Apoptosis induction using ultraviolet type B (UV-B) radiation in peripheral blood lympchotytes

In this stage, different exposition times to UV-B radiation were evaluated to obtain apoptotic cells from lymphocytes from peripheral blood from initially healthy individuals and afterwards from patients with SLE. To corroborate the apoptosis induction, Propidium Iodide (PI) and Annexin V (Anex) were used as dyes and the samples were analyzed using flow cytometry. Said cells in apoptosis status are later used to pulse DCs with the aim to provide autoantigens (Figure 2). Figure 3 shows that an exposition time of 1.5 hours to UV-B radiation is suitable to induce apoptosis (PI + Anex +) in these cells.

Evaluation of the capacity of DCs of patients with SLE to endocytose apoptotic cells.

Afterwards, it was evaluated if DCs generated in the previous Example had the capacity to endocytose apoptotic cells to process antigens and then present them to self-reactive T lymphocytes. Apoptotic cells generated through exposition to UV-B radiation were labeled with cellular dye carboxyfluorescein succinimidyl ester (CFSE) (Invitrogen, Carlsbad, CA, USA) to detect them using flow cytometry. DCs were incubated for 24 hours with apoptotic cells labeled with CFSE. This experiment selected a population CD11c+ allowing to identify DCs. CFSE detection in a CD11c+ population (39.5%) is indicative of phagocytosis of apoptotic cells by DCs (Figures 4 and 5). Briefly, apoptotic cells generated by UV-B radiation were labeled with CFSE and co-cultured for 24 hours with DCs. DCs were labeled with vital dye BODIPY-TR Ceramide (Life Technologies), and as observed in Figure 6, the composition of the image shows that DCs are able to endocytose apoptotic cells in a control individual (panel B) as well as SLE patients (panel C).

Efecto de co-cultivo de células apoptóticas y DCs en inmunofenotipo de DCs.

It is widely reported that DNA molecules play a role as autoantigen in lupus patients and that are exposed to extracellular space during apoptosis associated to proteins, such as histones

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Effect of co-culture of apoptotic cells and DCs on DCs immunophenotype.

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