SUPPLEMENTARY METHODS

Quantitative RT-PCR primers

ITGAM

5′-TCCAAGAGAACGCAAGGGGCT-3′

5′-CAGGGACAGGCCCAGGGACA-3′;

RPL27 (housekeeping gene 1)

5′-ATCGCCAAGAGATCAAAGATAA-3′

5′-TCTGAAGACATCCTTATTGACG-3′

OAZ1 (housekeeping gene 2)

5'-GGATCCTCAATAGCCACTGC-3'

5'-TACAGCAGTGGAGGGAGACC-3'

GAPDH (housekeeping gene 3)

5′-CATGAGAAGTATGACAACAGCCT-3′

5′-AGTCCTTCCACGATACCAAAGT-3′

SRP14 (housekeeping gene 4)

5'-CAGATGGCTTATTCAAACCTCCT-3'

5'-ATGCCCTTTACTGTGCTGCT-3'.

ΔCt-levels were calculated in triplicates

Preparation of Opsonised Sheep Erythrocytes

Sheep erythrocytes (sRBCs) were washed and resuspended in Gelatin Veronal Buffer (Sigma) and coated in rabbit anti-sheep erythrocyte IgM (Cedarlane) for 1 hour on a rotator

wheel at room temperature. For the additional opsonisation with iC3b the IgM coated sRBC were further incubated in 10% C5-depleted human serum for 20minutes at 37°C before washing and resuspending in serum-free RPMI medium. The presence of high concentration iC3b using this technique was confirmed by flow cytometry (using anti-human iC3b primary antibodies (AbD Serotec) – data not shown).

For COS7 phagocytic assay sRBC's were opsonised with rabbit anti-sheep IgG fraction (Sigma). These had better secondary staining characteristics than the IgM antibody and could be used as COS7 cells do not express Fc-gamma receptors.

For cytokine studies sRBCs were prepared in an identical way to that described above except the rabbit anti-sRBC IgM was dialysed in endotoxin-free PBS and filter sterilised and sterile endotoxin PBS substituted for the gelatin veronal buffer.

Differential Staining of Internalised and External sRBC's in Phagocytic Assay

Following incubation of phagocytes with opsonised sRBC, phagocytosis was halted in ice and an external stain using Alexa 488 conjugated goat anti-rabbit IgG secondary antibody was added for 7 minutes (also capable of binding the light chain of the IgM rabbit anti-sheep sRBC primary antibody). Cells were then fixed in 4% paraformaldehyde for 15 minutes, permeabilised in 0.2% Triton X-100, and rewashed before adding the internal stain (Alexa 555 conjugated goat anti-rabbit IgG secondary antibody + DAPI for nuclear localisation) for 45 minutes. Cells were then rewashed and mounted on glass slides using Mowiol resin).

Quantification of Adherent Monocytes in Adhesion Assay

Monocytes adhering to ligand coated flat bottomed 96-well plates were fixed for 20 minutes with 1% gluteraldehyde. Fixed cells were the stained with 0.05% crystal violet (in 20% methanol) and then solublised with 1% SDS, before measuring absorbance at 595nm (Thermo Multiskan ascent). Preliminary titration (not shown) using firmly adherent COS7 cells to uncoated plastic wells demonstrated a linear relationship between 595nm absorbance and cell number over the range for values reported in this study so data is presented as a percentage difference in adhesion.