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Lymphocyte Hybridomas

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Expression of Thy 1.2 Antigen on Hybrids of B Cells and a T Lymphoma

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We and others reported that hybrids of spleen cells and the lymphoma line BW5147 express the Thy1.2 antigen of the splenic donor (1-3). Whether only T cells were the splenic parent of these hybrids could not be concluded from the hybrid phenotypes. We report here that either Ig^+ (B) cells or Ig^- (T and other) cells give hybrids with BW5147 that express the Thy1.2 allele in the genome of the spleen cell whether or not that allele was expressed on the spleen cell parent.

Our specific purpose was to find out if any hybrids resulting from the fusion of B cells with BW5147 express the Thy-1 antigen coded for in the B cell. This could be accomplished on non-cloned populations. Therefore, we did not clone the hybrids but rather tested them as populations of hybrid cells.

Spleen cells from (BALB/c x SJL)F_1 mice (Thy1.2) were stained with rhodamine-labeled rabbit anti-mouse immunoglobulin (R-anti-MIg) that had been adsorbed with a 10% volume of normal BALB/c thymocytes. The brightly stained cells, Ig^+ B cells, were separated from the non-stained cells, Ig^-, T cell-enriched populations, by means of the fluorescence-activated cell sorter (FACS) (4) (see Fig. 1). Each separated population was fused, with the aid of polyethylene glycol after the method of Pontecorvo (5) as adapted by Goldsby (1), with BW5147 (Thy1.1) at a ratio of 4:1 (spleen cells to BW5147). After fusion the cells were plated at 1.2×10^5, 0.3×10^5, and 0.17×10^5 cells/ml and cultured in HAT medium. Approximately 4 wks later each well that had visible cell growth was transferred to a new culture vessel and kept as a unique hybrid population.

The fusion of BW5147 with purified B cells did not result in fewer hybrid populations than fusion of BW5147 with the T cell-enriched population (see Table 1). Therefore, we conclude that BW5147 does not preferentially hybridize with T cells.

Table 1. Hybrids between BW5147 and separated T or B spleen cells

	No. of wells with hybrids / Total no. of wells plated
BW x Ig^+	37/72
BW x Ig^-	21/72

The hybrid populations were tested for the expression of the Thy-1 allele of each parental type -- Thy1.1 from BW5147 and Thy1.2 from the (BALB/c x SJL)F_1 spleen cells. Each hybrid population was tested by direct cytotoxicity using anti-Thy1.1 and anti-Thy1.2, produced in congenic strains of mice, plus rabbit complement. All of the hybrid populations were lysed with anti-Thy1.1 plus complement. Approximately 80% of thy hybrid populations showed significant cytotoxic indices with anti-Thy1.2 plus complement (see Fig. 2).

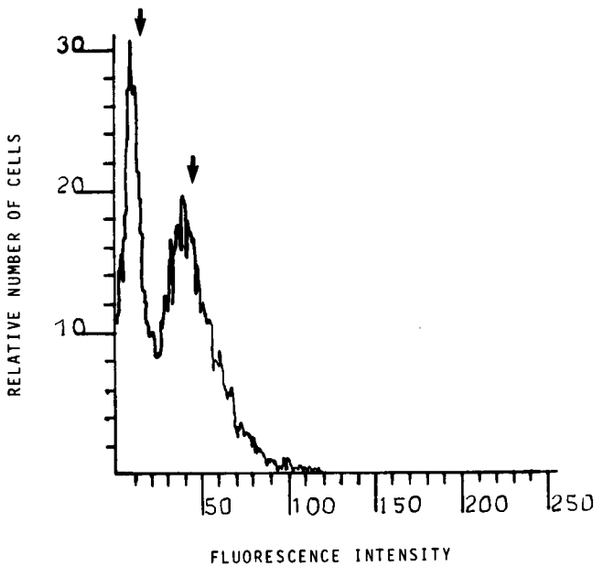
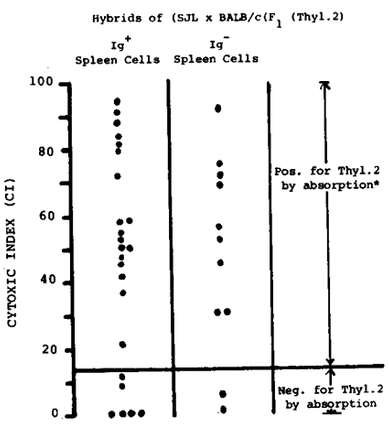


Fig. 1. FACS fluorescence profile of (BALB/c x SJL)F_1 spleen cells stained with rhodamine-labeled R-anti-MIg. The arrows indicate the gates used to separate the Ig^+ (the brightest 38%) from the Ig^- (the dullest 40%).

Fig. 2. The expression of Thy1.2 on hybrids between BW5147 (Thy1.1) and separated Ig^+ and Ig^- spleen cells of Thy1.2$^+$ mice



All hybrids were Thy1.1 positive by cytotoxicity.

$$CI = \frac{(\%\ \text{kill}\ \bar{c}\ \text{Ab} + c') - (\%\ \text{kill with c' only})}{100 - (\%\ \text{kill with c' only})} \times 100$$

*Tested back on Thy1.2 thymocytes. No absorption by BW5147 was found.

The background cytotoxicity of rabbit complement alone was constant for any given hybrid population but was quite variable from one population to another. The background cytotoxicity varied from as low as 10% with some hybrid populations to as high as 50% with others. This was true for six different lots of rabbit complement.

To ensure the specificity of the direct cytotoxcity test, all of the hybrids were tested for their ability to specifically adsorb anti-Thy1.2 activity against BALB/c thymocytes. Normal BALB/c thymocytes absorbed and BW5147 did not absorb the anti-Thy1.2 activity. Absorption of anti-Thy1.2 was observed for a given hybrid population only if a cytotoxic index (CI) above 20% had been found for that population. None of the hybrid populations had detectable surface Ig by immunofluorescence and FACS analysis nor secreted Ig by radioimmune assay.

From these experiments we conclude that BW5147 can and does fuse with both splenic B and T cells. In about 80% of the hybrid populations the Thy-1 antigen of the normal B or T cell donor is expressed. Also, the surface Ig characteristics of the splenic B cell are lost in the hybrid with BW5147.

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