

Drosophila genetics should lead to the isolation of mutants that will allow us to establish the function of the antigens.

We thank Richard Smith for technical help, Danny L. Brower for the PS2 antibody (CF2C7) and Nick Brown and many of our colleagues for criticism of the manuscript. M.L. is the recipient of an EMBO long-term fellowship.

Received 27 February; accepted 9 May 1985.

1. Kemler, R., Babinet, C., Eisen, H. & Jacob, F. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4449-4452 (1977).
2. Gerisch, G. in *Current Topics in Developmental Biology* Vol. 14, Pt 2 (eds Moscona, A. A. & Monroy, A.) 243-269 (Academic, New York, 1980).
3. Edelman, G. M. *Science* **219**, 450-457 (1983).
4. Damsky, C. H., Richa, J., Solter, D., Knudsen, K. & Buck, C. A. *Cell* **34**, 455-466 (1983).
5. Kruse, J. et al. *Nature* **311**, 153-155 (1984).
6. Rutishauser, U. *Nature* **310**, 549-553 (1984).
7. Wilcox, M., Brower, D. L. & Smith, R. J. *Cell* **25**, 159-164 (1981).
8. Brower, D. L., Wilcox, M., Piovant, M., Smith, R. J. & Reger, L. A. *Proc. natn. Acad. Sci. U.S.A.* **81**, 7485-7489 (1984).
9. Wilcox, M., Brown, N., Piovant, M., Smith, R. J. & White, R. A. H. *EMBO J.* **3**, 2307-2313 (1984).
10. Brower, D. L. *Nature* **310**, 496-498 (1984).
11. Brower, D. L., Piovant, M. & Reger, L. A. *Dev. Biol.* **108**, 120-130 (1985).
12. Fullilove, S. L. & Jacobson, A. G. in *The Genetics and Biology of Drosophila* Vol. 2c (eds Ashburner, M. & Wright, T. R. F.) 105-227 (Academic, New York, 1978).
13. Hawkes, R. *Analyt. Biochem.* **123**, 143-146 (1982).
14. Towbin, H., Staehelin, T. & Gordon, J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4350-4354 (1979).
15. Cheney, C. M. & Shearn, A. *Dev. Biol.* **95**, 325-330 (1983).
16. Bonner, W. M. & Laskey, R. A. *Eur. J. Biochem.* **46**, 83-88 (1974).

Specific targeting of cytotoxic T cells by anti-T3 linked to anti-target cell antibody

Pilar Perez, Robert W. Hoffman, Stephen Shaw, Jeffrey A. Bluestone & David M. Segal*

Immunology Branch, National Cancer Institute, Bethesda, Maryland 20205, USA

The specificity of cytotoxic T lymphocytes (T_c) cells is conferred by an antigen-specific receptor, T_i , which in humans is physically associated with an invariant cell-surface glycoprotein, T3 (refs. 1-3). Monoclonal antibodies specific for either T3 and T_i are able to elicit a variety of T-cell responses such as lymphokine production, mitogenesis and cytotoxicity¹⁻¹¹. For example, human T_c cells lyse anti-T3-expressing hybridoma cells, but not cells of other specificity, presumably because anti-T3 on the hybridoma cells binds to T3 on the T_c cells and triggers lysis¹². Here, we have adapted approaches used in a different cytotoxic effector system, antibody-dependent cellular cytotoxicity (ADCC), to alter the specificity of T_c cell. Studies of ADCC¹³ showed that heteroaggregates containing anti-Fc receptor ($Fc\gamma R$) antibody cross-linked to a second antibody bind to $Fc\gamma R$ on ADCC effectors and cause them to kill target cells bearing antigen recognized by the second antibody. The present studies use anti-T3-containing heteroaggregates to re-target human T_c cells to cells for which we have appropriate antibodies, including xenogeneic tumour cells and chicken erythrocytes. These results extend previous observations on the role of T3 in triggering cytotoxicity and suggest that effector cell re-targeting could be used for *in vivo* treatment of neoplasms and other pathogens that express distinctive surface antigens.

Four different human anti-HLA-DPw2 (SB2) cytotoxic T-cell clones, 8.2, 8.4, 8.5 and 8.9 (ref. 14), were used in this study. All four express the T3 and T4 antigens and target-cell lysis mediated by three of the clones (8.4 being the exception) is inhibited by anti-T3 antibody¹⁴. Clones 8.9 and 8.5 were negative for $Fc\gamma R$ (as determined by flow cytometry¹⁵) and therefore cannot mediate ADCC (clones 8.2 and 8.4 were not tested).

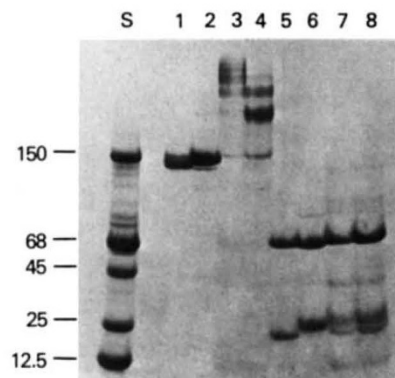


Fig. 1 SDS-polyacrylamide gel electrophoresis of cross-linked antibodies. Lanes 1-4, non-reduced; lanes 5-8, reduced. Lanes 1, 5, OKT3 (anti-T3); lanes 2, 6, 36-7-5 (anti- K^k); lanes 3, 7, OKT3 cross-linked to 36-7-5 (anti-T3 $\times$ anti- K^k), heavier fraction containing mainly trimers and larger oligomers; lanes 4, 8, lighter fraction of cross-linked material, containing mainly dimers and trimers. The cross-linked material reduces to light and heavy chains because a reducible cross-linking reagent was used in their preparation. Note that the cross-linked preparations contain light chains from both antibodies, as expected. Lane S, standards: IgG (relative molecular mass (M_r) 150,000), bovine serum albumin (68,000), ovalbumin (45,000), chymotrypsinogen (25,000) and cytochrome c (12,500).

Methods. OKT3 (Ortho) and 36-7-5²⁰ antibodies were purified from ascites by ion-exchange chromatography and gel filtration²¹. Each protein (5 mg) was incubated with a threefold molar excess of N-succinimidyl-3-(2-pyridyldithiol) propionate (Pharmacia). OKT3 was then reduced and coupled to 36-7-5 as described previously¹³. The hetero-cross-linked antibodies were eluted on a 1.6 $\times$ 90-cm Ultrogel AcA 22 (LKB) column, fractions taken at relative elution volume K_d = 0.08-0.33 (heavy fraction) and 0.38-0.54 (lighter fraction). Monomeric IgG elutes at (K_d) 0.63-0.71. Samples were concentrated with a CX10 immersible membrane (Millipore), and aliquots were denatured and run on a 2-16% gradient slab gel (Pharmacia). Other antibodies used were affinity-purified rabbit anti-DNP antibodies²² and anti-T4 monoclonal antibody^{1,3} (Ortho) purified as described above. In cross-linking anti-T3 to anti-DNP, an eightfold molar excess of SPDP to each antibody was used. This gave larger heteroaggregates than the threefold excess used in the other combinations, but also partially inactivated the antibodies, as shown by the inability of anti-T3 $\times$ anti-DNP to inhibit lysis of M16 cells by clone 8.9 (Table 2).

Cross-linked antibodies were prepared as described in Fig. 1 legend. Two cross-linked fractions were separated from monomeric IgG by gel filtration (Fig. 1). Preliminary experiments showed that both were equally active and they were therefore used interchangeably. In the present study, hetero-cross-linked antibodies are designated 'antibody 1 $\times$ antibody 2', for example, anti-T3 $\times$ anti- K^k .

The four clones were tested for their ability to lyse M16, a human lymphoblastoid cell line bearing the HLA-DPw2 antigen, RDM4, an H-2^k mouse tumour line, and EL-4, an H-2^b mouse tumour line (Table 1). As expected, these clones lyse the M16 target but not the murine tumour cells. Moreover, lysis of M16 by clones 8.9, 8.2 and 8.5 was blocked by anti-T3 $\times$ anti- K^k , showing that anti-T3 remained active in the heteroaggregate. The main finding of Table 1 is that anti-T3 $\times$ anti- K^k causes each of the T_c -cell clones to lyse the K^k -positive RDM4 targets, but not the K^b -expressing EL-4 cells. RDM4 was also lysed if either effector or target cells were preincubated with the heteroaggregates and washed before the experiment (data not shown).

These results suggest that anti-T3 $\times$ anti- K^k cross-links K^k on RDM4 to the T3 portion of the T-cell receptor complex on the T_c -cell clones and triggers the T_c cells to lyse the RDM4 targets. Figure 2 confirms specific intercellular cross-linking, as reflected in the formation of multicellular conjugates between effectors and targets. Figure 2a, b shows that in the absence of the

* To whom correspondence should be addressed at: Bldg 10, Rm 4B17, NIH, 9000 Rockville Pike, Bethesda, Maryland 20205, USA.

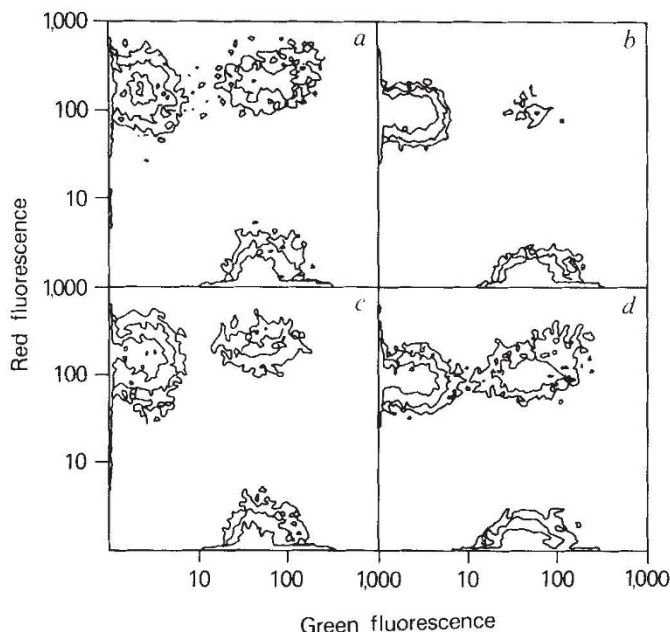


Fig. 2 Conjugate formation between T_c -cell clone 8.9 and tumour lines M16 (a, c) and RDM4 (b, d) in the absence (a, b) or presence (c, d) of anti-T3 $\times$ anti- K^k . Clone 8.9 cells were labelled with fluorescein isothiocyanate as described elsewhere²³. M16 and RDM4 cells (10^7 per ml) were incubated for 10 min at 37 °C with $10 \mu\text{g ml}^{-1}$ *N*-hydroxysuccinimidobiotin (Sigma) in PBS, pH 8.0, washed and incubated with phycoerythrin-avidin (20 μl of an appropriate dilution of *R*-phycoerythrin-avidin was added to 10^7 cells in 100 μl of PBS, pH 7.2, and incubated for 30 min at 24 °C). Conjugates were formed by mixing 8.9 cells with M16 or RDM4 cells at a 1:1 ratio, centrifuging and incubating for 20 min at 24 °C. In c and d, effectors were preincubated for 30 min at 0 °C with $10 \mu\text{g ml}^{-1}$ anti-T3 $\times$ anti- K^k before adding the target cells. After the 20-min incubation, cells were gently resuspended with a Pasteur pipette in 1 ml PBS at 0 °C and analysed with a Becton-Dickinson FACS analyser. Free 8.9 cells appear green only, free target cells appear red only and conjugates appear both red and green. 2×10^4 particles were analysed in each sample and contours were drawn through points containing 10, 20 and 40 particles. The percentages of effectors in conjugates were: a, 28%; b, 8%; c, 25%; d, 31%.

heteroaggregate, clone 8.9 forms the expected large number of conjugates with M16, but few conjugates with RDM4. By contrast, in the presence of heteroaggregates, the number of RDM4-8.9 conjugates increases dramatically while the number of M16-8.9 conjugates remains relatively unaffected. The latter observation is consistent with previous studies^{16,17} showing that anti-T3 blocks T_c -cell mediated lysis at a step following conjugate formation.

To test the specificities required for cross-linked antibodies to mediate lysis, we coupled anti-T3 to anti-dinitrophenyl (DNP), anti-T4 to anti- K^k and anti-DNP to anti- K^k , using procedures similar to those described for Fig. 1. Table 2 shows that the anti-T3 $\times$ anti-DNP confers anti-DNP specificity to T_c cell clones; in the presence of this heteroaggregate, T_c cells lyse TNP-RDM4 and TNP-EL4, but not unmodified EL4 or RDM4. Moreover, DNP hapten in solution strongly inhibits lysis of the haptenated targets. Interestingly, T_c cells will even lyse hapten-modified chicken red blood cells (CRBC) in the presence of anti-T3 $\times$ anti-DNP. Thus, the antibody heteroaggregates seem to override the normal requirement for lysis that T_c cell receptors bind to molecules of the major histocompatibility complex (MHC) on target cells, as it is unlikely that either CRBC or the murine T_c -cell tumour lines EL4 and RDM4 express MHC molecules, which could be recognized by the class II-specific T_c -cell clones used here.

We next investigated whether anti-T3 must be a component of the antibody heteroaggregate, or whether monoclonal antibodies against any effector cell-surface molecule would trigger lysis. For this, we tested the effect of anti-T4 $\times$ anti- K^k on lysis

Table 1 Anti-T3 $\times$ anti- K^k heteroaggregates re-target T_c cells to K^k -expressing cells

Clone	Anti-T3 $\times$ anti- K^k	% Specific lysis of target cell		
		M16	RDM4	EL-4
8.9	—	47.0	6.6	0.8
8.9	+	11.5	53.3	0.2
8.2	—	44.9	9.1	3.3
8.2	+	20.4	43.9	—0.7
8.4	—	49.8	7.2	4.3
8.4	+	54.1	32.9	0.8
8.5	—	35.7	1.5	0.6
8.5	+	8.0	30.0	0.4

Lysis was measured in the presence or absence of $5 \mu\text{g ml}^{-1}$ anti-T3 $\times$ anti- K^k at a 10:1 effector: target ratio in a 3-h ^{51}Cr -release assay. Antibody was preincubated with effectors and targets for 30 min at 0 °C, before warming to 37 °C. Human T_c -cell clones 8.2, 8.4, 8.5 and 8.9 are specific for the HLA-DPw2 antigen^{14,18}. M16 (ref. 19) is a human Epstein-Barr virus-transformed lymphoblastoid cell line that expresses HLA-DPw1 and -DPw2 antigens. RDM4 and EL4 are H-2^k and H-2^b murine T_c -cell tumour lines. Per cent lysis was calculated as described elsewhere¹³, and spontaneous release was 8.9, 5.2 and 6.8% for M16, RDM4 and EL-4, respectively.

of M16 and RDM4. Results from experiments similar to those shown in Fig. 2 confirmed that anti-T4 $\times$ anti- K^k caused conjugates to form between clone 8.9 and RDM4 (21% of effectors were conjugated in the presence of heteroaggregate, 9% in its absence). However, this cross-linked preparation neither promoted lysis of RDM4 nor significantly blocked lysis of M16 by clone 8.9 (it has previously been demonstrated¹⁸ that anti-T4 does not inhibit lysis mediated by clone 8.9) (Table 2). In a second experiment (not shown in Table 2), T_c -cell clone 8.9 was treated with 1.5 mM trinitrobenzene sulphonate and tested for lysis against RDM4 in the presence of anti-DNP $\times$ anti- K^k . Control experiments showed that anti-DNP $\times$ anti- K^k promoted specific conjugate formation between TNP-8.9 and RDM4 and that the TNP-8.9 cells were able to lyse M16. However, TNP-8.9 cells did not lyse RDM4 cells in the presence of anti-DNP $\times$ anti- K^k (<2% lysis over a concentration range of 5–40 $\mu\text{g ml}^{-1}$ heteroaggregate). Therefore, cross-linking of target cells specifically to the T4 molecule and to a presumably large number of TNP-modified components on the effector cells did not lead to lysis. These data suggest that the cross-linking of target cells directly to the T_c -cell receptor complex is a requirement for lysis. In support of this view, earlier experiments¹² showed that several hybridoma cells expressing antibodies against effector cell-surface components other than T3 are not lysed by T_c -cell clones. In addition, Kranz *et al.*¹¹ have recently shown that a mouse T_c -cell clone will lyse targets to which anti-Ti antibodies were attached, but not targets bearing antibodies that bind to other effector cell-surface components.

From current results, there seems to be a striking parallel in the way in which ADCC effector cells and T_c cells lyse target cells; in both cases, direct attachment of the target cells to the appropriate receptor (Fc γ R on ADCC¹³ effectors or T_c -cell receptor complex on T_c cells) leads to target cell lysis, and attachment to other cell-surface components on the effector cell does not. These data strongly suggest that the target-specific receptors in both systems not only mediate conjugate formation, but also have an essential role in the post-conjugate phase of the lytic process. T3 is one cell-surface component that is clearly involved in the post-conjugate phase of T_c -cell mediated lysis, because anti-T3 blocks lysis but does not inhibit conjugate formation. Nevertheless, binding of the target cell directly to T3 results in target cell death. This suggests that the local aggregation of T3 at the cell/cell interface may be instrumental in triggering lysis, and that soluble anti-T3 blocks normal T_c -cell lysis by preventing local T3 aggregation.

We have demonstrated here that the specificities of T_c cells can be altered *in vitro* simply by treating them with antibody heteroaggregates. In fact, we have now been able to direct T_c

Table 2 Specificity of T_c-cell lysis in the presence of hetero-cross-linked antibodies

Target	% Specific lysis of target cells by clone 8.9					
	Antibody added					
	None	Anti-T3 × anti-K ^k	Anti-T3 × anti-K ^k + anti-T3	Anti-T3 × anti-DNP	Anti-T3 × anti-DNP + DNP	Anti-T4 × anti-K ^k
M16	37.8	-7.0	-29.7	22.3	—	29.9
RDM4	-8.7	38.2	2.0	-8.6	—	2.1
EL-4	-4.9	-4.2	—	-4.3	—	—
CRBC	1.1	—	—	1.3	0	—
M16-TNP	55.1	—	—	32.3	43.8	—
RDM4-TNP	-8.1	—	—	40.5	7.4	—
EL-4-TNP	0.4	—	—	49.1	0.3	—
CRBC-TNP	0.8	—	—	63.9	0	—

Experiments were performed as described in Table 1 legend using clone 8.9 cells as effectors. Clone 8.5 also lysed TNP-CRBC in the presence of anti-T3 × anti-DNP in a hapten-specific manner (data not shown). In all experiments shown, 10 µg ml⁻¹ cross-linked antibody was used, but 20 and 40 µg ml⁻¹ of anti-T4 × anti-K^k were also tested, with results similar to those shown here. Anti-T3 (50 µg ml⁻¹, column 4) and DNP-ε-aminocaproate (0.1 mM, column 6) were used as inhibitors. DNP-ε-aminocaproate did not inhibit lysis of M16 by clone 8.9 (data not shown). Target cells were modified with TNP by incubating them for 10 min at 37 °C in phosphate-buffered saline (PBS), pH 7.4, containing 3 mM trinitrobenzene sulphate. (—) Indicates experiment not done. Negative values for per cent lysis indicate that chromium release was less than spontaneous release. In the test sample, spontaneous release values for the various targets were: M16, 28.6%; RDM4, 11.4%; EL-4, 12.4%; CRBC, 2.9%; M16-TNP, 7.4%; RDM4-TNP, 15.4%; EL-4-TNP, 4.5%; and CRBC-TNP, 3.0%.

cells to lyse every target cell for which we have had an appropriate anti-target cell antibody. Based on these observations, it is reasonable to propose that T_c cells could be directed against various pathogenic cells *in vivo*, for example against tumour cells. By using anti-T3-containing heteroaggregates, presumably all T_c cells, and not a limited number of clonotypes, would be targeted to the tumour. In addition, such heteroaggregates could also target T-helper cells to a tumour (or other lesion) where they could serve as a source of lymphokines. Finally, appropriate heteroaggregates could be used to target both T cells (using anti-T3-containing heteroaggregates) and macrophages, granulocytes and natural killer cells (using anti-FcγR heteroaggregates) to the same lesion. Such strategies might greatly augment the normal immune response against tumours and other pathogenic cells, and could also provide insight into the normal role of various effector cells in combating infections and neoplasms.

We thank Drs Alfred Singer, Pierre Henkart and Ralph Quinones for helpful suggestions during the preparation of the manuscript, Ms Julie Titus for help in isolating antibodies and Ms Judy Kress for typing the manuscript. P. P. is the recipient of a Fulbright fellowship awarded by the Spanish Department of Education and Culture.

Note added in proof: Staerz *et al.*²⁴ have recently shown that anti-Ti-containing heteroaggregates will re-target murine T_c-cells.

Received 17 April; accepted 21 May 1985.

- Meuer, S. C., Acuto, O., Hercend, T., Schlossman, S. F. & Reinherz, E. L. *A. Rev. Immun.* **2**, 23-50 (1984).
- Haskins, K., Kappler, J. & Marrack, P. *A. Rev. Immun.* **2**, 51-66 (1984).
- Reinherz, E. L., Meuer, S. C. & Schlossman, S. F. *Immun. Rev.* **74**, 83-112 (1983).
- Lancki, D. W., Ma, D. I., Havran, W. L. & Fitch, F. W. *Immun. Rev.* **81**, 65-94 (1984).
- von Wussow, P., Platsoucas, C. D., Wiranowska-Stewart, M. & Stewart, W. E. *J. Immun.* **127**, 1197-1200 (1981).
- Meuer, S. C. *et al. J. exp. Med.* **158**, 988-993 (1983).
- van Wauwe, J. P., De May, J. R. & Goossens, J. G. *J. Immun.* **124**, 2708-2713 (1980).
- Tax, W. J. M., Hermes, F. F. M., Willems, H. W., Capel, P. J. A. & Koene, R. A. P. *J. Immun.* **133**, 1185-1189 (1984).
- Kaneko, H., Perez-Rojas, G., Sasasuki, T., Benike, C. J. & Engelman, E. G. *J. Immun.* **131**, 158-164 (1983).
- Kan, E. A. R., Platzer, E., Welte, K. & Wang, C. Y. *J. Immun.* **133**, 2979-2985 (1984).
- Kranz, D. M., Tonegawa, S. & Elsen, H. N. *Proc. natn. Acad. Sci. U.S.A.* **81**, 7922-7926 (1984).
- Hoffman, R. W., Bluestone, J. A., Leo, O. & Shaw, S. *J. Immun.* (in the press).
- Karpovsky, B., Titus, J. A., Stephany, D. A. & Segal, D. M. *J. exp. Med.* **160**, 1686-1701 (1984).
- Biddison, W. E., Rao, P. E., Talle, M. A., Goldstein, G. & Shaw, S. *J. exp. Med.* **159**, 783-797 (1984).
- Titus, J. A., Sharrow, S. O., Connolly, J. M. & Segal, D. M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 519-523 (1981).
- Tsoukas, C. P., Carson, D. A., Fong, S. & Vaughan, J. H. *J. Immun.* **129**, 1421-1425 (1982).
- Landegren, U. *et al. J. exp. Med.* **155**, 1579-1584 (1982).
- Shaw, S., Goldstein, G., Springer, T. A. & Biddison, W. E. *J. Immun.* **134**, 3019-3026 (1985).
- Shaw, S., Johnson, A. H. & Shearer, G. M. *J. exp. Med.* **152**, 565-580 (1980).
- Sachs, D. H., Mayer, N. & Ozato, K. in *Monoclonal Antibodies and T-Cell Hybridomas* (eds Hammerling, G., Hammerling, U. & Kearny, J. F.) 95-101 (Elsevier, New York, 1981).
- Unkeless, J. C. *J. exp. Med.* **150**, 580-596 (1979).
- Segal, D. M. & Hurwitz, E. *Biochemistry* **15**, 5253-5258 (1976).
- Perez, P., Bluestone, J. A., Stephany, D. A. & Segal, D. M. *J. Immun.* **134**, 478-485 (1985).
- Staerz, U. D., Kanagawa, O. & Bevan, M. J. *Nature* **314**, 628-631 (1985).

Pre-B cells in κ-transgenic mice

Ursula Storb, Kathleen A. Denis*, Ralph L. Brinster† & Owen N. Witte*

Department of Microbiology and Immunology, University of Washington, Seattle, Washington 98195, USA

* Department of Microbiology and Molecular Biology Institute, University of California, Los Angeles, California 90024, USA

† Laboratory of Reproductive Physiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

Recent experiments have shown that the microinjected κ-chain gene of transgenic mice is expressed in a tissue-specific fashion only in B lymphocytes^{1,2}. The next step was to determine whether, within the B-lymphocyte lineage, the κ-chain gene was expressed in a normal developmental fashion. Normally, only μ heavy(H)-chain genes, and not κ-chain genes, are expressed in pre-B cells³. To obtain cloned cell lines derived from early cells of the B-cell lineage, we transformed bone marrow cells from κ-transgenic mice with Abelson murine leukaemia virus (A-MuLV) and tested the resultant cell lines for the retention of the κ transgene and its expression in RNA and protein. We found that cells with the pre-B phenotype exist in κ-transgenic mice. We further observed that in A-MuLV-transformed cell lines from a κ-transgenic mouse with a high copy number of the transgene, the proportion of cell lines expressing κ (transgenic κ) was higher than in cell lines from normal or low copy number transgenic mice.

Bone marrow was obtained from two 4-week-old transgenic mice and one normal sibling from the same litter. The parents were a male transgenic mouse (no. 48, ref. 2) and a normal female C57BL mouse. A portion of the bone marrow cells was directly transformed by A-MuLV⁴; the rest was first cultured for 3-4 months *in vitro*⁵ and then transformed with A-MuLV.

Kidney DNA from the three donor mice was tested for the presence of the transgene by dot hybridization with a constant region (C_κ) probe and pBR322 as described previously¹. One donor had not inherited the transgene (normal littermate), whereas the two transgenic donors had different copy numbers of the transgene (see below). Apparently the father, no. 48 (ref. 2), contained mosaic germ cells, whereas only the high copy transgenes were observed in the tail DNA (R. L. O'Brien and U.S., unpublished).

All the A-MuLV-transformed cell lines from normal and transgenic mice produce μ-chain RNA (Fig. 1, Table 1). The levels of μ RNA are variable: some cells produce very low

Dana-Farber EX1061

©1985 Nature Publishing Group Dana-Farber v. BMS, IPR2023-00501

Page 3 of 3