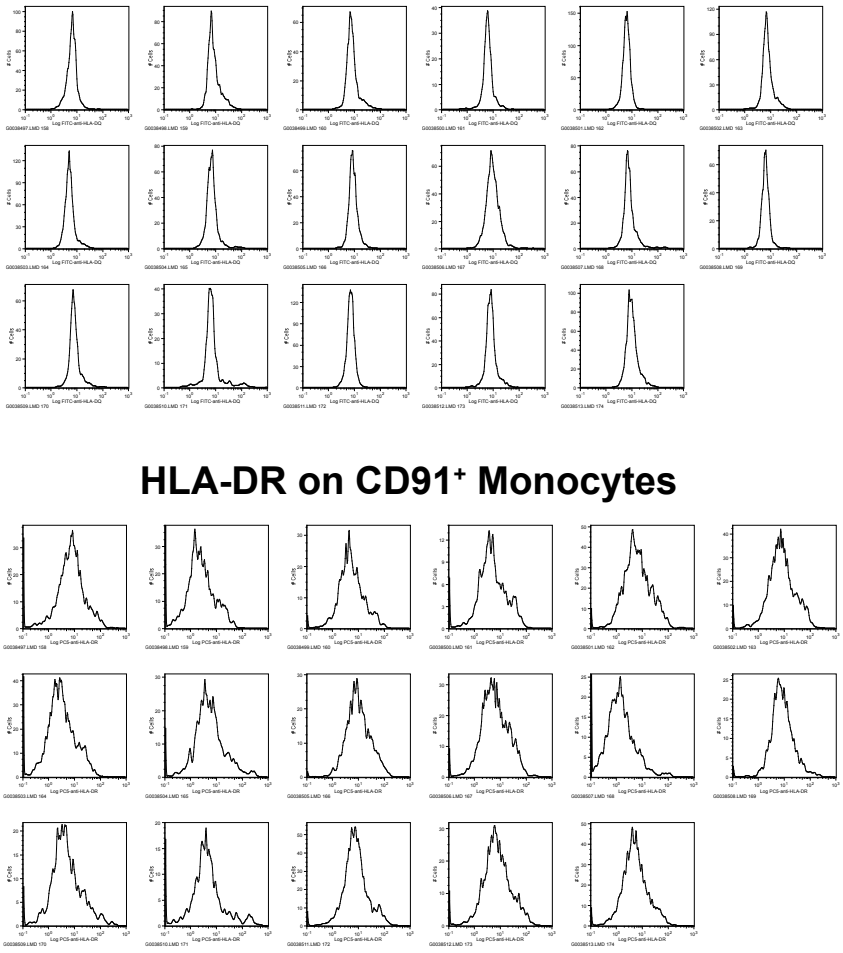


Supplemental Figure S3. Expression of CD33 on monocytes from 199 subjects. Aliquots of blood from the subjects were labeled with antibody combinations including either clone WM53 or clone D3HL60.251 PC5-anti-CD33. The $CD33^+$ monocytes were identified and the PC5 MFI values were determined and plotted *vs.* subject age as shown above. There was considerable heterogeneity in the amounts of CD33 expressed per monocyte among these subjects as depicted here and described elsewhere but there was no apparent association between the age of the subject and amount of CD33 expressed as illustrated. Likewise, there were no systematic differences between these two cross-competing clones reactive with the lectin-like V domain of CD33.



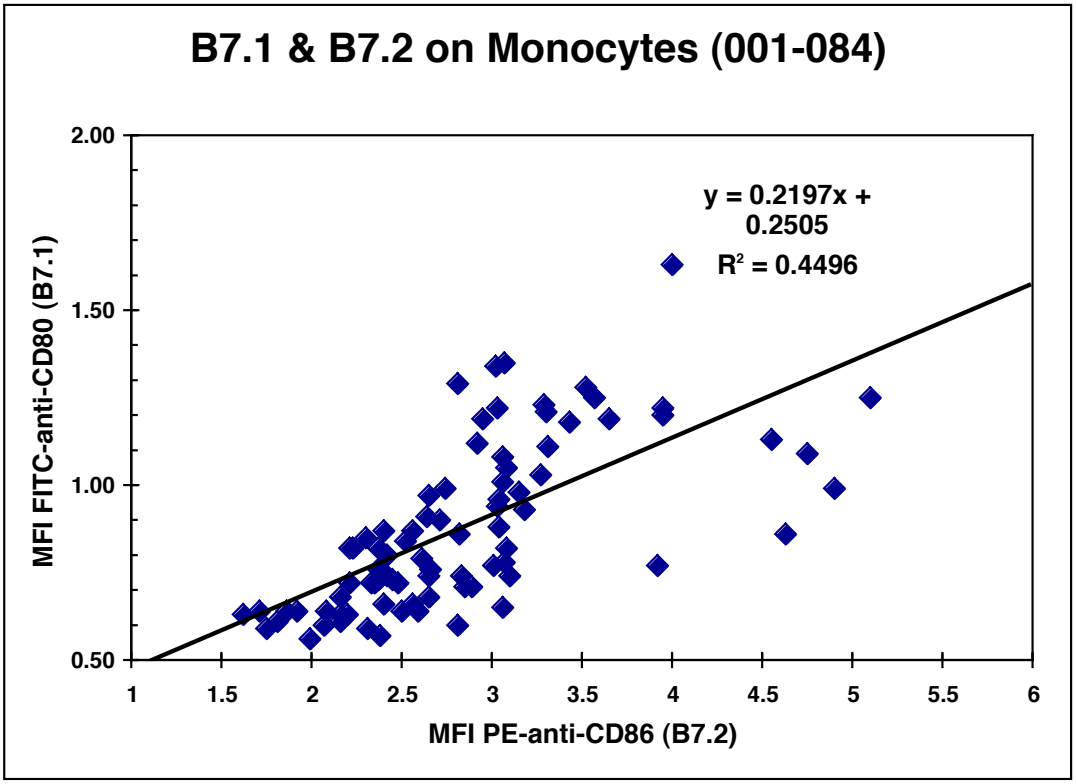
Supplemental Figure S4. Expression of CD38 on blood monocytes from subjects #104 - #200.

Aliquots of blood from subjects #104 - #200 were surface labeled with antibody combinations including PC5-anti-CD38, fixed, rendered permeable and then labeled with FITC-anti-PRF1. The primary goal with these antibody combinations was to examine CD38 on T lymphocytes and natural killer (NK) cells. However, monocytes could be identified by a combination of CD38 expression and SSC so that the the MFI of PC5-anti-CD38 could be determined. As shown in the figure, the MFI values mostly varied by ±~16% and there was no apparent association between the amount of CD38 detected and the age of the subjects that were examined.



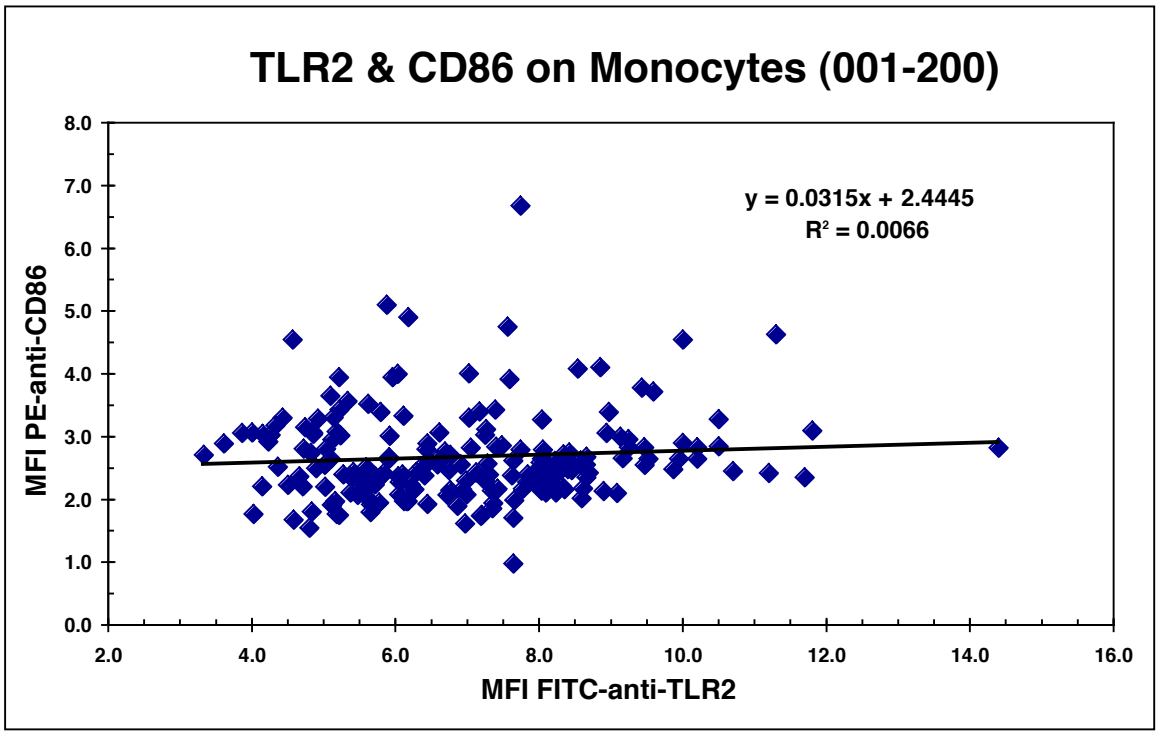
Supplemental Figure S5. Expression of HLA-DQ and HLA-DR on CD91+ Monocytes

Blood samples from a group of 17 subjects (158 – 174) were labeled with a mixture of FITC-anti-HLA-DQ, PE-anti-CD91, PE-anti-CD3e, PC5-anti-HLA-DR and PC7-anti-CD19. The CD91+ monocytes were identified as depicted in Figure 2 and the univariate distributions of HLA-DQ and HLA-DR expression are depicted above.



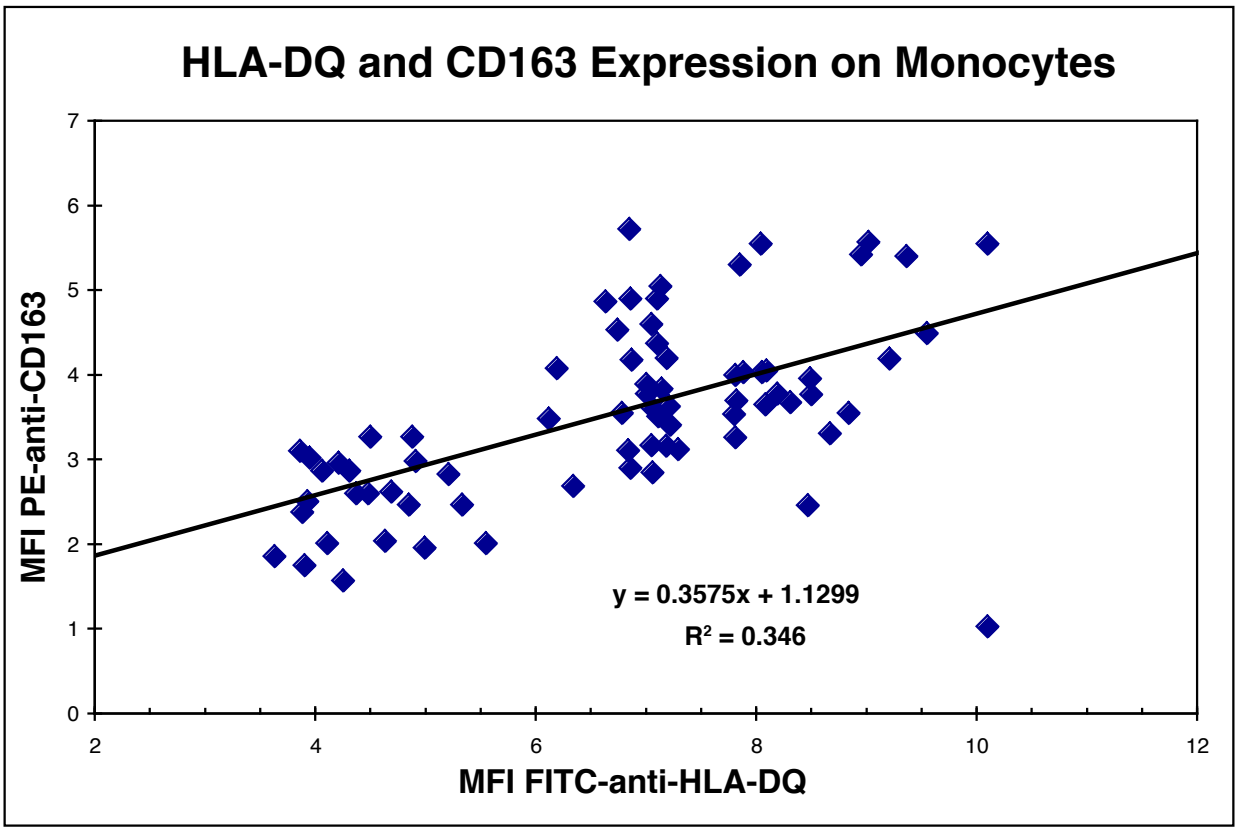
Supplemental Figure S6. Expression of CD80 (B7.1) and CD86 (B7.2) on CD33⁺ monocytes.

Replicate aliquots of blood from subjects #001 - #084 were labeled with a mixture of 1) FITC-anti-CD25 (or anti-PD-1), PE-anti-CD86, PC5-anti-CD3e/CD33 and PC7-anti-CD19 or of 2) FITC-anti-CD80, PE-anti-TLR1, PC5-anti-CD3e/CD33 and PC7-anti-CD19. The CD33⁺ monocytes were identified and the MFI values for FITC-anti-CD80 or for PE-anti-CD86 were determined from each labeled aliquot and plotted as depicted. CD80 was expressed at low levels on the monocytes from these subjects but there was a modest association between the amounts of CD80 and CD86 detected on these monocytes.



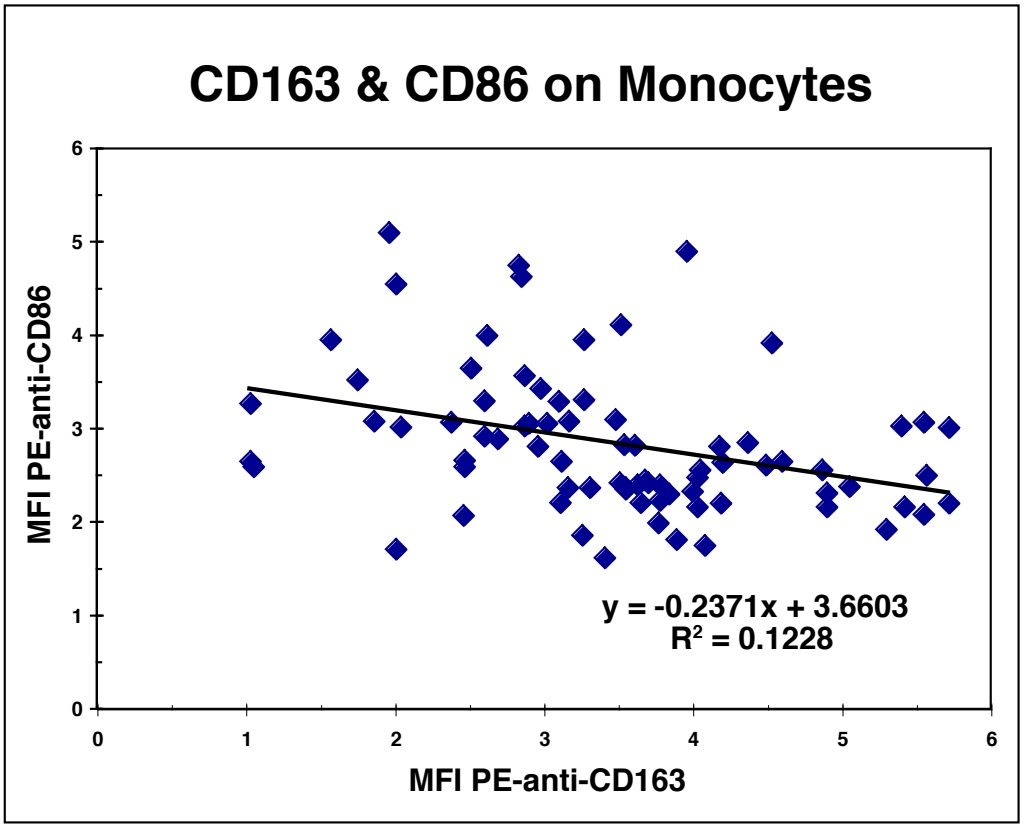
Supplemental Figure S7. The expression of CD86 and Toll-like Receptor 2 (TLR2) vary independently on monocytes

Replicate aliquots of blood from all 200 subjects were labeled with a mixture of 1) FITC-anti-TLR2, PE-anti-TLR4, PC5-anti-CD3e/CD33 and PC7-anti-CD4 or of 2) FITC-anti-CD25 or FITC-anti-PD-1, PE-anti-CD86, PC5-anti-CD3e/CD33 and PC7-anti-CD19. The $CD33^{+}$ monocytes were identified and the MFI values for FITC-anti-TLR2 and PE-anti-CD86 were determined and plotted as shown. The expression of both molecules varied among individuals but there was no detectable association as indicated.



Supplemental Figure S8. HLA-DQ and CD163 expression on monocytes are modestly associated.

Replicate aliquots of blood from 74 subjects (#011 - #084) were labeled with antibody mixtures of 1) FITC-anti-HLA-DQ, PE-anti-CD3e/CD91, PC5-anti-HLA-DR and PC7-anti-CD19 and of 2) FITC-anti-CD57, PE-anti-CD163, PC5-anti-CD3e/CD33 and PC7-anti-CD8a. The $CD91^+$ or $CD33^+$ monocytes were identified and the MFI values for FITC-anti-HLA-DQ and PE-anti-CD163, respectively, were determined and plotted as shown. There was a modest association between the levels of HLA-DQ and CD163 expressed per cell as indicated.



Supplemental Figure FS9. Expression of CD163 and CD86 on monocytes is negatively associated.

Replicate aliquots of blood from 74 subjects (#011 - #084) were labeled with antibody mixtures containing FITC-anti-CD57, PE-anti-CD163, PC5-anti-CD3e/CD33 and PC7-anti-CD8a and with with other mixtures containing FITC-anti-PD-1, PE-anti-CD86, PC5-anti-CD3e/CD33 and PC7-anti-CD19. $CD33^{+}$ monocytes were identified and the MFI values for PE-anti-CD163 and for PE-anti-CD86 were determined and plotted as shown. There was a modest negative association such that monocytes from subjects with higher levels of CD163 tended to have lower levels of CD86.