[A287] Evaluation of apoptosis in peripheral human lymphocytes exposed to Rapamycin.

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The influence of immunosuppressive drugs in the regulation of apoptosis of activated lymphocytes and their role in the control of immune response to human allografts is still not well determined. The aim of this study is to evaluate the induction of apoptosis by rapamycin in peripheral blood human lymphocytes.
Methods: Mononuclear cells were isolated by gradient density centrifugation of peripheral blood obtained from five healthy volunteers. Non stimulated and phytohemagglutinin (PHA) stimulated cells were suspended in RPMI 1640 and were cultured for 24 and 48 hours on 24-well plates with and without rapamycin (Wyeth, Brazil). Apoptosis was determined by Annexin V-EGFP staining (Alexis Biochemicals, Switzerland) to externalized phosphatidylserine and analysed by flow citometry. Five experiments were performed in triplicates for each experimental condition (with and without PHA and with and without rapamycin). Results are shown as average standard deviation and Stundents t test was used for statistical analyses.
Results: In non-stimulated cultures there was no statistically significant difference in the percentage of apoptotic cultured lymphocytes with and without rapamycin both after 24 hours (7.1% 3.8% x 6.6%2.6%; P=1.0) and after 48 hours (6.1%1.9% x 6.2% 1.8%; P=1.0). In PHA stimulated cell cultures in the absence of rapamycin there was a statistically significant increase in the percentage apoptotic cells at 24 hours (39.0%12.6% x 6.6% 2.6%; P=0.002) and 48 hours (24.3%11.0% x 6.2%1.8%; P=0.033) respectivelly, as compared with non-stimulated cultures. The adition of rapamycin to PHA stimulated cultures did not modify the percentage of apoptotic cells at 24 hours (49.5%11.0% x 39.0%12.6%; P=0.69 and at 48 hours (30.2%8.7% x 24.3%11.0%; P=0.73).
Conclusion: These data demonstrate that there is increased apoptosis upon stimulation of peripheral blood lymphocytes in vitro and that rapamycin does not induce apoptotic cell death in such conditions.