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Hybrid cell lines with T-cell characteristics

THE hybridisation of established cell lines with differentiated cells provides a useful strategy for the production of cell lines having differentiated properties and unlimited growth potential. Clones of these hybrids could be particularly useful in the dissection of cellular interactions of the immune system, a network involving numerous specific cell types which display characteristic ensembles of surface and internal markers. We describe, here, the production and partial characterisation of hybrid cell lines with surface antigens characteristic of T lymphocytes.

Köhler and Milstein[1,2] have shown that the fusion of mouse myeloma cells cultivated *in vitro*, with spleen cells from immunised mice, gives rise to B-cell hybrid cell lines which secrete monoclonal immunoglobulins with antibody activity to the immunising antigen. Although 30–40% of the spleen cells used for fusion were T cells, none of the hybrids expressed the Thy-1 surface antigen, a characteristic marker for mouse T cells. Without expression of this (or some other) T-cell marker, it is difficult to determine in retrospect whether the non-producing hybrids were T cells-myeloma fusion products in which Thy-1 is repressed.[3]

In contrast with results from the myeloma fusion studies, we found that fusion of spleen cells with a mouse T-lymphoma cell line (BW 5147) gives rise to hybrid cell lines which express T-cell and not B-cell markers. Hybrid populations were obtained by fusing spleen cells from $(BALB/c \times SJL)F_1$ mice with HAT- sensitive BW 5147-AzG^R, Ou^R cells (kindly provided by Dr Robert Hyman). The BW 5147 cells were HGPRTase negative and resistant to 1×10^{-3} M ouabain. Spleen donors were immunised by intraperitoneal injection of 100 μg of DNP-KLH on alum with 2×10^7 *Bordetella pertussis*, and boosted 3 months later by intravenous injection of 20 μg of DNP-KLH. Cells were harvested from the spleens 4 days later. Hybrids were also obtained by fusing BW 5147 with spleen cells from non-immunised CBA and AKR donors which had been stimulated for 4 or 5 d in mixed lymphocyte cultures (MLC) by irradiated allogeneic spleen cells.

For the fusion, a mixture of 10^8 spleen or MLC-derived cells and 10^7 BW 5147 cells in Dulbecco's Modified Eagles medium plus 10% foetal calf serum (DME-FCS) was pelleted at 400*g*. The medium was removed and fusion initiated by the gradual addition of 2 ml of 50% (w/w) polyethylene glycol (PEG) 1,000 or 1,500 in serum-free DME. After 2 min at 37 °C, the suspension was then gradually diluted to 50 ml (over the course of 10 min) with serum-free DME and then pelleted by centrifugation[4].

The pelleted cells were next resuspended and cultured in 0.2- or 2-ml volumes at $1–2 \times 10^6$ cells ml^{-1} in selective medium (DME-FCS, 100 μM hypoxanthine, 10 μM aminopterin, 30 μM thymidine and 3.3×10^{-4} ouabain). Preliminary experiments have shown that neither spleen cells nor BW 5147 cells survive for longer than a few days in this medium; therefore, after 14 d, selective medium was removed and replaced with DME-FCS. All populations surviving after 2 weeks were presumed to be hybrids since survival requires that the gene for HGPRTase from the spleen cell parent and the (genetic) information for continuous growth from the lymphoma parent both be present in each cell.

In later experiments, cell numbers per well were adjusted to allow one or a few hybrid clones to be seeded. Hybrid populations were transferred when adequate growth was observed. Clonal populations were obtained either by limiting dilution in 0.25-ml wells or by soft agar cloning. Only the $(BALB/c \times SJL)F_1$ hybrid populations were cloned at the time of this report.

The putative hybrids were analysed for DNA content per cell using flow micro fluorometry in a fluorescence activated cell sorter (FACS-II, Becton-Dickinson Electronics Laboratory, Mountain View, California) after fluorescent straining of the DNA with propidium iodide in hypotonic (0.1%) sodium citrate[5]. Approximately 40 hybrid populations were tested within the first 2 weeks after hybridisation. All had G_1 DNA contents which were about the

Table 1 Expression of Thy-1 antigens on hybrid cells

Experiment	Hybridisation: Parental populations			Number of hybrid populations expressing Thy-1 alleles*			
				1.1+1.2	1.1	1.2	—
921	AKR T Lymphoma BW 5147 (Thy-1.1)	×	$(BALB/c \times SJL)F_1$ Spleen (Thy-1.2)	18	7	0	2
C.2	AKR T Lymphoma BW 5147 (Thy-1.1)	×	CBA Spleen MLC† (Thy-1.2)	11	1	0	0
A.2	AKR T Lymphoma BW 5147 (Thy-1.1)	×	AKR Spleen MLC (Thy-1.1)	0	13	0	0
Kohler and Milstein[4]	BALB/c Myeloma MOPC 21 (X63) (Thy-1 negative)	×	BALB/c Spleen (Thy-1.2)	0	0	0	>10

*Populations from experiment 921 are individual clones, populations from experiments C.2 and A.2 are isolates from different fusion hybrids and may contain one or more clones. Thy-1 phenotype was determined by direct cytotoxicity using congenic anti-Thy 1 antisera (experiment 921) and non-congenic antisera (experiments C.2 and A.2). Thy-1 phenotype of several clones in experiment 921 was confirmed by quantitative absorption studies.

†Cells from a 5-d mixed lymphocyte culture (MLC) of spleen cells stimulated with irradiated allogenic cells. Nearly all surviving cells in this culture are T cells from the responder spleen.

sum of the DNA contents of spleen cell (diploid mouse) and the near tetraploid lymphoma used for hybridisation.

The expression of both parental Thy-1 alleles on 29/39 hybrid cell populations (and clones) obtained from hybridisation of Thy-1 differing cells demonstrates directly that the hybrids arose from fusion of normal spleen cells and the BW 5147 lymphoma (see Table 1). Specificity of typing was confirmed by quantitatively absorbing the anti Thy-1.2 with hybrid cells and then testing the absorbed antiserum on normal thymocytes to demonstrate removal of anti Thy-1.2 activity. The demonstration that hybrids of BW 5147 with a Thy-1.1 carrying strain, AKR, express only Thy-1.1 rules out expression of a cryptic Thy-1.2 allele by the hybrids (Table 1).

Identification of the splenic lymphocyte subpopulation which fused with BW 5147 to give rise to hybrids expressing both parental Thy-1 alleles is difficult in retrospect; however the increased frequency of such hybrids obtained by fusion of MLC cells, which are nearly all T cells, suggests that the Thy-1.1, 1.2 hybrids are probably derived from fusions of normal T cells with the lymphoma (Table 1). Whether the fusions which gave rise to hybrids expressing only the BW 5147 Thy-1.1 allele were also T cell–lymphoma fusions may become clear as the hybrids are tested for more T-cell markers. Interestingly, the spleen cell hybridisation gave rise to two clones which express neither parental Thy-1 allele, that is in which the BW 5147 Thy-1.1 is lost or repressed.

Preliminary tests for Ly 1,2,3, and Ia markers on the hybrids are consistent with hybrids being derived from fusions of normal T cells and lymphoma cells. The BW 5147 lymphoma comes from a strain which carries Ly-1.2 and Ly-2.1; however it expresses neither of these characteristic T-cell markers. T cells from $(BALB/c \times SJL)F_1$ mice carry Ly-1.2 and Ly-2.2 determinants. Some of the clones derived from the $(BALB/c \times SJL)F_1$ hybridisation selectively express Ly-1.2 determinants, some selectively express Ly-2.2 determinants while the majority expresses neither. Similarly, Ia determinants selectively expressed on a very small subpopulation of lymphocytes (most likely T) in Ia^s haplotype mice and not on BW 5147 (Ia^k) are expressed in some of the hybrid clones derived from the $(BALB/c \times SJL)F_1$ Ia^d/Ia^s haplotype spleen cells. Among normal T lymphocytes, selective expression of Ly and Ia determinants defines supopulations responsible for the various T-lymphocyte functions[6,7].

From the above, it is clear that the hybridisation of spleen cells with an appropriate established cell line (such as the BW 5147 T lymphoma) can yield continuous cell populations expressing characteristic T-cell surface markers. The preliminary evidence for expression of functional T-cell subset markers by these hybrid populations encourages us to examine them further for the presence of T-cell functions such as helper, suppressor and cytotoxic activity. It is apparent that the expression of any of these functions by clonal populations of continuous cell lines would greatly facilitate the analysis of these T cell-mediated functions.

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