Materials and Methods

To the wells in a volume of 180 µL, a total of 1 x 10^5 cells were then brought to a concentration of 3 x 10^5 to 4.875 x 10^5 cells in RPMI+10%FBS and counted manually. The cells were washed twice with PBS+2%FBS. Cells were suspended in human blood was lysed with ammonium chloride for 10 minutes on ice to the relatively higher density of rat lymphocytes. Instead, the cells were processed using the same method as the human blood.

Canine blood was obtained from multiple donors (SNBL USA). Canine blood was processed using the same method as the human blood. Mouse and rat cells couldn't be processed over ficoll and mouse samples and stored in the gaseous phase of liquid nitrogen. Mouse and rat cells couldn't be processed over ficoll but were instead lysed with an ammonium chloride buffer. Consequently, viability post-thaw was greatly diminished and the baseline level of Annexin V expression was unacceptably high for use in a predictive assay (Figure 3).

Results

Flow cytometry proved to be a useful platform in evaluating T and B cell apoptosis using species-specific antibodies. For Rodent T cells, Annexin V and CD45R(B220)+ were used to detect T and B cells, respectively. For rat, B cells were detected by gating on CD3-CD45R(B220)+, and for mouse, B cells were detected by gating on CD3-CD45(R/B220)+. Examples of the gating used to identify cells of a specific lineage, and to discriminate apoptotic from non-apoptotic cells, are shown in Figures 1A through 1E.

After establishing the appropriate antibody panels, gating strategy and positive control for each species, we then compared two MAPK kinase inhibitors, Losmapimod and VX-702, for their ability to trigger apoptosis.

T cell populations were not affected by Losmapimod in any species. In contrast, B cell apoptosis was detected in human and dog PBMCs only in response to Losmapimod at 10, 1 and 0.1 µM. VX-702 had no effect on T cell apoptosis in any species, but there was an increase in B cell apoptosis in both human (100, 10 and 1 µM), cyno (100 and 10 µM) and dog (1, 0.1 and 0.01 µM) PBMCs (Figure 2).

For both Losmapimod and VX-702, there was a hook effect in the dog cells indicating that this species’ cells are particularly sensitive to these compounds. Cells were frozen down from a number of fresh human, NHP, dog, and rat samples and stored in the gaseous phase of liquid nitrogen. Mouse and rat cells couldn't be processed over ficoll but were instead lysed with an ammonium chloride buffer. Consequently, viability post-thaw was greatly diminished and the baseline level of Annexin V expression was unacceptably high for use in a predictive assay (Figure 3).

Conclusions

Fresh peripheral blood from human, NHP, dog and rat provide an appropriate cell type to evaluate potential lymphotoxicity. Fresh spleen cells from a mouse provide an adequate supply of cells to assess lymphotoxicity in the mouse, where blood volumes may be too low for same.

Staurosporin at 3 µM caused both T and B cell apoptosis in all species tested. Losmapimod did not affect T cell apoptosis in any species, however B cell apoptosis was detected in human and dog PBMCs. VX-702 had no effect on T cell apoptosis in any species, but there was an increase in B cell apoptosis in human, NHP and dog.

Although banking cells and having a ready supply for drug screening is optimal, thawed PBMCs do not function appropriately in this assay.

This specialized primary cell based platform can evaluate novel targeted therapies on species-specific lymphoid toxicity prior to or concurrent with in vivo testing and may be of use pre-clinically to identify potential discrepancies between human and animal models.

References


Table 1. Staurosporin induced T and B cell apoptosis in all species tested when used on freshly isolated samples.