

Table 1 Specific binding of murine ^{125} I-G-CSF to human normal and leukaemic cells

Type of patient (F.A.B. classification)	Cell source	No. of patients	Specific binding (% of WEHI-3B D ⁺)	
			Mean ($\pm$ s.d.)	Range
Normal	Bone marrow	5	34 $\pm$ 16	17–55
	Blood buffy coat	4	12 $\pm$ 11	1–27
	Purified blood neutrophils	5	86 $\pm$ 29	45–114
Preleukaemia*	Bone marrow	4	103 $\pm$ 55	62–184
Acute promyelocytic leukaemia (M3)†	Bone marrow	2		163, 82
	Blood	1		19
Chronic myeloid leukaemia‡	Bone marrow	5	66 $\pm$ 26	27–97
	Blood	3	56 $\pm$ 6	52–63
Acute myeloblastic leukaemia (M1 + M2)†	Bone marrow	9	56 $\pm$ 41	18–140
	Blood	9	30 $\pm$ 17	12–56
Acute myelomonocytic leukaemia (M4)†	Bone marrow	2		4, 7
	Blood	2		5, 13
Acute lymphoblastic leukaemia†	Bone marrow	3	4 $\pm$ 4	1–8
	Blood	1		<1
Chronic lymphocytic leukaemia§	Blood	3	4 $\pm$ 6	–1–10

Leukaemic cells of density $<1.077 \text{ g cm}^{-3}$ were prepared by centrifugation over Ficoll/Paque (Pharmacia) at 700g for 20 min. Normal blood cells comprised unfractionated buffy coat cells. Normal granulocytes were purified from blood by density centrifugation in hypertonic metrizamide gradients²⁰ and were 95–100% pure. Cells, usually 5×10^6 per incubation, were incubated in duplicate with 90,000–300,000 c.p.m. of ^{125} I-G-CSF with or without a 20-fold excess of unlabelled G-CSF in Dulbecco's modified Eagle's medium (DMEM)/ fetal calf serum (FCS) (70–130 μl total volume) at 37 °C for 1 h. Specific binding (total binding – binding in the presence of unlabelled G-CSF) is expressed as a percentage of the specific binding to an equivalent number of WEHI-3B D⁺ cells using the same preparation of ^{125} I-G-CSF on the same day.

* Including a patient with refractory anaemia with excess blast cells.

† Acute leukaemic patients were newly diagnosed or in relapse and their density-fractionated cells contained $>80\%$ leukaemic blasts.

‡ Cells taken at diagnosis before any treatment.

§ All showed $>90\%$ leukaemic lymphocytes.

line HL60 (ref. 18). The frequency of binding sites on leukaemic cells was similar to that on the equivalent normal cells and the apparent binding affinity for ^{125} I-G-CSF appeared to be approximately the same on all the cell types studied. Finally, other studies have shown that G-CSF is able to stimulate various functional activities of post-mitotic neutrophil granulocytes of mouse and man¹⁹.

The strong conservation of G-CSF/CSF- β and their receptors through evolution signals their importance for the maintenance of granulocytic cells in the animal. The conservation of receptors on human granulocytes and myeloid leukaemias indicates that G-CSF and its human analogue, CSF- β , merit further study as regards their possible therapeutic usefulness.

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Hybrid antibodies can target sites for attack by T cells

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It would be advantageous in the case of certain diseases to be able to focus a strong T-cell response at a chosen target, for example, in treating cancer or infections that have escaped the normal host response. At present, it seems inconceivable that we could use antigen-specific lines or clones of effector T cells for this purpose because of complications due to the major histocompatibility restriction of T-cell specificity and the problem of rejection of transplanted effector cells. Here we describe a novel technology which combines the power of T lymphocytes in eliminating unwanted cells and causing beneficial inflammatory reactions with the great advantages of monoclonal antibodies (their specificity and availability). We show that heteroconjugates of monoclonal antibodies (referred to hereafter as hybrid antibodies), in which one of the component binding sites is anti-T-cell receptor and the other component binding site is directed against any chosen target antigen, can focus T cells to act at the targeted site. Monoclonal antibodies directed against the T-cell receptor, such as the anti-allotype used here, are mitogenic for resting T cells and can be used to induce effector T cells carrying the T-cell receptor determinant which can then be directed against the target by a hybrid antibody.

Monoclonal antibodies directed against determinants of the T-cell receptor complex of cytotoxic T lymphocytes (CTL) or T-helper cells are able to mimic the effect of antigen in many ways. For example, anti-idiotypes in an appropriate form can

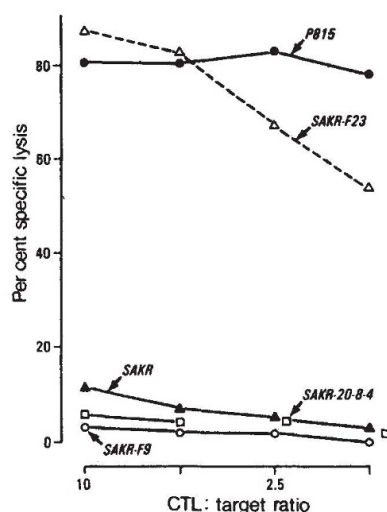


Fig. 1 Anti-receptor allotype monoclonal antibody F23.1 coupled to the target cell surface renders the cell susceptible to lysis by an allotype-positive CTL clone, OE4. F23.1 (IgG2a)⁸, F9 (IgG1) monoclonal antibody specific for an idiotype determinant on CTL clone G4 (ref. 3) and the anti-H-2^b monoclonal antibody 20-8-4 (IgG2a)¹⁵, produced in C3H mice, were purified from ascites fluids on protein A-Sepharose. Antibodies were reacted with the heterobifunctional crosslinker SPDP (Sigma)¹⁶; a threefold molar excess of SPDP was used to introduce two to three groups per 7S antibody. The modified antibody was then reacted with S.AKR lymphoma cells (H-2^k, Thy-1.1) which had been pretreated with 0.5 mM dithiothreitol to introduce free sulphhydryl groups at the cell surface^{17,18}. After washing, 1×10^6 pre-labelled (⁵¹Cr-labelled sodium chromate) target cells were incubated at 37 °C with twofold serial dilutions of the CTL clone OE4 at 37 °C for 4 h (ref. 3). Per cent specific lysis of these target cells and of P815 (DBA/2, H-2^d) targets was calculated as described elsewhere³. The CTL clone OE4 was isolated by limiting dilution from a C57BL/6 (H-2^b) anti-DBA/2 (H-2^d) mixed lymphocyte culture and is maintained by weekly subculture with irradiated DBA/2 spleen cells plus IL-2. OE4 reacts with the anti-allotypic antibody F23.1 and with the anti-H-2^b antibody 20-8-4 but not with the anti-idiotype F9 antibody. Target cells were: P815 (●), S.AKR (▲), S.AKR coupled to F23.1 (△), S.AKR coupled to F9 (○) and S.AKR coupled to 20-8-4 (□).

stimulate idiotype-positive T-cell clones to release soluble factors such as interleukin-2 (IL-2)^{1,2} or γ -interferon³ (IFN- γ) and to proliferate^{1,2,4}. In addition, recent reports suggest that anti-idiotype on the surface of a cell renders that cell sensitive to recognition and lysis by an idiotype-positive CTL clone: B-cell hybridomas producing anti-idiotype serve as targets for idiotype-positive CTL^{5,6}, and anti-T3 antibodies (T3 is associated with the T-cell receptor) fixed covalently to cells convert them to sensitive CTL targets (H. Spitz, cited in ref. 7).

Recently, we have produced a murine monoclonal antibody specific for an allotypic determinant present on the T-cell receptor of ~25% of peripheral T lymphocytes⁸. The antibody was generated from a C57L/J mouse that had been hyperimmunized with BALB.B peripheral T cells. This monoclonal antibody, F23.1, seems to be very similar in specificity to a rat monoclonal antibody directed against a T-cell receptor isolated from a T hybridoma^{9,10}. To determine whether F23.1, the anti-allotypic monoclonal antibody, could focus CTL onto a target cell, we directly coupled the surface of various cell types to the isolated monoclonal antibody using a heterobifunctional crosslinker. OE4 is a CTL clone of C57BL/6 (H-2^b) origin with lytic specificity for H-2^d-bearing cells. The antigen receptor of clone OE4 bears the allotypic determinant recognized by F23.1. Figure 1 shows that OE4 does not kill cells from the lymphoma S.AKR (derived from AKR/J (H-2^k) mice) in a 4-h cytotoxicity assay, but does efficiently lyse P815 (H-2^d) targets. However, S.AKR

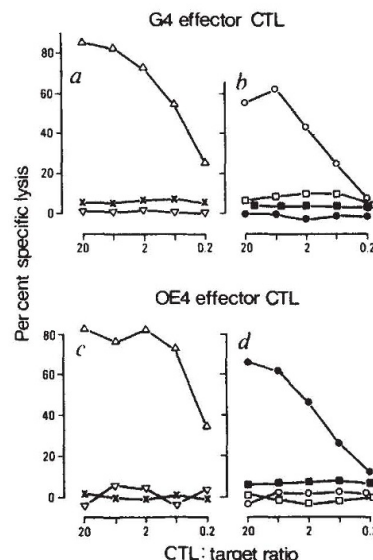


Fig. 2 Heteroconjugates of monoclonal antibodies (hybrid antibodies) can specifically target cells for CTL-mediated lysis. Effector CTL clones were: *a* and *b*, G4 [BALB.B (H-2^b) anti-BALB/c (H-2^d)], which is positive for the idiotype determinant recognized by F9 (ref. 3), and unreactive with F23; *c* and *d*, OE4 [C57BL/6 (H-2^b) anti-DBA/2 (H-2^d)] CTL clone which is F23⁺, F9⁻. Target cells were: P815 (H-2^d, △), S.AKR (H-2^k, ▽), EL4 (H-2^b, ×), S.AKR incubated with 19E12/F23 (●), S.AKR incubated with 19E12/F9 (○), EL4 incubated with 19E12/F23 (■), and EL4 incubated with 19E12/F9 (□).

Methods. The monoclonal alloantibody 19E12 (ref. 11) (IgG2a) specific for the Thy-1.1 alloantigen was purified from ascites fluid on protein A-Sepharose, modified with SPDP¹⁶ and coupled to either F23.1 or F9 monoclonal antibody according to the manufacturer's handbook (Pharmacia). The hybrid antibodies were used to coat ⁵¹Cr-labelled EL4 (H-2^b, Thy-1.2) and S.AKR (H-2^k, Thy-1.1) lymphoma cells by incubation at 37 °C for 20 min followed by washing. These target cells plus uncoated ⁵¹Cr-labelled S.AKR, EL4 and P815 (H-2^d) targets were assayed for lysis by serial threefold dilutions of CTL at 37 °C for 4 h.

cells that had been chemically coupled to F23.1 monoclonal antibody were efficiently lysed by OE4. Target cells coupled to the anti-idiotype monoclonal antibody F9 (which does not recognize OE4), were insensitive to OE4 effector cells, as were S.AKR cells coupled with an anti-H-2^b monoclonal antibody, 20-8-4, which does react with the H-2^b molecules expressed by OE4. Reciprocal results were obtained with the F9 idiotype-positive, allotype-negative CTL clone G4 (BALB.B (H-2^b) anti-H-2^d). In this case, F9-coupled cells were sensitive targets whereas F23.1- and 20-8-4-coupled cells were not killed (data not shown). Thus, we confirmed that monoclonal antibodies directed against determinants on the T-cell antigen receptor can target cells for attack by CTL when coupled to the surface of the cells. Furthermore, an anti-H-2 monoclonal antibody which reacts with the CTL did not trigger lysis.

We next asked whether heteroconjugates of monoclonal antibodies would be able to specifically target cells for destruction by CTL. In the hybrid antibodies that we constructed, one of the monoclonal antibodies in the hybrid is directed at the T-cell receptor (anti-idiotype or anti-allotype) while the other monoclonal antibody is directed at a surface antigen expressed by the targeted tumour cell but not by the CTL. We chose as the targeting monoclonal antibody 19E12, which is specific for the Thy-1.1 alloantigen¹¹. The CTL clones G4 and OE4 both express Thy-1.2 but not Thy-1.1 and do not react with this monoclonal antibody. The target cell was S.AKR (H-2^k, Thy-1.1). We

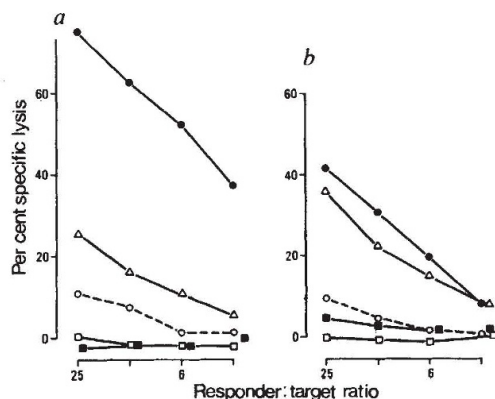


Fig. 3 Cytotoxic effects of Con A-induced (a) and F23.1-induced (b) spleen cells on various targets. Spleen cells from normal BALB.K (H-2^k) mice were cultured for 4 days at 1×10^6 cells ml⁻¹ in RPMI 1640 medium plus 10% fetal calf serum and 10% Con A-induced rat spleen cell supernatant, with either 2.5 µg ml⁻¹ Con A (a) or F23.1 monoclonal antibody coupled to Sepharose beads (10^3 beads per ml; b). F23.1 cultures also contained 50 mM α-methyl-D-mannoside to block the action of residual Con A present in the rat spleen supernatant. Cells were collected, washed and serial twofold dilutions assayed for lysis of the following ⁵¹Cr-labelled target cells: P815 (○), P815 in the presence of 10 µg ml⁻¹ PHA (●), AKR.A1 (□), AKR.A1 cells pre-incubated with 19E12 (anti-Thy-1.1)/F23.1 conjugate (△), and AKR.A1 cells pre-incubated with the 19E12/F9 conjugate (■). AKR.A1 is a T-cell lymphoma derived from an AKR/J mouse which expresses H-2^k and Thy-1.1.

constructed two hybrid monoclonal antibodies using the cross-linker *N*-succinimidyl-3-(1-pyridyldithio) propionate (SPDP). Both hybrid A (19E12/F9) and hybrid B (19E12/F23) worked efficiently and specifically (see Fig. 2). Hybrid A targeted S.AKR cells for lysis by G4 effector cells (Fig. 2b) while hybrid B targeted S.AKR cells for lysis by OE4 effector CTL (Fig. 2d). Neither hybrid antibody targeted EL4 (H-2^b, Thy-1.2) cells for lysis. Thus, targeting via an antibody combining site works extremely well. Mixtures of unconjugated monoclonal antibodies did not allow target lysis under any circumstances and fractionation of the heteroconjugated monoclonal antibodies on a Sephacryl S-400 column revealed a major peak of specific targeting activity eluting at a relative molecular mass (*M_r*) of ~300,000, a smaller peak at *M_r* ~500,000 and no targeting activity at lower or higher *M_r* ranges (data not shown). Other experiments have shown that EL4 cells, which served as the negative control in Fig. 2, can themselves be targeted for lysis by the syngeneic CTL clone OE4 by first infecting them with vesicular stomatitis virus and using an F23/anti-virus G protein monoclonal antibody heteroconjugate.

With regard to potential therapeutic applications of this hybrid antibody targeting technique, it would be beneficial to be able to generate effector T cells carrying a monoclonal antibody-defined receptor determinant from any individual. The anti-allotypic monoclonal F23.1 reacts with ~25% of peripheral T cells (both CTL and non-cytolytic T cells^{8,12}), is mitogenic for resting, peripheral T cells from mouse strains which are allotype-positive, and induces effector CTL which bear the allotypic determinant (U.D.S. and M.J.B., unpublished). Figure 3 shows the cytotoxic activity of BALB.K (H-2^k) spleen cells that were induced with either the pan-T-cell mitogen concanavalin A (Con A) or with F23.1 monoclonal antibody coupled to Sepharose beads for 4 days in the presence of exogenous IL-2. Con A induces CTL and T-helper cells regardless of their antigen specificity. The CTL activity can be assayed in an antigen-independent fashion by their ability to lyse any target cell coated with an agglutinant such as Con A or phytohaemagglutinin (PHA)¹³. Figure 3a shows that Con A-induced BALB.K cells do not specifically lyse P815 or AKR.A1 (H-2^k, Thy-1.1) targets but do lyse such targets in the presence

Table 1 Monoclonal antibody-induced cytotoxic cells express Thy-1

Target cells	Con A-induced effectors		F23.1-induced effectors	
	C'-treated	Anti-Thy-1 + C'	C'-treated	Anti-Thy-1 + C'
P815 + PHA	76.5	0.4	39.8	-0.6
AKR.A1 + 19E12/F23	24.2	-0.8	36.7	-0.9

Spleen cells from normal BALB.K mice were cultured for 4 days in the presence of 2.5 µg ml⁻¹ Con A, or F23.1 monoclonal antibody covalently coupled to Sepharose beads plus 10% supernatant of rat spleen cells which had been cultured with Con A for 24 h as a source of IL-2. Cultures not containing Con A also included 50 mM α-methyl-D-mannoside to block the effect of Con A. Cells were collected and treated with complement (C') alone or with monoclonal anti-Thy-1.2 (ref. 14) plus C', and surviving cells were assayed for 4 h on ⁵¹Cr-labelled target cells. Target cells were P815 in the presence of 10 µg ml⁻¹ PHA or AKR.A1 cells that had been incubated with a hybrid antibody (19E12/F23) for 20 min and washed. AKR.A1 is a T-cell lymphoma from AKR/J mice which expresses H-2^k and Thy-1.1. Spleen cells cultured without a stimulus had <1% of the cytotoxic activity shown here on either target. Assays were performed at a killer/target cell ratio of 25:1 for the C'-treated cells and for the equivalent number of surviving cells from the anti-Thy-1-treated groups. Values are per cent specific lysis of the target cells³.

of PHA. A fraction of the lytic activity revealed by PHA can also be measured on AKR.A1 cells targeted by the 19E12/F23 hybrid antibody. In the case of F23.1-induced spleen cells (Fig. 3b), PHA plus P815 and the hybrid antibody targeting AKR.A1 cells reveal approximately equal amounts of lytic activity. The levels of lytic activity correlate well with other data showing that the bulk of F23.1-induced T cells express the F23.1 determinant whereas only ~20% of Con A-induced T cells express the F23.1 determinant. As has been shown previously for lectin-induced, lectin-revealed cytotoxicity¹³, all of the monoclonal antibody-induced cytotoxicity is sensitive to treatment with anti-Thy-1 antibody plus complement, that is, is due to T cells (Table 1).

Although we do not know whether target cell destruction in this hybrid antibody-directed system proceeds via the same lytic mechanism as in antigen-specific, CTL-mediated killing, we suspect that the mechanism is very similar for the following reasons: first, the T-cell clones that we used are conventional CTL clones (Thy-1⁺, Lyt-2⁺ cells, whose growth is dependent on a supply of exogenous IL-2 plus periodic antigen stimulation). Second, in the case of Con A-induced effector cells, we have also shown that all of the antibody-directed cytotoxicity is mediated by Lyt-2⁺ T cells. Finally, target cell death in this system has the same kinetics and requirement for cell contact as in antigen-specific CTL-mediated lysis.

The results presented here suggest that it is possible to focus a strong T-cell response to a particular target antigen using hybrid antibodies; this could have clinical significance in the rejection of tumour cells or viral infections. One requirement is an antibody against the chosen target, for example, a tumour-associated or viral antigen; the other requirement is an antibody against the T-cell receptor complex. This technique circumvents the failure of the patients' T cells to respond in an effective way to the antigenic challenge (if any) with a specific, histocompatibility locus antigen (HLA)-restricted response. One can envisage inducing the patient's own T cells *in vitro* with an antibody against a receptor determinant, then transferring them back to the patient in the presence of an anti-target/anti-receptor hybrid antibody. We are presently assessing the *in vivo* applications in a murine lymphoma model system, by growing AKR/J (Thy-1.1) T lymphomas in AKR/Cum (Thy-1.2) hosts and using the hosts' own effector T cells and a 19E12/F23 hybrid antibody.

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Gene rearrangement in cells with natural killer activity and expression of the β -chain of the T-cell antigen receptor

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The mammalian host defence system can be divided broadly into adaptive and non-adaptive immunity. Adaptive immunity is acquired and is mediated by B and T lymphocytes. Non-adaptive immunity is mediated in part by a small subclass of heterogeneous peripheral blood mononuclear cells. This population, termed null cells, consists of haematopoietic precursors and cells mediating natural killer (NK) activity and antibody-dependent cellular cytotoxicity (ADCC). NK cells are a class of non-adherent, non-phagocytic, rapidly cytotoxic lymphocytes which can efficiently lyse a wide variety of tumour cells¹, virally infected cells² and immature cell types of normal origin³. Despite the broad range of targets, only a limited number of specificities are thought to be involved in target-cell recognition⁴⁻⁶. Morphologically, NK cells are large granular lymphocytes^{7,8}, but they have been shown to exhibit cell-surface markers characteristic of both T cells⁹⁻¹¹ and monocytes¹², raising doubt over their lineage. The recent cloning of the β -chain of the T-cell antigen receptor^{13,14} has now allowed us to investigate whether some NK cells are T-cell-related. We have examined rearrangement and expression of the β -chain of the T-cell receptor in cloned murine NK cell lines and fresh murine NK cell populations, and our results support the hypothesis that a subpopulation of NK cells is related to T cells and provide a basis for examining whether some NK activity is mediated by a small number of T-cell receptors.

DNA from five independent NK cell lines, HY-1 (ref. 15), HY-3 (ref. 15), NKB61A2 (ref. 16), NKB61B10 (ref. 16) and BALB/c NK (ref. 17), was examined for rearrangement of the genes coding for the β -chain of the T-cell receptor. DNA was digested with six restriction enzymes (*EcoRI*, *PstI*, *PvuII*, *HpaI*, *HindIII* or *XbaI*) and examined by Southern gel analysis using a radioactive probe derived from the joining ($J_{\beta}1$; *EcoRI*, *PvuII*

Fig. 1 *a*, Southern blot analysis of DNA from NK cell lines using restriction enzyme *XbaI*. Lane 1, C57BL/6 liver DNA; lane 2, HY-1 DNA; lane 3, HY-3 DNA; lane 4, NKB61A2 DNA; lane 5, NKB61B10 DNA; lane 6, BALB/c NK DNA. *b*, Southern blot analysis of DNA from NK cell lines using T-cell receptor β -chain constant- and joining-region probes. DNA (10 μ g) was digested overnight with a 10-fold excess of *HindIII* (top panel), *EcoRI* (second panel), *PvuII* (third panel) or *HpaI* (bottom panel) (all restriction enzymes from Boehringer Mannheim) and treated as described for *a*, with the exception of the probes. Lane 1, C57BL/6 liver DNA; lane 2, HY-1 DNA; lane 3, HY-3 DNA; lane 4, NKB61A2 DNA; lane 5, NKB61B10 DNA; lane 6, BALB/c NK DNA. Symbols: ●, germline band; ○, bands which have been deleted; ▲, a new, rearranged band.

Methods. *a*, DNA was extracted from the cells using the procedure of Blin and Stafford²⁴. DNA (10 μ g) was digested overnight with a 10-fold excess of *XbaI* (Boehringer Mannheim) and run on a 6-mm 1% agarose gel (Bio-Rad) at 1.0 V cm⁻¹ for 18 h. The DNA was then hybridized with 10⁶ c.p.m. ml⁻¹ (1 μ g of DNA per filter) of nick-translated²⁵ $C_{\beta}1A$ probe of the T-cell antigen receptor β -chain genes for 18 h. $C_{\beta}1A$ is the 3.5-kb *HindIII/SstI* fragment of $C1A$ ¹⁸. This probe contains most of the $C_{\beta}1$ coding domains and some 5'-noncoding sequences. Filters were washed and exposed to Kodak XAR-5 films. *b*, *HindIII*-digested DNA was hybridized to $C_{\beta}1A$ probe (described in *a*) and DNA digested with *EcoRI*, *PvuII* and *HpaI* was hybridized to $J_{\beta}1$, the insert from the pUCBJ1 subclone of β -chain genes described by Malissen *et al.*¹⁸.

and *HpaI*) or constant ($C_{\beta}1$; *HindIII*, *HpaI*, *PstI* and *XbaI*) regions of the mouse genomic T-cell receptor β -chain genes¹⁸.

Comparison of the banding pattern of C57BL/6 liver DNA as a control to the NK DNA showed that DNA from all five NK cell lines was rearranged. This result was most evident after digestion by *XbaI* (Fig. 1*a*). There were three germline bands of 5.0, 4.8 and 1.8 kilobases (kb), respectively (Fig. 1*a*, lane 1), and in all cases the 4.8-kb band was retained and the 5.0-kb band deleted. The cell lines HY-1 (Fig. 1*a*, lane 2) and HY-3 (lane 3) had also lost the 1.8-kb band, whereas cell lines

