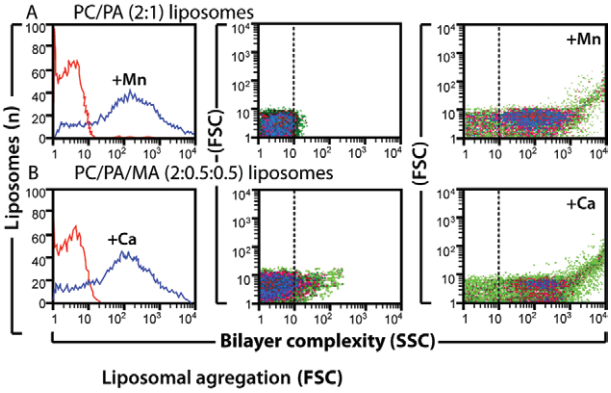
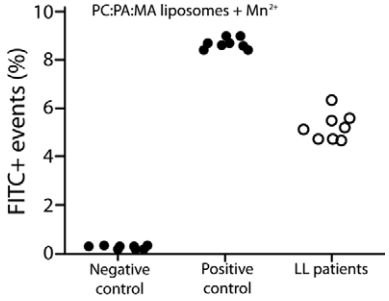


Detection of non-bilayer lipid arrangements on liposomes by flow cytometry. Liposomes made of phosphatidylcholine (PC)/phosphatidic acid (PA) (2:1 molar ratio) or PC/PA/mycolic acid (MA) (2:0.5:0.5) were incubated at 37ºC for 30 min with 5 mM MnCl2 or with 3 mM CaCl2. Changes in bilayer complexity [side scatter (SSC)] and liposomal aggregation [forward scatter (FSC)] were evaluated. Bilayer complexity is represented in histograms (red lines represent liposomes alone, blue lines represent liposomes with $Mn2^{+}$ or $Ca2^{+}$); bilayer complexity and aggregation of liposomes alone or with $Mn2^{+}$ or $Ca2^{+}$ are represented in density plots.



Sera of lepromatous leprosy (LL) patients bind specifically to non-bilayer lipid arrangements. Eight sera of LL patients were assayed by flow cytometry using Mn2+-treated phosphatidylcholine (PC)/phosphatidic acid (PA)/mycolic acid (MA) liposomes as antigens. The eight sera were previously immune adsorbed using cardiolipin-coated ELISA plates (Baeza et al. 2004). Mn2+-treated liposomes incubated with fluorescein isothiocyanate (FITC)-labelled goat anti-human polyvalent antibody or with the monoclonal antibody (H-308) that binds non-bilayer phospholipid arrangements were used as the negative and positive control, respectively. Immune adsorption assays were made as was previously described. Sera samples at 1:50 dilution in 8% foetal bovine serum in TS buffer were added to cardiolipin-coated ELISA plates. After 1 h incubation at 37ºC, sera were removed and incubated again in new cardiolipin-coated ELISA plates, a procedure repeated three times to achieve complete anti-cardiolipin antibodies adsorption.