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Journal of Immunological Methods, 144 (1991) 1–10

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JIM 06092

CD4 rat × rat and mouse × rat T cell hybridomas produced by fusion of established T cell lines and clones to W/Fu (C58NT)D

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(Received 26 March 1991, revised received 22 May 1991, accepted 21 June 1991)

Previously, fusion of established T cell lines or clones has been claimed to be difficult. We now report our experiences in the fusion of both long term cultures of rat T cell clones and mouse T cell lines to rat W/Fu (C58NT)D. Upon fusion of rat T cell clones the hybrids obtained expressed antigen specificities identical to those of the parent clones. In addition, C58 was used for interspecies hybridisation of murine T cell lines. The specificity of intra- and inter-species hybrids was maintained by subcloning.

We conclude that the C58 cell line can be used to generate continuously growing monoclonal T-cell reagents of sufficient stability using both intra- and inter-species hybridisation.

Key words: CD4$^+$; T cell hybridoma; Intra- and inter-species hybridisation

Introduction

The understanding of T cell function has been greatly advanced by the study of T cell clones and T cell hybridomas (Möller, 1989). In comparison to T cell clones, T cell hybridomas display a number of attractive practical qualities. Antigen specific T cells are immortalized by fusion to T cell tumour lines and hence cell expansion is independent of regular restimulation with antigen thereby excluding contamination with feeder cells. Antigen-specific reactivity is independent of the cell cycle and a continuous source of well characterized monoclonal cellular reagents is available. Cell activation of CD4 positive hybridomas can be measured easily by determining lymphokine production (e.g., IL-2). One possible disadvantage which is experienced in working with T cell hybridomas is their relative instability, a phenomenon which is common to hybridomas in general and is due to chromosome loss (Taussig, 1985). Because hybridomas can be frozen and thawed easily, this problem can be overcome by freezing multiple aliquots of cells.

To the best of our knowledge there is only one report on the generation of rat × rat T cell hybridomas by fusion to C58 (Curling et al., 1988). Freshly isolated rat spleen cells were used to generate T suppressor hybridomas. Established, long term cultured T cell clones are reportedly more difficult to fuse than fresh lymph node T cells (Ozaki et al., 1988). In fact, fusion of estab-

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lished rat T helper lines or clones to rat C58 has not been reported. This paper describes the successful immortalization of the established rat T cell clones A2b, A2c (Cohen et al., 1985) and Z1a (Ben Nun and Cohen, 1982) by fusion to C58. The resulting T cell hybrids were shown to possess T cell antigen specificities which were identical to the parental clones, a feature which was reported earlier by others for mouse × mouse T cell hybridomas (Schreier et al., 1982; Krammer et al., 1983).

In addition, C58 was used successfully in the generation of interspecies T cell hybridomas from various, virus-specific mouse T cell lines providing us with monoclonal systems to study the fine specificity and restriction of T cell recognition.

Materials and methods

Antigen source

Rat × rat hybridomas. In order to select antigen-specific hybridomas following fusion of the A2b and A2c clones to C58, the mycobacterial heatshock protein 65 kDa was used. Additionally, we selected hybridomas with the 180–188 peptide of the 65 kDa protein which was shown to be the epitope recognized by both A2b and A2c (Van Eden et al., 1988). To select antigen-specific hybridomas after fusion of the Z1a clone (Ben Nun and Cohen, 1982) to C58, we used crude myelin basic protein (MBP) extracted from guinea pig spinal cord as described by Hirshfeld et al. (1970).

Mouse × rat hybridomas. To generate infectious bronchitis virus (IBV)-specific T cells and to select IBV-specific T cell hybridomas, a sucrose gradient purified preparation of IBV, strain M41 was used (Niesters et al., 1986). The synthetic peptide which contains the T cell epitope recognized by T cell hybridomas MJB100 and MJB101 (IBV N 67-83) was synthesized at TNO MBL Rijswijk, The Netherlands (Boots et al., 1991). Foot and Mouth disease virus (FMDV) of serotype A10 Holland (Meloen and Briaire, 1980), grown on monolayers of BHKZ1 cells and purified as described elsewhere was used to stimulate FMDV-specific mouse T cells and to select FMDV-specific mouse × rat T cell hybridomas.

Cell lines

Fusion partner. The cell line W/Fu(C58NT) D, a rat thymoma (Geering et al., 1966) HGPTR deficient and Ouabain resistant, was donated by Dr. Curling, Oxford, U.K., and was used as fusion partner. C58 cells were cultured in Iscove's modification of Dulbecco's medium (Gibco, Breda, The Netherlands) supplemented with 10% fetal calf serum (Gibco), penicillin (100 IU/ml) and streptomycin (100 μg/ml). 3 days prior to fusion C58 cells were seeded at 10^5 cells/ml into four 75 cm^2 flasks (50 ml). 24 h prior to fusion the cells were diluted to 10^5 cells/ml to obtain logarithmic growth which was required for optimal fusion efficiency.

Rat T cell clones. The rat T cell clones A2b and A2c were isolated in the rat model of adjuvant arthritis and were shown to modulate disease activity in vivo (Cohen et al., 1985). Both clones were shown to be specific for the 180–188 epitope of the 65 kDa heatshock protein (Van Eden et al., 1988). The rat T cell clone Z1a was isolated in the rat model of experimental autoimmune encephalomyelitis (EAE) and was shown to respond to an epitope of MBP (Ben Nun and Cohen, 1982). A2b, A2c and Z1a were used as parent clones for fusion to C58. As a preparation for fusion, cells were stimulated with specific antigen in the presence of antigen presenting cells (APCs). 3 days after stimulation IL-2 was added as this was reported to augment hybridization frequency (Ozaki et al., 1988). Cells were fused on day 4 after stimulation.

Murine T cell lines. The murine T cell lines were obtained after i.p. immunization of BALB/c mice, according to institutional guidelines at 6–8 weeks of age (Harlan CPB, Zeist, The Netherlands) using either a mixture of RIBI (Immunochemical Research, Hamilton, MT, U.S.A.) and a gradient-purified UV-inactivated (Jacobs et al., 1981) IBV M41 preparation or with a mixture of RIBI and inactivated FMDV-A10 Holland. Each mouse received approximately 20–50 μg of protein. 6 weeks after immunization, mice were killed to obtain the spleens. Spleen cells were incubated for 3–4 days at 2×10^6 cells/ml in Iscove's modification of Dulbecco's medium (Gibco) supplemented with 10% fetal calf serum (Gibco, Breda, The Netherlands), penicillin (100

IU/ml), streptomycin (100 μg/ml) and 2-mercaptoethanol (2×10^{-5} M) in the presence of M41 or FMDV-A10 Holland (1–4 μg/ml). Subsequently, cells were washed and medium containing 1% of supernatant of Concanavalin A-activated rat spleen cells was added (Kast et al., 1984). 7 days later viable cells were isolated on a density gradient (Lympholyte M; Cederlane Laboratories, Hornby, Canada), washed and cultured with irradiated (2500 rad) syngeneic spleen cells as APCs (10^7 cells/ml) and M41 or FMDV-A10 Holland (1–4 μg/ml) for another 3–4 days. This stimulation cycle was repeated and antigen-specific reactivity was determined. The IBV-specific T cell line and the FMDV-specific T cell line were fused on day 48 and day 26 of culture (fusion 4 and 5) respectively.

Generation of T cell hybridomas

The rat T cell clones (A2b, A2c and Z1a) and the virus specific mouse T cell lines were added to an equal number of logarithmically expanding C58 cells (Table I). The culture medium of the C58 cells was harvested and used as a source of conditioned medium. To accomplish fusion (Fazekas de St.Groth and Scheidegger, 1980; Silva et al., 1983; Curling et al., 1988) the mixture of T cells and tumour cells was incubated with 40% PEG 4000 (Merck 9727) at 37°C for 1 min. The PEG medium was diluted with 8 ml of medium which did not contain serum during the next 4 min. Then 6 ml of conditioned medium containing 15% fetal calf serum (FCS, Boehringer, Mannheim) were added slowly over 1 min and the cells were left to stabilize for 10 min at room temperature. Subsequently, cells were pelleted and resuspended in 10 ml conditioned medium. After fusion, cells were plated in flat-bottomed microtitration plates; 2–5x10^4 cells/well in 200 μl HAT selection medium containing 15% FCS. On days 7, 10 and 13 of culture 50% of the HAT medium was renewed. Within 14–28 days, growth positive wells were scored and hybrid cells were cultured in HT medium (without aminopterin). To ensure monoclonality antigen-specific hybridomas were subcloned by seeding 0.5 cell/well in flat-bottomed microtitration plates in the presence of 10^5 feeder cells/well. The feeder cells consisted of either rat thymocytes or mouse spleen cells.

Selection and characterization of antigen-specific rat × rat T cell hybridomas

Selection. Hybridomas were screened for their ability to produce IL-2 in the presence of antigen and APCs. Hybrid cells (10^4/well) were cultured in 200 μl of medium in flat-bottomed microtitration plates with 10^6 irradiated (2500 rad) syngeneic thymocytes per well and 5–10 μg 65 kDa or MBP antigen. After 24–48 h, 100 μl of the supernatant of these cultures were harvested and assayed for IL-2 (Gillis et al., 1978). This was accomplished by the addition of 5x10^3–10^4 IL-2-dependent CTLL-16 cells (The CTLL-16 is a

TABLE I

FUSION PROTOCOLS USED

Source	Cells: C58	Cells/well	Wells [a]	Hybrids [b]
Rat × rat fusion:				
1 rat T cell clone A2b	1:1	5×10^4	192	6/7 = 86%
2 rat T cell clone A2c	1:1	5×10^4	192	15/22 = 68%
3 rat T cell clone Z1a	1:1	5×10^4	192	25/40 = 62.5%
Mouse × rat fusion:				
4 IBV mouse T cell line fused at day 48	1:1	2–5×10^4	384	2/28 = 8%
5 FMDV mouse T cell line fused at day 26	1:1	2–5×10^4	480	3/6 = 50%

[a] Number of wells seeded.

[b] Relative number of antigen-specific hybrids.

cloned murine cell line, which was kindly donated by Dr. L. Aarden, CLB, Amsterdam, The Netherlands). After a further 24 h, [^{3}H]thymidine (0.4 μCi/well, SA 1.0 Ci/mmol) was added and 18 h thereafter the incorporated radioactivity was measured. Results were measured as stimulation indices (SI = antigen-specific counts per minute (cpm)/control cpm).

Antigen specificity. Antigen-specific hybridomas of fusion 1, 2 and 3 were subcloned (Table I). Subcloning was achieved by the distribution of cells in two microtitration plates (0.5 or 1.0 cell per well). Subclones with the highest IL-2 responses were selected for further characterization.

Cell surface phenotype. Cells (10^6) were tested for the expression of Ox19 (CD5), W3/25 (CD4), Ox8 (CD8), Ox6 (MHC class II RT1.B), Ox17 (MHC class II RT1.D) and R73 (T cell receptor (TCR) α and β chain; Hunig et al., 1989). Supernatants of the Oxford monoclonal antibodies (mAb) were used (McMaster and Williams, 1979; Fukumoto et al., 1982). Cells were incubated in undiluted supernatant of the specific antibody for 30 min at 4°C, washed three times with phosphate-buffered saline (PBS) + 1% BSA + 0.1% NaN_3, incubated for another 30 min with a 1/50 dilution of goat anti-mouse-FITC conjugate (Becton Dickinson, CA, U.S.A.), washed ×3 in PBS + 1% BSA and analysed with the FACSCAN (Becton Dickinson).

MHC restriction. Antigen-specific proliferative responses of T cell clones A2b, A2c and Z1a were measured in the presence of Ox6 or Ox17 antibody supernatant dilutions ranging from 1/40 to 1/200. The antigen-specific IL-2 responses of T cell hybridomas A2bH, A2cH and Z1aH were measured in the IL-2 induction assay in the presence of Ox6 or Ox17 antibody dilutions of 1/40 to 1/1000.

Selection and characterization of virus-specific mouse × rat T cell hybridomas

Selection. The screening of antigen-specific mouse × rat hybridomas was essentially as described above. Hybrid cells (10^4/well) were cultured in 200 μl of medium in flat-bottomed microtitration plates with 2×10^5 irradiated murine spleen cells in the presence of 0.5–1.0 μg IBV M41 or FMDV-A10 Holland antigen. Supernatants of 24 h cultures were harvested and assayed for IL-2.

Antigen specificity. IBV- and FMDV-specific hybridomas were subcloned as described above. Of the subcloned cells, hybridomas with the highest IL-2 responses were selected for further characterization.

Cell surface phenotype. IBV-specific hybridomas (MJB100 and MJB101), the FMDV-specific T cell hybridoma FM1.2 and controls: C58 (fusion partner) and the A2cH hybridoma (rat T cell clone A2c fused to C58) were tested for the expression of Thy-1 (general T cell marker), L3T4 (CD4) and Ly 2 (CD8). Monoclonal antibodies from Becton Dickinson (California, U.S.A.) i.e., L3T4 conjugated to phycoerythrin and Ly 2 and Thy1 conjugated to FITC, were used in a direct staining protocol. Cells (10^6) were incubated in a 1/50 dilution of the antibody conjugates for 30 min at 4°C, washed three times with phosphate-buffered saline (PBS + 1% BSA + 0.1% NaN_3) and analysed with the FACSCAN (Becton Dickinson).

Cell activation with anti-CD3. The anti-CD3 mAb was used to activate T cell hybridomas to produce IL-2 (Bluestone et al., 1987). 10^4 T hybridoma cells were incubated with a 1/200–1/1000 dilution of the mAb supernatant and 24 h later IL-2 production was determined as described above.

MHC restriction. The MHC restriction of the mouse × rat T cell hybrids was determined by substitution of BALB/c APCs (H-2^d) for C57bl/6 APCs (H-2^b) (Harlan CPB, Zeist, The Netherlands). Furthermore the restricting element was determined in detail with the use of L cell transfectants expressing different MHC class II molecules as APCs (Germain et al., 1985; Germain and Quill, 1986; Ronchese et al., 1987).

Results

Selection and characterization of antigen-specific rat × rat T cell hybridomas

Selection. Following fusion of the A2b clone to C58, 192 wells were seeded. Within 4 weeks six out of seven hybrid cell lines showed a 65 kDa

TABLE II

ANTIGEN-SPECIFIC RESPONSES OF RAT × RAT AND MOUSE × RAT T CELL HYBRIDOMAS

IL-2 responses of all hybridoma cell lines are shown to 5 μg/ml antigen. Responses are presented as SI values

T cell hybridomas	Antigens		Peptide 180–188	Control
	MBP	65 kDa protein		
Rat × rat				
A2bH	1	20	17	1
A2cH	1	25	24	1
Z1aH	21	1	1	1
Mouse × rat	IBV M41	IBV N 67-83	FMDV A	Control
MJB100	71	57	ND	< 2
MJB101	28	88	ND	< 2
FM1.2	< 2	ND	41	< 2

specific IL-2 response (fusion 1, Table I). Three clones were subcloned and the best responding clone, A2bH, was selected for further characterization.

After fusion of the A2c clone, 192 wells were seeded. Within 4 weeks 22 wells contained hybridomas. 15 out of these 22 showed a 65 kDa specific IL-2 response (fusion 2, Table I). The highest responding clone, A2CH, was subcloned for stabilization. it was shown that A2bH and A2cH recognized the same epitope as recognized by the parent clones (Table II).

Following fusion of the Z1a clone to C58, 25 out of 40 hybrid cell lines were MBP specific (fusion 3, Table I). The Z1aH hybrid was selected for further characterization.

Cell surface phenotype. FACS analysis showed that the A2bH, A2cH and Z1aH hybrids expressed the cell surface markers Ox19, W3/25 and R73. The C58 cell line showed an intermediate expression of Ox19 (Table III). These cell surface markers were also expressed at the cell surface of the parent clones A2b, A2c and Z1a (Table III). All parental T cell clones expressed the class II antigens RT1.B and RT1.D as detected after staining with the Ox6 and Ox17 mAbs. Of the three hybridomas none expressed class II (Table III).

MHC restriction. The MHC restriction of the parent clones A2b, A2c and Z1a and the A2bH, A2cH and Z1aH cell lines was determined with the use of the anti-class II RT1.B (Ox6) and RT1.D (Ox17) mAbs (Table IV). Antigen-specific proliferative responses of A2b, A2c and Z1a were abolished in the presence of a 1/40 dilution of Ox6 antibody supernatant, indicating RT1.B restricted recognition of antigen. Higher dilutions resulted in concentration dependent inhibition of proliferative responses. The antigen-specific response of either of the clones was not affected in the presence of a 1/40 or higher dilution of the Ox17 antibody supernatant. The T cell hybridomas A2bH, A2cH and Z1aH showed the same

TABLE III

SURFACE MARKER EXPRESSION ON RAT CELL LINES

Expression of cell surface markers on rat cell lines before and after fusion to C58. The T cell clones A2b, A2c and Z1a were stained 7 days after restimulation with antigen and 3 days after the addition of IL-2 to the culture medium. The hybridomas A2bH, A2cH and Z1aH were cultured in medium not containing IL-2. Fluorescence was measured by FACS analysis. + indicates the presence of the marker tested; – indicates no expression of the cell surface marker; +/– indicates an intermediate expression

mAb	specificity	T cell clones			T cell hybridomas			Control
		A2b	A2c	Z1a	A2bH	A2cH	Z1aH	C58
Ox 19	T cell	+	+	+	+	+	+	+/–
Ox 8	CD8	–	–	–	–	–	–	–
W3/25	CD4	+	+	+	+	+	+	–
R73	TCR	+	+	+	+	+	+	–
Class II								
Ox 6	RT1B	+	+	+	–	–	–	–
Ox 17	RT1D	+	+	+	–	–	–	–

RT1.B restricted recognition of antigen as the parent clones A2b, A2c and Z1a. The antigen specific IL-2 responses of A2bH, A2cH and Z1aH were inhibited in the presence of Ox6 dilutions which varied between 1/40 and 1/200 while the same dilutions of the Ox17 antibody supernatant did not have any effect on IL-2 production.

Selection and characterization of virus-specific mouse × rat hybridomas

Selection. After fusion of the IBV-specific T cell line (fusion 4, Table I), 384 wells were seeded and within 4 weeks 28 wells contained hybridomas. Two out of these 28 (MJB100 and MJB101) showed an IBV-specific IL-2 response in the CTLL assay (SI values of 2.5).

Following fusion of the FMDV-specific T cell line (fusion 5, Table I), 480 wells were seeded and within 4 weeks 6 wells contained hybridomas. Three out of six hybrid cell lines showed a FMDV-specific IL-2 response detected in the CTLL assay with SI values above 2. The best responding antigen-specific hybridomas (IBV: MJB100 and MJB101 and FMDV: FM1.2) were subcloned for stabilization and characterization.

Cell surface phenotype. FACS analysis showed that all hybridomas (MJB100, MJB101 and FM1.2) stained with the anti-L3T4 (T helper phenotype) monoclonal antibody and Thy1 monoclonal antibody, whereas the C58 and A2cH cell lines were negative for L3T4 (Table V). The hybridomas MJB100, MJB101 and FM1.2 could be activated to produce IL-2 by the anti-CD3 mAb (Table V).

MHC restriction. The IL-2 response of T cell hybridoma MJB100, MJB101 and FM1.2 was MHC class II restricted: allogeneic spleen cells used as APCs were not able to elicit a response.

TABLE IV

MHC RESTRICTION OF RAT CELL LINES BEFORE AND AFTER FUSION TO C58

The MHC restricted recognition of A2b, A2c and Z1a and T cell hybridomas A2bH, A2cH and Z1aH to the 65 kDa, 180–188 peptide or to MBP antigen was tested in the presence of class II specific antibodies Ox6 (anti-RT1B) and Ox17 (anti-RT1D). The proliferative responses of the T cell clones in the presence and absence of Ox6 and Ox17 antibody dilutions are presented in c.p.m. $\times 10^{-3}$. The values represent the mean of triplicate measurements. The ability of the T cell hybridomas to produce IL-2 as detected in the IL-2 assay tested in the presence and absence of Ox6 and Ox17 antibody dilutions are also presented in c.p.m. $\times 10^{-3}$ as incorporated by IL-2 dependent CTLL. BG = Background values. ND = Not done

Antigen + mAb	T cell clones				T cell hybridomas		
	A2b	A2c	Z1a		A2bH	A2cH	Z1aH
MBP (10 μg/ml)	ND	ND	72.01	MBP (5 μg/ml)	ND	ND	56.87
+ Ox6 (1:40)	ND	ND	0.24	+ Ox6 (1:40)	ND	ND	1.66
+ Ox6 (1:80)	ND	ND	0.18	+ Ox6 (1:200)	ND	ND	14.48
+ Ox17 (1:40)	ND	ND	74.57	+ Ox17 (1:40)	ND	ND	48.24
+ Ox17 (1:80)	ND	ND	79.69	+ Ox17 (1:200)	ND	ND	58.99
65 kDa (1 μg/ml) protein	35.82	ND	ND	65 kDa (5 μg/ml) protein	52.57	18.81	ND
+ Ox6 (1:40)	0.12	ND	ND	+ Ox6 (1:40)	6.04	2.80	ND
+ Ox6 (1:200)	2.37	ND	ND	+ Ox6 (1:200)	32.51	11.26	ND
+ Ox17 (1:40)	33.18	ND	ND	+ Ox17 (1:40)	61.44	20.60	ND
+ Ox17 (1:200)	35.98	ND	ND	+ Ox17 (1:200)	72.82	19.87	ND
Peptide 180–188 (10 μg/ml)	37.04	14.13	ND	Peptide 180–188 (10 μg/ml)	55.20	9.62	ND
+ Ox6 (1:40)	0.25	0.02	ND	+ Ox6 (1:40)	12.65	4.18	ND
+ Ox6 (1:100)	0.11	0.02	ND	+ Ox6 (1:200)	36.86	8.97	ND
+ Ox6 (1:1000)	17.63	ND	ND	+ Ox6 (1:1000)	56.80	10.61	ND
+ Ox17 (1:40)	25.68	17.51	ND	+ Ox17 (1:40)	57.45	9.34	ND
+ Ox17 (1:100)	28.43	14.49	ND	+ Ox17 (1:200)	47.32	9.19	ND
+ Ox17 (1:1000)	31.23	ND	ND	+ Ox17 (1:1000)	51.11	7.36	ND
BG	0.12	0.02	0.14		1.09	3.32	0.74

TABLE V

SURFACE MARKER EXPRESSION ON MOUSE × RAT T CELL HYBRIDOMAS

Expression of mouse surface markers on MJB100, MJB101, FM1.2, C58 and the rat × rat hybridoma A2cH. The presence of the CD3 molecule was shown by activation of an IL-2 response in MJB100, MJB101 and FM1.2 by the anti-CD3 antibody (2). Results are presented as SI values

Anti-mouse markers		T cell hybridomas			Controls	
mAb	specificity	MJB100	MJB101	FM1.2	A2cH	C58
Thy1	T cell	+	+	+	–	–
L3T4	CD4	+	+	+	–	–
Ly 2	CD8	–	–	–	–	–
Class II						
anti I-A^d	I-A^d	–	–	–	–	–
IL-2 production						
anti-CD3 (1/200)		425	614	12	ND	1

The restriction element was determined with the use of L cells transfected with various class II molecules. The IBV-specific MJB100 and MJB101 hybridomas showed an I-E^d restricted recognition of antigen. Only L cell transfectants expressing E^d molecules were able to induce antigen-specific stimulation of MJB100 and MJB101. In contrast, the FMDV-specific hybridoma FM1.2 could only be stimulated with antigen in the presence of L cells expressing I-A^d molecules, indicating an I-A^d restricted recognition of antigen (Table VI).

TABLE VI

MHC RESTRICTION OF MOUSE × RAT T CELL HYBRIDOMAS

The MHC class II restricted response of T cell hybridoma MJB100, MJB101 and FM1.2 was determined in the presence of 5 μg/ml IBV M41 or FMDV A10 Holland. Spleen cells of BALB/c and C57BL/6 or L cell transfectants were used as APC. Results are presented as SI values

APCs	IL-2 response (SI) of		
	MJB100	MJB101	FM1.2
Spleen cells			
BALB/c, H-2^d	140	125	65
C57BL/6, H-2^b	1	1	1
L cells			
RT 10.3 (I-E^d)	158	83	1
RT 2.33 (I-A^d)	1	1	41

Discussion

T cell hybridomas have proven to be efficient research tools in immunology (Möller, 1989). This paper evaluates the use of the W/Fu(C58NT)D fusion partner in the generation of T-T hybrids from established, well characterized, rat T cell clones and from mouse T cell lines. C58 had been used previously for the generation of mouse × rat cytotoxic T cell hybridomas (Silva et al., 1983) and rat × rat suppressor T cell hybridomas (Curling et al., 1988).

T cell clones are generally believed to be more difficult to fuse than fresh lymph node T cells (Ozaki et al., 1988). In fact only a few cases of cloned T cells and derived hybridomas with identical T cell receptors have been reported in the mouse system (Schreier et al., 1982; Krammer et al., 1983). It is possible that in some cases lack of success was due to mycoplasma infection during long term maintenance in vitro as suggested by Ozaki (1988).

Here we show three examples of immortalization of established, well defined, long term cultured rat T cell clones A2b, A2c and Z1a by fusion to C58. The frequencies of specific hybridomas obtained after fusion 1, 2 and 3 were good, ranging from 62% to 86%. This was to be expected considering the monoclonal status of

the parental T cell clones. The hybridomas A2bH and A2cH showed no differences in antigen-specific reactivity when compared to the parental clones: they recognized the 180–188 epitope of the 65 kDa heatshock protein of *Mycobacterium tuberculosis* within the context of MHC class II RT1.B. This indicated the presence of an identical T cell receptor on the surface of the immortalized T cell hybridoma. This finding was further substantiated by analysis of the reactivity of the hybridoma cell lines A2bH and A2cH to a set of PEPSCAN peptides covering the 180–188 sequence. The reactivity of both hybridomas was indistinguishable from the reactivity of the parent clones (Van Eden et al., 1989). Although we were not able to analyse the reactivity of the Z1a and Z1aH cell lines at a peptide level, both cell lines showed the same antigen-specificity within the context of MHC class II RT1.B.

The Ox19, W3/25 and R73 markers expressed by the parental clones A2b, A2c and Z1a were also expressed at the cell surface of clones A2bH, A2cH and Z1aH. Expression of MHC class II determinants on long term propagated rat T cells has been reported (Reske et al., 1987). Indeed the cell surface display of class II RT1.B and RT1.D determinants was seen on all parental rat T cell clones. Notably, none of the hybrid cell lines showed expression of class II determinants. As yet the function of class II expression on T cells is unclear and future investigations will be required to determine whether expression of MHC class II on T cells plays a role in the amplification or down-regulation of immune responses.

In addition to the intraspecies hybridization, our results demonstrate the possibility of interspecies hybridisation of murine, virus-specific, T cell lines to C58. Interspecies cell hybrids have proven to be useful in several research fields. More precisely, interspecies hybrids may permit the determination of the origin of chromosomes or the origin of specific markers in the hybridoma (Simon et al., 1988). In some instances, e.g., generation of novel monoclonal antibodies, it may be advantageous to express antigens in the context of a different immunogenic background.

The mouse T cell lines were obtained using a protocol which supported generation of CD4 positive helper T cells (Klaus, 1987). Indeed, the T cell lines expressed the CD4 phenotype (not shown). The hybridomas subsequently generated were shown to express mouse surface markers CD3 (shown by activation of IL-2 production) and L3T4 (CD4). Furthermore, antigen-specific responses were MHC class II restricted. The preservation of the CD4 molecule at the surface of these hybridoma cell lines supports the argument that CD4 is a physical component of the T cell receptor as proposed by Janeway (1989).

The IBV- and FMDV-specific T cell hybrids were generated to provide monoclonal reagents for the delineation of T cell epitopes on proteins of IBV and FMDV. For the purposes of T cell epitope mapping, T cell hybridomas, due to their constant availability, can be a more convenient tool than T cell clones or T cell lines. The IBV-specific hybridomas were successfully used to precisely localize a T cell epitope within the nucleocapsid protein of IBV using expression products (Stanley and Luzio, 1984) and synthetic peptides (Boots et al., 1991). The fine specificity of the FM1.2 hybrid is currently being investigated. The fusion of early T cell lines could have another advantage. The number of clonalities represented within an early line are certain to decrease by further restimulation. By fusion of early lines, different clonalities could be sustained which would allow further analysis of determinants recognized by T cells.

The MJB100, MJB101 and FM1.2 hybridomas were cultured for prolonged periods of time (over 4 months) without loss of antigen-specific IL-2 production. Inter-species hybrids between C58 and cytotoxic T cells were previously found to be unstable (Conzelmann et al., 1982; Erard et al., 1984; Kanagawa and Chiller, 1985; Lefrancois and Kanagawa, 1986). As we now have shown for our CD4 positive T cell lines and clones it appears that adaptation to cell culture conditions before fusion facilitates the generation of T cell hybridomas.

In conclusion, we have preserved, propagated and characterized rat and mouse T cells by fusion to C58 and conclude that this cell line can be used to create monoclonal tools with sufficient stability in both intra- and inter-species hybridisation.

Acknowledgements

We gratefully acknowledge the contribution of K. Delsman, E. Hogervorst and M. Holewijn (Department of Immunology, Institute of Infectious Diseases and Immunology, Utrecht, The Netherlands) to the work described above.

We would like to thank Dr. E. Curling (Oxford, U.K.) for donation of the C58 cell line and Dr. A.F. Williams of the MRC Cellular Immunology Unit, University of Oxford, U.K. for the Oxford hybridoma cell lines. The R73 cell line was kindly donated by Dr. T. Hunig (Munich, F.R.G.). In addition, we are grateful to Dr. R. Germain (Bethesda, U.S.A.) for the donation of mouse class II transfectants.

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