Supplemental Data

Mechanism of endosomal TLR inhibition by antimalarial drugs and imidazoquinolines

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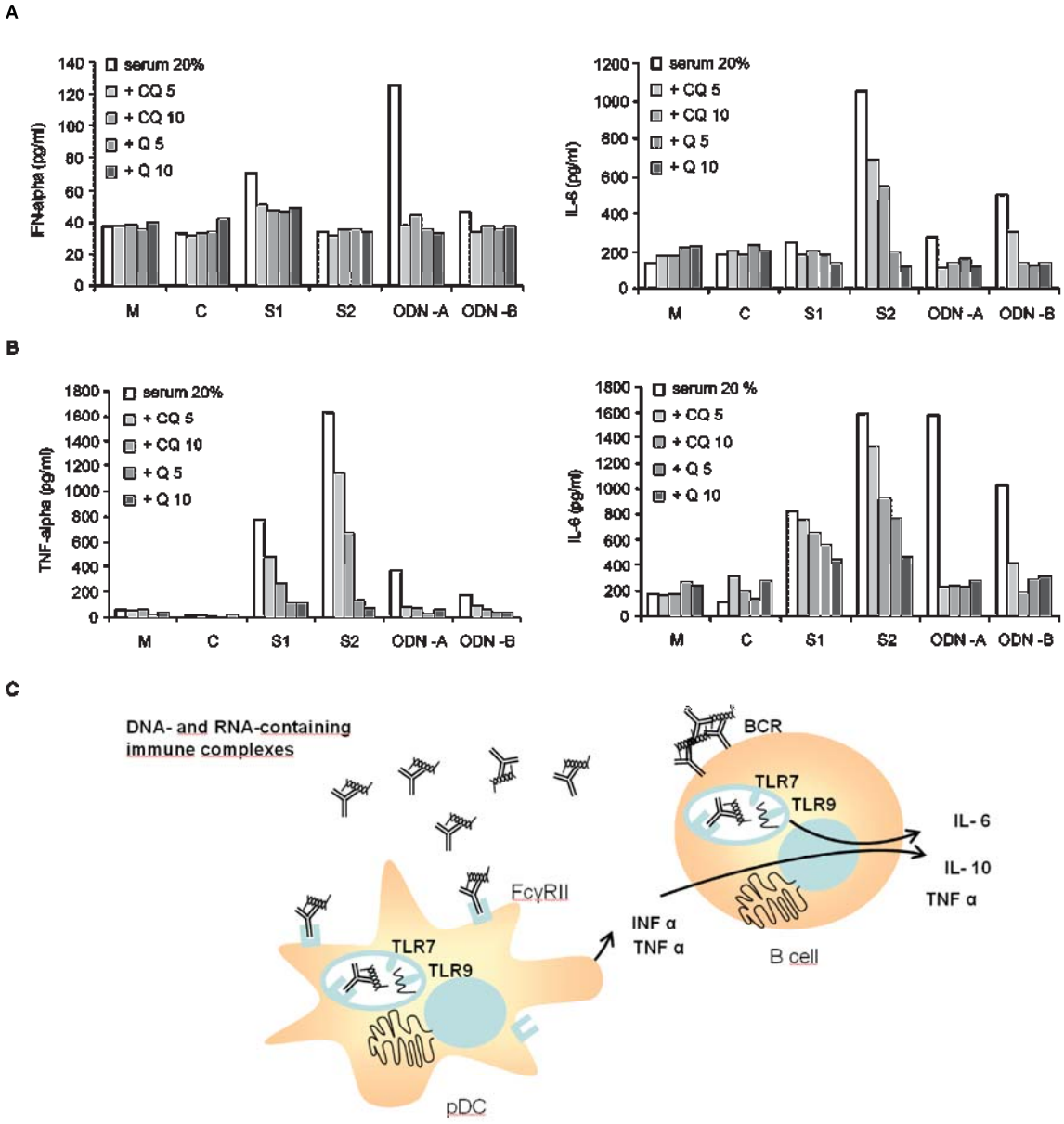
SUPPLEMENTAL FIGURE 1



SUPPLEMENTAL FIGURE 1. Chloroquine and quinacrine reduce the production of proinflammatory cytokines in pDC and PBMC treated with anti-DNA antibody positive sera of SLE patients.

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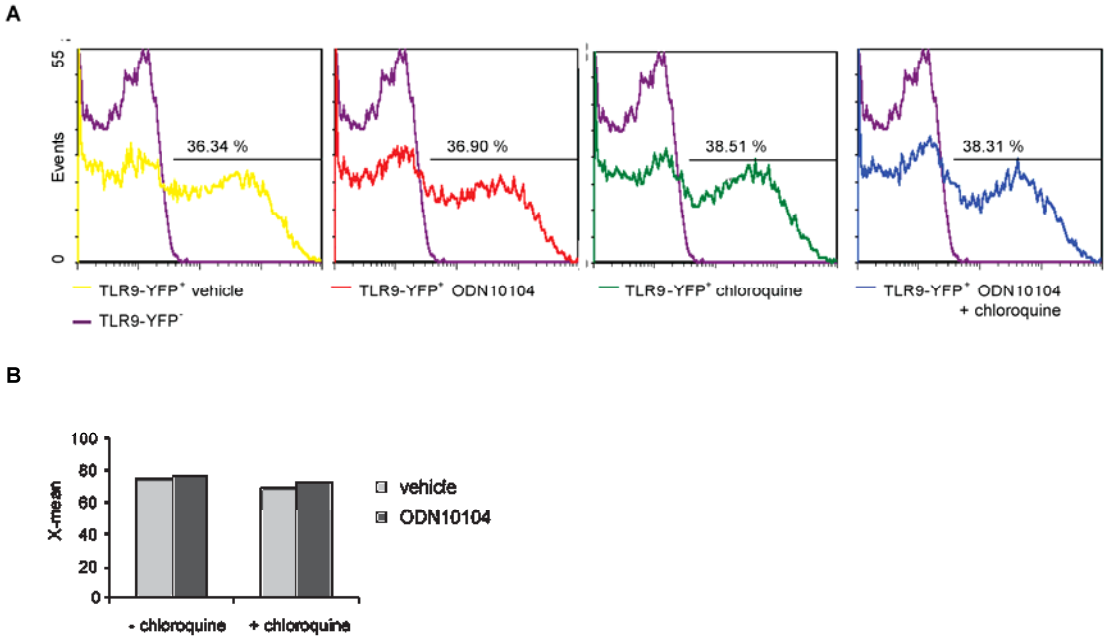
A, pDCs were treated for 24 hours with 20% serum of SLE patients, negative (negative control, C) or positive for anti-DNA antibodies (positive samples S1, S2), which binding activities were greater that 0.70 according to the Farr technique. Chloroquine (CQ) or quinacrine (Q) was added at final concentration of 5 or 10 μg/ml. Human IFN-α protein and IL-6 levels in the pDC supernatants were measured by ELISA. *B*, PBMCs were treated for 24 hours with 20% serum of SLE patients, negative (negative control C) or positive for anti-DNA antibodies (positive samples S1, S2), which binding activities were greater that 0.70 according to the Farr technique. Chloroquine (CQ) or quinacrine (Q) was added at final concentration of 5 or 10 μg/ml. Human TNF-α and IL-6 protein level in the supernatants of PBMC cultures was measured by ELISA. M means X-vivo15 Medium. *C*, DNA- or RNA-containing immune complexes from SLE patient sera activate human pDCs and B cells. They are internalized in pDCs mainly via Fc receptors (FcγRII) and in B cells predominantly via the B cell receptor (BCR). In endosomes DNA or RNA stimulate TLR9 or TLR7, which results in the activation of pDCs and B cells. pDCs produce cytokines, which regulate B cell differentiation and antibody production, as they synergistically enhance B cell responses toward stimulatory immune complexes (Lamphier et al., 2006; Means et al., 2005).

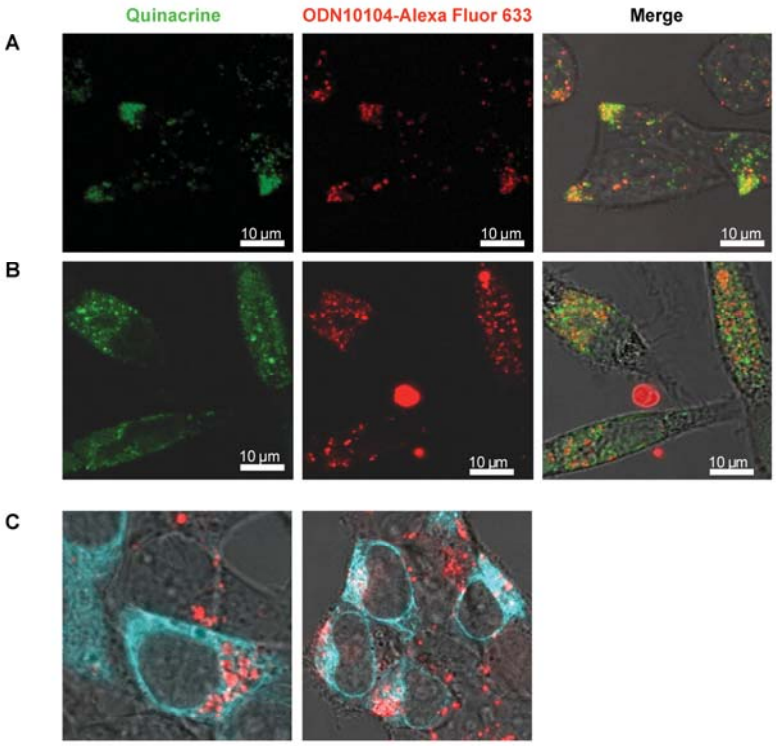
SUPPLEMENTAL FIGURE 2



SUPPLEMENTAL FIGURE 2. Chloroquine has no effect on the TLR9 expression.

A, *B*, Flow cytometry of HEK293 cells transfected with TLR9-YFP and stimulated with ODN10104 (3 μM) in the absence or presence of chloroquine (10 μg/ml). After 20 hours the fluorescence of TLR9-YFP was measured.

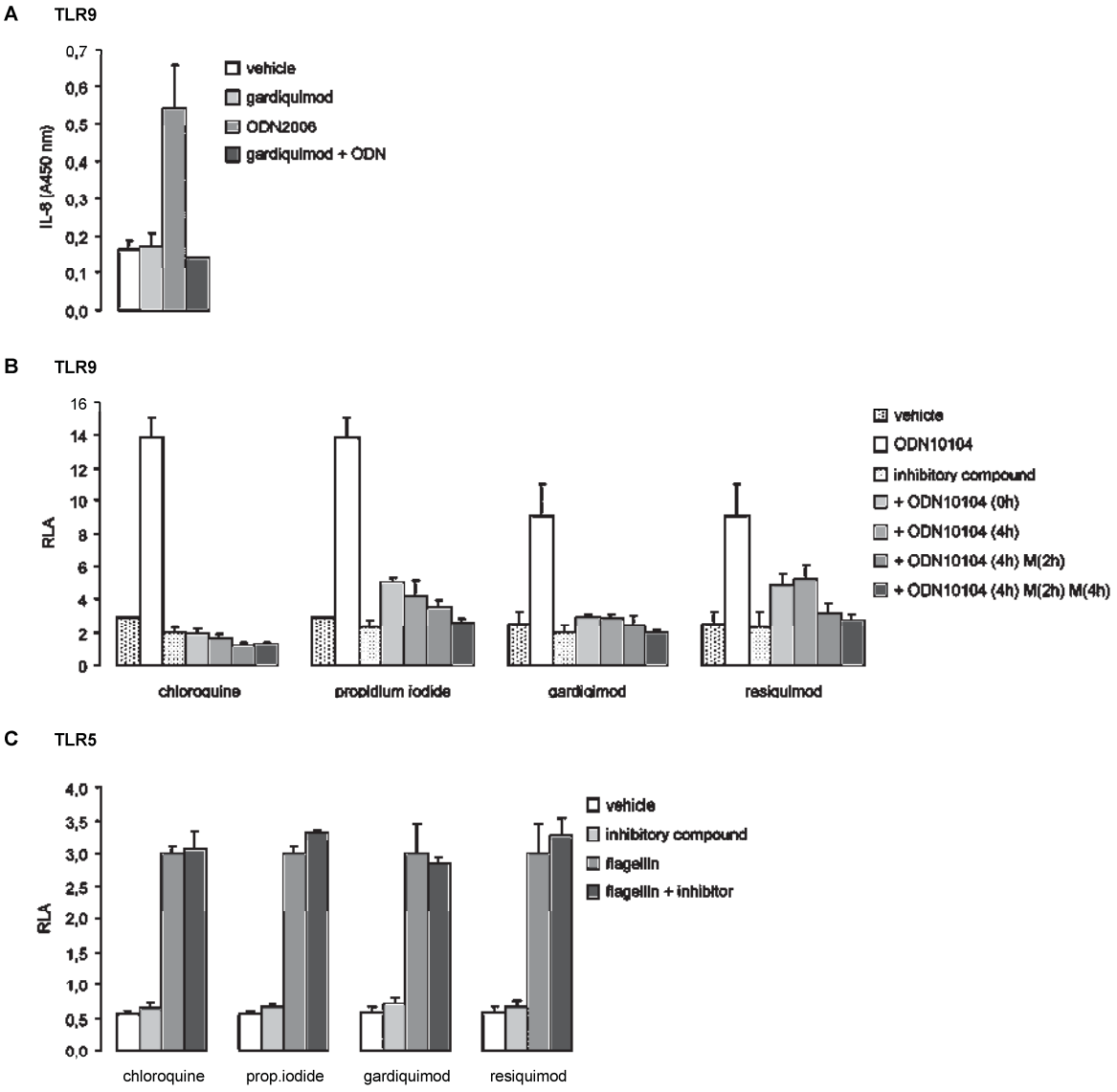
SUPPLEMENTAL FIGURE 3



SUPPLEMENTAL FIGURE 3. Colocalization of quinacrine and ODN with TLR9.

A, HEK293T cells and *B*, PBMCs 18-hours after incubation with quinacrine (1 µg/ml) (green) and ODN10104-Alexa Fluor 633 (2 µM) (red). The colocalisation of quinacrine and ODN10104-Alexa Fluor 633 is shown in yellow on the overlay image. *C*, HEK293T cells transfected with TLR9–YFP (blue) 18-hours after stimulation with propidium iodide (1 µg/ml) and ODN10104 (6 µM) (left) or with ODN10104-Alexa Fluor 633 (2 µM) (right). ODN is important for visualization of propidum iodide (red). Colocalization of TLR9-YFP and ODN10104-Alexa Fluor 633 is shown as white.

SUPPLEMENTAL FIGURE 4



SUPPLEMENTAL FIGURE 4. Inhibition of TLR9 by propidium iodide, gardiquimod and resiquimod.

A, Interleukin-8 (IL-8) synthesis was inhibited with gardiquimod. HEK293 cells, transfected with hTLR9, were incubated with ODN10104 in the absence or presence of gardiquimod (1 μg/ml). After 20 hours, IL-8 was determined using hIL-8 ELISA. *B*, Inhibition of TLR9 by chloroquine, propidium iodide, gardiquimod and resiquimod at concentration of 3 μM. Stimulation of HEK293 cells transfected with TLR9 was achieved by ODN10104 at concentration 3 μM, which was added (i) together with an inhibitory compound (+ ODN10104 (0h)), (ii) 4-hours after addition of an inhibitory compound (ODN10104 (4h)), (iii) 4-hours after addition of an inhibitory compound, when the medium was changed 2-hours after inhibitor's addition (+ ODN10104 (4h) M(2h)) and (iv) 4-hours after addition of an inhibitory compound, when the medium was changed 2- and 4-hours after inhibitor's addition (+ ODN10104 (4h) M(2h) M(4h)). *C*, TLR5 was not inhibited with chloroquine, propidium iodide, gardiquimod and resiquimod. HEK293 cells expressing TLR5 were treated with 10 ng/ml flagellin in the absence or presence of TLR9 inhibitors at concentration of 3 μM.