

THE EFFECT OF PRONASE ON HLA AND CD20 ANTIGENS



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Introduction

As a prelude to establishing flow cytometry-based crossmatching for patients treated with Rituximab we evaluated the effect of different concentrations of pronase (protease Type XIV from *Streptomyces griseus*, Sigma Aldrich, UK) on the expression of HLA-Class I (CI), HLA-Class II (CII) and CD20.

Methods

15 fresh peripheral blood lymphocyte (PBL) and 5 fresh splenic lymphocyte preparations were assessed. Each cell pellet was mixed and incubated for 30 mins at 37°C with 1ml of pronase at concentrations of 0.5, 1.0, 1.5 and 2.0 mg/ml in PBS. PBS only was used as a control.

Antigen expression at each pronase concentration was evaluated using fluorescent antibodies, viz. anti-HLA-ABC/FITC (IM1838U), anti-HLA-DR, DQ, DP/RD1 (6604366) and anti-CD20/PC5 (A07773) (Beckman-Coulter, UK).

Results

See the two figures below. For both PBL and splenic cell types, average CD20 median channel fluorescence (MCF) with 0.5 mg/ml of pronase dropped to approximately 10% of control values and was negligible with increasing concentrations of pronase thereafter.

Average CI MCF steadily decreased with increasing pronase concentration with an average CI MCF of

56% for PBLs and 41% for splenic cells with 2.0 mg/ml pronase.

Average CII MCF, measured on PBL 'B-cells' (those cells which fell into a HLA-CI and CII positive gate), initially decreased to 79% at 0.5 mg/ml pronase, but then rose steadily to 97% MCF at 2.0 mg/ml pronase.

Of the 15 PBL samples, with increasing pronase concentration, 7 had an increase in CII MCF, 6 a decrease and 2 showed an initial decrease followed by an increase in MCF. For spleen derived 'B-cells', average CII MCF increased 30-40% above control MCF at all concentrations of pronase.

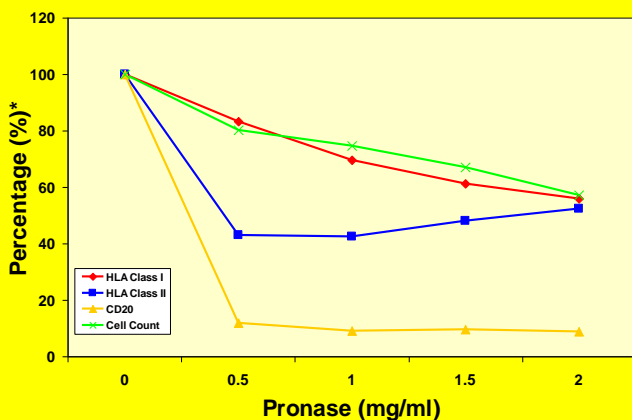
PBL cell counts decreased with increasing concentrations of pronase - down to 57% of the control count at 2.0 mg/ml. The total spleen cell count was seemingly unaffected but splenic B-cells decreased with increasing concentrations of pronase similar to PBLs.

Conclusions

Pronase treatment is clearly suitable for removing lymphocyte CD20. However, HLA antigen expression and cell viability are also significantly affected with increasing concentrations of the enzyme.

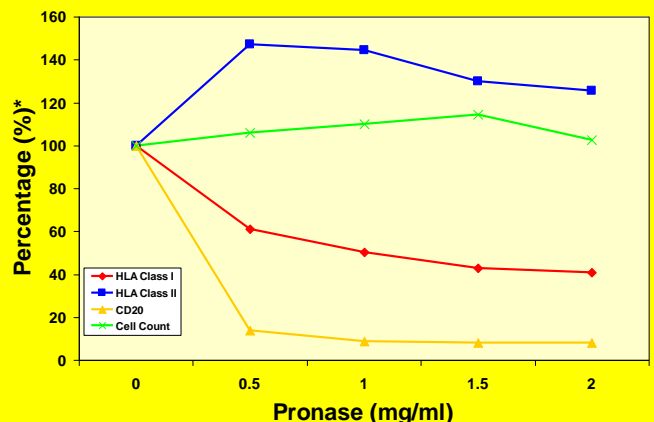
It is clearly vital that pronase concentrations - and other technical parameters - are well validated for flow cytometry-based donor/recipient crossmatching.

**Pronase treated PBLs - All cell types:
Antigen and cell count percentages**



* Percentage median channel fluorescence for antigens, percentage cell number for cell count.

**Pronase treated spleen - All cell types :
Antigen and cell count percentages**



* Percentage median channel fluorescence for antigens, percentage cell number for cell count.