

Induction of Cytolytic Natural Killer Cells and Tumour Rejection in Multiple Myeloma Using Ultraviolet Light Irradiated Tumour Cells for Lymphocyte Priming

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SUMMARY

Eradication of tumour cells is most effectively performed by cytolytic T lymphocytes (CTL).¹ This is mediated by the proapoptotic protein, granzyme A, and the pore-forming protein, perforin.² Since expression of heat shock proteins (HSP) is induced via ultraviolet (UV) irradiation, and HSPs are known to mediate tumour rejection;³ we investigated whether multiple myeloma (MM) cells could be eradicated by CTLs primed using UV-irradiated MM cells. We used UV-irradiated MM cells as immunogens to prime (*ex vivo*) normal peripheral blood (PB) mononuclear cell (MNC) responder cells. Initiating co-cultures contained 50,000 non-UV-irradiated PBMNCs and 500,000 UV-irradiated MM cells per well (i.e. 1:10 responder cell:stimulator cell ratio). The resulting CTLs were co-cultured with MM cells, and analysed for tumour cell eradication as well as CTL expansion using indirect immunofluorescence flow cytometric analysis. The various T cell and natural killer (NK) cell subsets were identified using CD4/CD8 and CD56 staining; and CTLs using granzyme A/perforin staining. We demonstrated that granzyme A plus perforin-positive CTLs were enriched (0.2% on Day 0; 25.5% on Day 7;

and 53.5% on Day 14), accompanied by enrichment (0.0% on Day 0; 26.3% on Day 7; and 54.3% on Day 14) of CD4-CD8-CD56+ NK cells in co-cultures of UV-irradiated RPMI 8226 MM stimulator cells. Moreover, priming of PBMNCs using UV-irradiated tumour cells resulted in significant (75 to 90%) eradication of MM cells. These data suggest that UV-irradiated tumour cells are effective immunogens that can be used to prime PBMNCs and generate tumour-specific CTLs. Our study, therefore, provides the framework for the development of anti-tumour CTL cellular vaccines for treating MM using UV-irradiated tumour cells as immunogens.

FIGURE LEGENDS

Co-cultures of PBMNCs (outside ovals) and UV-irradiated RPMI 8226 MM cells (within ovals) were assayed using flow cytometry (forward scatter = cell size; side scatter = cell granularity). Significantly ($p < 0.001$) fewer (5.4% and 5.6% for Days 7 and 14, respectively) MM cells remained in cell co-cultures that contained primed CTLs (Figs. 1a and 1c) as compared to those containing unprimed PBMNCs (52.3% and 22.1%) (Figs. 1b and 1d).

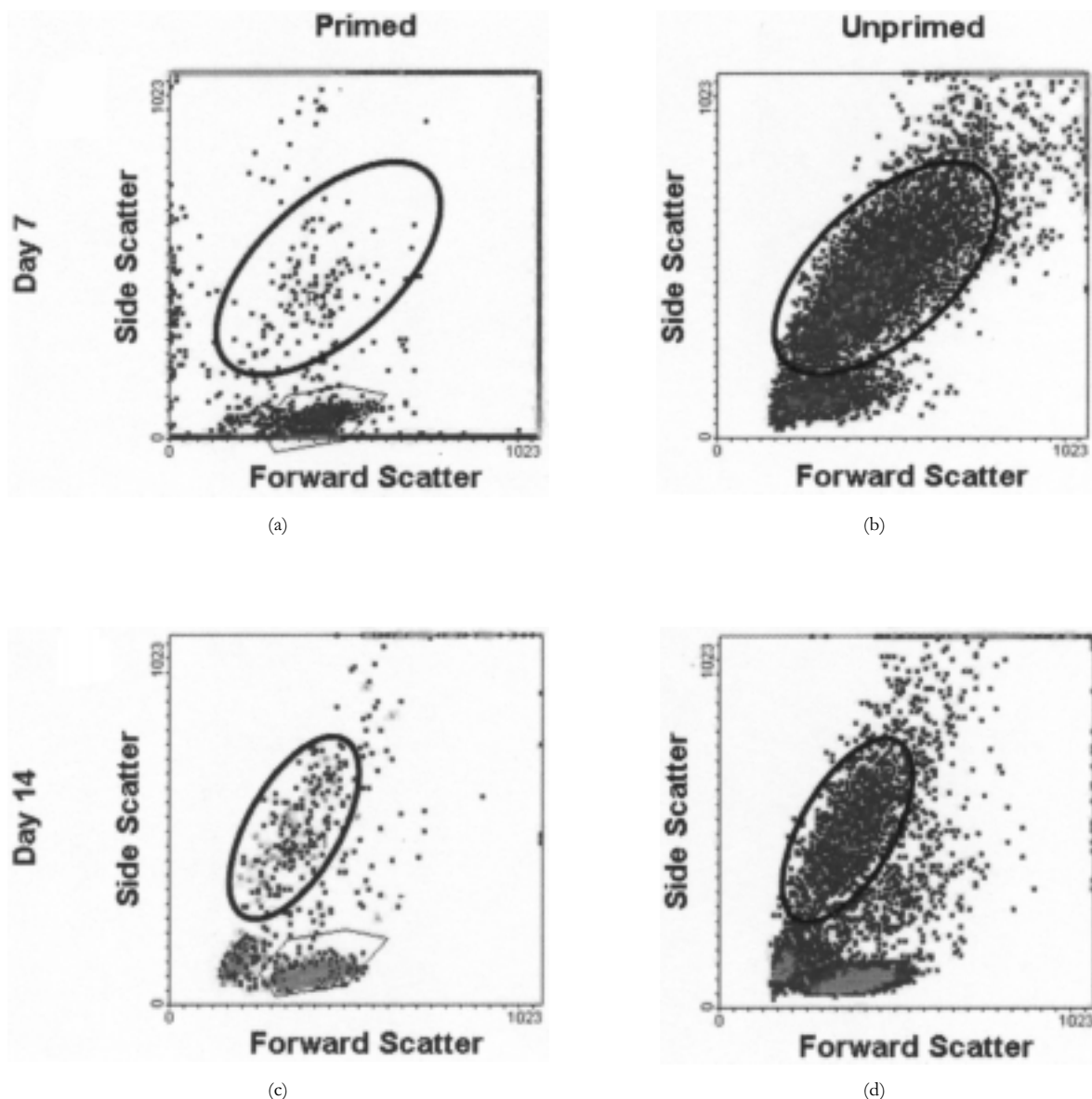


Fig. 1. Eradication of MM cells by CTLs primed with UV-irradiated MM cells.

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