APOPTOSIS: A CAUSE OF POSITIVE LYMPHOCYTE CROSSMATCHES?

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Introduction

Apoptosis is the controlled disassembly of cells that have been signaled for death. The apoptotic pathway results in the movement of the phospholipid, phosphatidylserine (PS) from the inner to the outer membrane of the cell, serving as a target for macrophage recognition and phagocytosis. Cells undergo apoptosis in response to a variety of stimuli, including physical, chemical, or nutritional stress.

Anti-phospholipid antibodies have been described in renal patients with systemic lupus erythematosus (SLE) or with thromboembolic events associated with anti-phospholipid syndrome. These patients are known to have a high incidence of positive lymphocyte crossmatches unrelated to HLA antibodies. Studies were conducted to determine whether apoptosis and outer membrane PS expression is induced during preparation and storage of donor lymphocytes and if so, whether this contributes to positive crossmatches for certain patients.

Materials and Methods

Lymphocyte Isolation, Purification and Storage:

Lymphocytes were isolated from anticoagulated whole blood using ficoll-hypaque density gradient separation. Standard lymphocyte purification included aggregation and removal of platelets with 0.1% adenine diphosphate and red blood cell lysis with hemolytic buffer. Modifications to the standard procedure included an additional hemolytic buffer wash or treatment with CD14/CD15 specific DynaBeads™ (Dynal) in 0% or 10% fetal calf serum (FCS). Lymphocytes were stored in RPMI or RPMI + 0.1% EDTA (FACS Media) and supplemented with 0% or 5% FCS at 4°C or room temperature.

Flow Cytometric Analyses:

Annexin V and propidium iodide (PI) staining were assessed by flow cytometry to determine the percentage of viable, apoptotic and necrotic donor lymphocytes prepared and stored under different conditions. Viable cells are identified by failure to bind Annexin V and PI, whereas apoptotic cells bind only Annexin V and necrotic cells bind both Annexin V and PI.

Lymphocyte preparations with >50% apoptotic cells and preparations with >95% viable cells were used in flow cytometric lymphocyte crossmatches with post

nephrectomy sera from 4 patients with SLE and/or a history of thrombotic complications. Sera were tested undiluted and diluted 1:2 with normal serum. All assays were acquired on a FACScan™ flow cytometer and analyzed utilizing CellQuest™ software (Becton-Dickinson).

Anti-phospholipid Antibody Analysis:

One patient serum was tested for the presence of anti-phospholipid antibodies, including anti-PS by ELISA, as described by McIntyre, et al (Clin Cardiol 1995; 18: 575).

Results

The effects of different preparation and storage conditions on induction of apoptosis and necrosis are shown in Figures 1 and 2. A higher degree of apoptosis is observed in the presence of Ca^{2+} as well as at $4^{o}C$ versus room temperature. The percent of necrotic cells increases when cells are purified and stored in the absence of a protein source such as fetal calf serum.

Flow cytometric crossmatches with >50% apoptotic donor cells were compared to the same donor cells prepared in such a way to minimize apoptosis. Significantly higher channel shifts were observed with apoptotic donor cells and the diluted serum of patient #4 than with viable donor cells and her undiluted serum (Table 1). However, there was no difference between crossmatches using apoptotic versus viable cells for the three other patients (data not shown).

Conclusion

We were intrigued by the crossmatch results of patient #4's serum and apoptotic donor cells, given that this patient lost her allograft due to thrombosis within one week of transplant. We initially speculated that the higher channel shifts observed in this patient were due to anti-phospholipid antibody binding to the complex of protein provided by normal serum and exposed PS on the apoptotic lymphocytes. However, no anti-phospholipid antibodies, including anti-PS were detected by ELISA. Furthermore, a serum with a known high titer of anti-PS antibodies did not react more strongly with apoptotic donor cells than with viable cells. Therefore, although the induction of apoptosis may result in a positive lymphocyte crossmatch in a few patients, it appears to be independent of anti-phospholipid antibodies.

Acknowledgement

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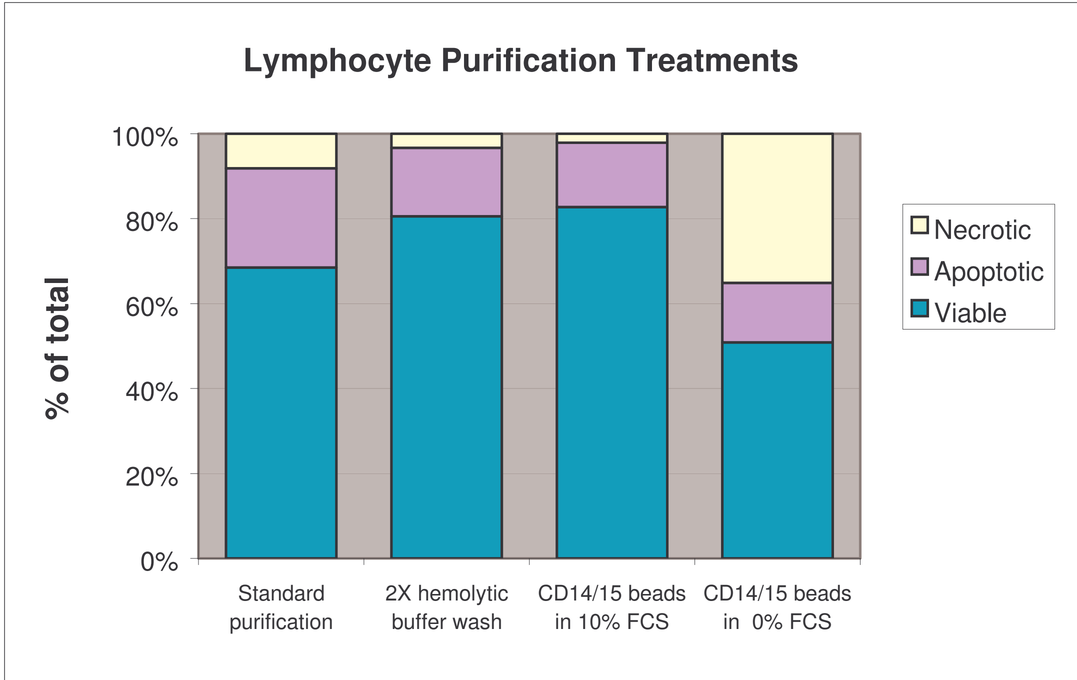

Figure 1. Effect of purification treatments on the percent of viable, apoptotic, and necrotic cells in a lymphocyte preparation

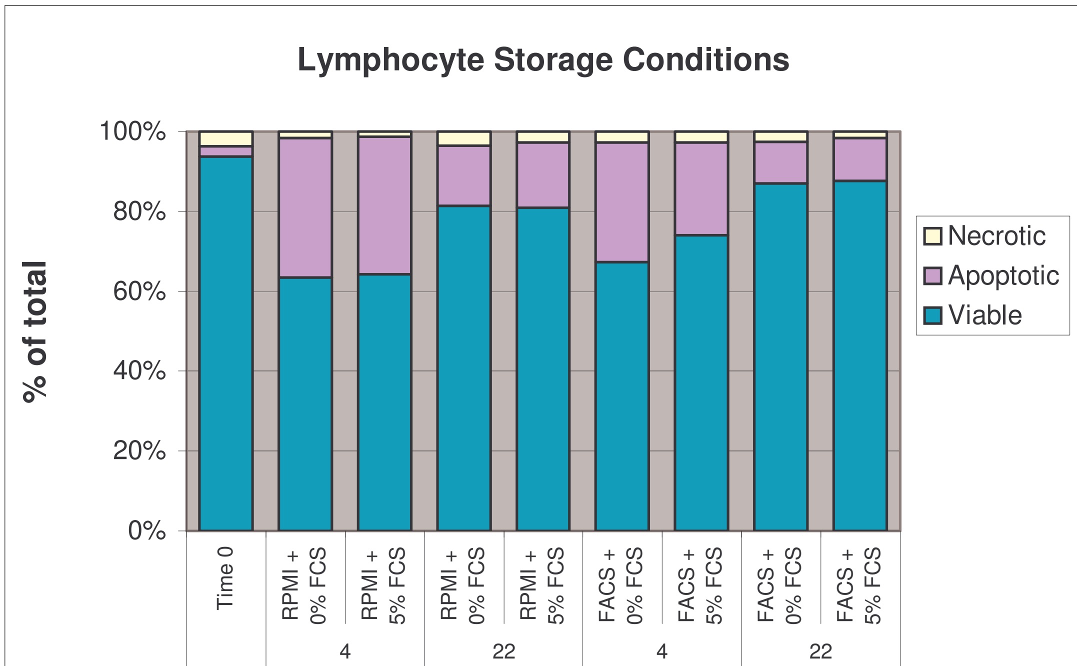

Figure 2. Effect of storage conditions (18-24 hour) on the percent of viable, apoptotic, and necrotic cells in a lymphocyte preparation.

	Channel Shifts			
	T-IgG neat	**T-IgG 1:2**	**B-IgG neat**	**B-IgG 1:2**
>95% Viable	14	11	7	**27**
>50% Apoptotic	**23**	14	**23**	**41**

Table 1. Patient #4's crossmatch results. Channel shifts $\geq$ 15 are considered positive.