Supplementary Online Content


This supplementary material has been provided by the authors to give readers additional information about their work.
Simple whole-blood staining was used to characterize leukocyte and B-cell subsets directly from the circulation. Triple-color immunofluorescent staining of whole-blood samples was performed within 60 min after blood was collected, using antibodies directed against CD14/CD3/CD19 and CD27/CD19 with isotype controls. This was followed by red blood cell lysis and immediate acquisition and analysis with flow cytometry.

Genomic DNA was prepared from peripheral blood samples using the QuickGene-mini80 nucleic acid isolation system (Fujifilm, Tokyo, Japan). The 158V/F polymorphism in FCGR3 was genotyped by direct Sanger sequencing method. PCR was performed in a mixture of 1.25 pmol of forward and reverse primers, 100 ng of genomic DNA, 250 µM dNTPs, 1X Band Doctor™ and 1 U Solg™ Taq DNA Polymerase (Solgent Co., LTD, Korea) in the buffer provided by the manufacturer. Amplification was performed in a GeneAmp PCR System 9700 thermal cycler (Life Technologies). The following PCR primers were employed; forward 5'-CTGTGTCCTTCAGCTGGC -3' and reverse 5'-AAATGACCAGAATAGTATCTCTGT-3'. The PCR conditions used were as follows: initial denaturation at 94°C for 2 min, followed by 7 cycles: 94°C for 30 s, annealing gradient at from 60°C to 57°C, 0.5°C decrement at each cycle for 40 s, extension at 72°C for 1 min 30 s, then 29 cycles as following: 94°C for 30 s, annealing at 57°C for 40 s, extension at 72°C for 1 min 30 s and final elongation at 72°C for 10 min. The PCR product was a fragment of 1.35kB, and that was checked by electrophoresis onto a 2% agarose gel. The PCR products were purified using a MultiScreen96-PCR Filter Plate (Milipore, Billerica, MA, USA). The purified products were then sequenced using a BigDye Terminator Cycle Sequencing Kit and an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA). The sequencing primer was done using the following primer: 5’ TGAGGTGTCACAGCTGGAAG-3’ and data analyses were performed using Chromas Lite 2.01.