Table 3 Expression of Lyt 1.1 and Lyt 2.1 antigens by FACS analysis

	% Lymph node cells expressing Lyt 1.1	Lyt 2.1
Control mice	83.6 ± 1.1	24.4 ± 1.4
VAA-fed mice	90.1 ± 0.6	24.2 ± 0.7
Student's *t*-test	$P < 0.001$	Non significant difference

Immunofluorescently stained CBA lymph node cells were analysed using FACS-II (Becton-Dickinson) as described previously[30,31], but with the argon ion laser set at 300 mW, 488 nm and the photomultiplier tube (EMI 9524A) at 750 V with a fluorescence gain of 8 and a light scatter gain of 4. Data for each individual mouse were derived from the analysis of 5×10^4 lymph node cells. The fluorescein isothiocyanate-coupled rabbit anti-mouse immunoglobulin (obtained from Nordic) was selected for its binding to viable cells expressing immunoglobulin at the cell surface while producing negligible staining of other cells[30]. The values in the table were calculated after subtracting the count for immunoglobulin-positive cells and represent the mean ±s.e. calculated from seven determinations. These results have been repeated in two subsequent experiments.

Our data are consistent with recent studies of Loveland and McKenzie[20,21] who have clearly shown that Lyt 1^+ T cells alone (depleted of Lyt 2^+ T cells) were sufficient to restore immune responses generated against H-2 and non-H-2 alloantigens in ATXBM (adult thymectomized, irradiated and bone-marrow reconstituted) mice. Our data also corroborate their view[21] that Lyt 1^+ cells have a central role in allograft responses. In either case, vitamin A acetate seems to be unique in that it enhances immune responses against an allograft when given by mouth and the mechanism of this action will require much further attention.

In summary, these findings support the hypothesis[17] that the anti-cancer action of vitamin A acetate [17,22] is mediated through an immunological process and we have shown that it acts by increasing the representation of the Lyt 1^+2^- phenotype in the T-cell population.

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Transfer of a cloned immunoglobulin light-chain gene to mutant hybridoma cells restores specific antibody production

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The expression of immunoglobulin (Ig) genes is regulated at several levels. For example, although κ-chain production requires a DNA rearrangement that juxtaposes variable and joining segments[1], this rearrangement is not sufficient for κ-chain gene expression; that is, some cell types do not permit immunoglobulin production[2–4]. The mechanisms responsible for the regulation of the expression of rearranged immunoglobulin genes are poorly understood. The technique of modifying cloned genes *in vitro* and transferring the modified genes to cells in culture provides a tool for identifying the structural features required for gene expression. To analyse immunoglobulin genes in this manner, however, it is first necessary to use, as recipients, cells that normally permit immunoglobulin production. We report here that a cloned κ-chain gene is expressed in immunoglobulin-producing hybridoma cells. Furthermore, the product of the transferred κ-chain gene is capable of restoring specific antibody production to the transformed cells.

The hybridoma Sp603 produces IgM(κ) specific for the hapten 2,4,6-trinitrophenyl (TNP)[5]. The rearranged gene encoding the TNP-specific κ chain (κ_{TNP}) has been cloned[6]. As recipient cells, we used the mutant cell line igk-14 which was derived from Sp603 and does not produce the κ_{TNP} chain. As shown in Fig. 3, the κ_{TNP} gene is apparently deleted from the igk-14 cell line. Because the igk-14 cells still produce the TNP-specific μ heavy chain, it would be expected that the expression of the κ_{TNP} gene in these cells would restore the production of TNP-specific IgM. To select for cells that have taken up the κ_{TNP} gene, the cloned κ_{TNP} gene, designated *Tκ1*, was inserted into the plasmid pSV2-*neo*, which contains a bacterial phosphotransferase gene (*neo*) and confers resistance to the aminoglycoside antibiotic G418 (ref. 7). In the present study, we have used two recombinant plasmids: in the plasmid pT-*Tκ1*, *Tκ1* was inserted so that the direction of transcription would be in the same direction as transcription initiated from the SV40 early promoter; in pR-*Tκ1*, the orientation of the gene is reversed (Fig. 1).

To transfer the DNA, bacteria harbouring the pT-*Tκ1* and pR-*Tκ1* plasmids were converted to protoplasts and fused with igk-14, after the method of Schaffner[8]. G418-resistant transformants of igk-14 were then selected as described in the legend to Table 1. The frequency of G418-resistant cells ranged from 5×10^{-4} to 10^{-5} per input igk-14 cell, and was comparable for the pT-*Tκ1*, pR-*Tκ1* and pSV2-*neo* plasmids. The resistant cells were then tested for the ability to make TNP-specific plaques. By this criterion about 10% of the G418-resistant transformants from pT-*Tκ1* and about 30% of the transformants from pR-*Tκ1* made TNP-specific IgM. By contrast, of 30 G418-resistant transformants from the pSV2-*neo* vector alone, none made TNP-specific plaques.

Representative transformants were selected, cloned by limiting dilution and studied further. The production of the κ_{TNP} chain has been assayed by TNP-specific haemagglutination and

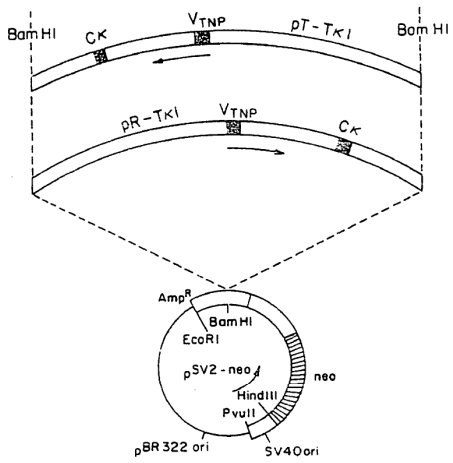

Fig. 1 Structure of *Tκ1* transducing plasmids. The *Tκ1* fragment (9.6 kb)[6] was inserted into the *Bam*HI site of pSV2-*neo* (donated by P. Berg)[7] and transfected into *Escherichia coli* cells (C600) to obtain the two types of recombinant, pT-*Tκ1* (*Tκ1* inserted in tandem with the SV40 early promoter) and pR-*Tk*1 (*Tk*1 orientation reversed with respect to the SV40 early promoter). The directions of transcription of the κ_{TNP} gene and SV40 early region are indicated by arrows.

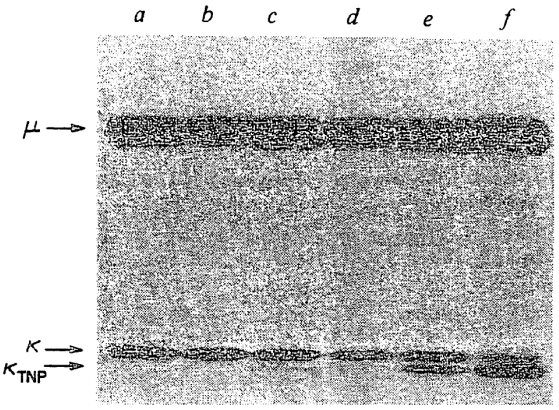

Fig. 2 Identification of κ_{TNP}-chain production in hybridoma cell lines and pT-*Tκ1* and pR-*Tκ1* transformants. Lane *a*, igk-14; lane *b*, T1L2; lane *c*, T3L2; lane *d*, R11L3; lane *e*, R31L4; lane *f*, Sp603. Secreted immunoglobulin was radiolabelled by incubating cells for 18 h in leucine-free Dulbecco's modified Eagle's medium containing ^{14}C-leucine (5 μCi ml^{-1}) and dialysed fetal calf serum (5%). Immunoglobulin was reduced and culture supernatants analysed by SDS-PAGE as described previously[5].

plaque formation (Table 1). Synthesis of the κ_{TNP} chain has also been measured by SDS-polyacrylamide gel electrophoresis (PAGE) (Fig. 2). The recipient cells igk-14 still produce the κ chain from the myeloma X63-Ag8 used to generate the parental Sp603 hybridoma, and this myeloma κ chain migrates more slowly than the κ_{TNP} protein. Low level production of the κ_{TNP} chain by R11L3 can be detected both as low titre haemagglutination and as a weak band on SDS-PAGE. In general, the intensities of the bands corresponding to the κ_{TNP} chain are roughly proportional to the TNP-specific haemagglutination titres.

To analyse the κ_{TNP} genes in the transformed cell lines, transformant DNA was digested with the restriction endonuclease *Bam*HI, fractionated by agarose gel electrophoresis and transferred to nitrocellulose. The blot was then probed with a cDNA clone of the κ constant-region gene segment (Fig. 3). Previous work has shown that this probe detects three κ-chain genes in DNA from the Sp603 hybridoma—the bands at 5.9 and 5.4 kilobases (kb) correspond to κ-chain genes donated by the myeloma parent; the band at 9.6 kb represents the κ_{TNP} gene[6], and this band is not observed in the case of igk-14. For all the G418-resistant transformants tested, including the T1L2 cells which do not make TNP-specific IgM, a band at 9.6 kb was observed with this probe. Results from DNA blot analysis of high molecular weight transformant DNA before and after digestion with the restriction endonuclease *Eco*RI (which cleaves the recombinant plasmids once) suggest that the transferred sequences are integrated into cellular DNA as tandem oligomers (data not shown). These results are consistent with those obtained by other investigators using the pSV2 transfer vectors[7,9,10].

We can estimate the copy number of the κ_{TNP} gene in the transformants by comparing the intensity of the band corresponding to the κ_{TNP} gene in DNA from the transformants with the intensity of the band corresponding to the κ_{TNP} gene in the DNA of Sp603 which apparently contains one copy of the κ_{TNP} gene per cell[6]. By this criterion, the transformants T3L2 and R31L4 have multiple copies of the κ_{TNP} gene per cell, while T1L2 and R11L3 contain about 1 copy per cell. It is interesting that the transformant R31L4 makes more κ_{TNP} chain than does T3L2, although T3L2 has more copies of the κ_{TNP} gene. Furthermore, R31L4 makes about 10-fold less κ_{TNP} chain per gene copy than does the wild-type hybridoma, although it should be pointed out that we do not know if all copies of the κ_{TNP} gene in R31L4 function equally efficiently. This variability in gene

Table 1 Secretion of TNP-specific IgM by G418-resistant transformants

	Cell lines	Haemagglutination titre	TNP plaque formation
	Sp603	1/1,280	+
	igk-14	<1/2	−
Vector	Transformants		
pT-*Tκ1*	T1L2	<1/2	−
	T3L2	1/160	+
	T4L1	<1/2	−
	T12e	<1/2	−
	T17L1	1/20	−
	T19L1	<1/2	−
	T21L2	<1/2	−
pR-*Tκ1*	R2L6	1/1,280	+
	R9L3	<1/2	−
	R10L18	<1/2	−
	R11L3	1/20	−
	R20L1	<1/2	−
	R22L1	1/640	+
	R31L4	1/320	+

The igk-14 cells were fused with bacterial protoplasts containing either the pT-*Tκ1* or pR-*Tκ1* plasmids. The methods for increasing the plasmid copy number and making protoplasts have been described elsewhere[14]. About 10^{10} protoplasts were collected by centrifugation. 10^7 igk-14 cells, grown as described previously[5], were washed and resuspended in Gibco H21 medium, and centrifuged onto the protoplast pellet. The protoplasts and igk-14 cells were then fused with polyethylene glycol 1000 (PEG), as described by Taniguchi and Miller for the production of T-cell hybridomas[15]. Briefly, after centrifugation, the cell–protoplast pellet was gently resuspended in 2 ml of H21 medium containing 40% PEG and 10% dimethyl sulphoxide. After 15 s, the mixture was added to an equal volume of H21 medium containing 50% PEG and mixing was continued for a further 15 s. The cells were then diluted to 50 ml with H21 medium containing 20% fetal calf serum, washed once in the same medium and plated in 24-well culture dishes at a cell density of 10^5 cells per well. After 24 h, the fused cells were selected in H21 medium containing 1 mg ml^{-1} of G418 (a gift of Schering Corporation). The transformants resistant to G418 were screened for the production of TNP-specific IgM by testing for TNP-specific plaque formation. Representative transformants were then cloned by limiting dilution. Culture supernatants were tested for haemagglutination of TNP-coupled sheep red cells (TNP-SRC) as described elsewhere[5]. The sensitivity of the assay was enhanced by including monoclonal rat anti-μ antibody C2-23, as described elsewhere (M. Potash *et al.*, in preparation). Plaquing was done on ~10^4 cells, also as described previously[5].

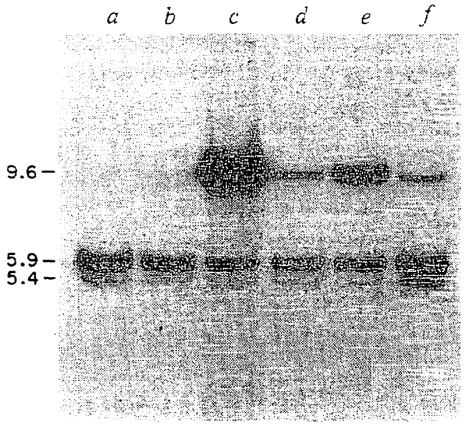

Fig. 3 Blot hybridization of DNAs from hybridoma cell lines and pT-*Tκ1* and pR-*Tκ1* transformants. Lane *a*, igk-14; lane *b*, T1L2; lane *c*, T3L2; lane *d*, R11L3; lane *e*, R31L4; lane *f*, Sp603. *Bam*HI-digested DNA samples (20 μg) were electrophoresed through a 1% agarose gel at 2 V cm^{-1} for 40 h. After transfer to nitrocellulose[16], the blot was hybridized with a ^{32}P-labelled cDNA clone of the κ constant-region gene segment (pL21-5, provided by R. Wall) by a method described elsewhere[6].

expression raises the question of whether all the regulatory elements of the normal κ_{TNP} gene are present or functioning on the cloned fragment. The messenger RNA cap sites have been identified for several immunoglobulin κ-chain genes[11]. In all cases, the cap site seems to be within 30 base pairs (bp) of the initiation codon. As the *Tκ1* fragment has 5 kb of DNA upstream of the initiation codon, it is likely that the DNA fragment carries the κ_{TNP} gene promoter. Similarly, the poly(A) addition site is estimated to lie 211 bp downstream of the constant-region gene segment[12], which is well within the 1.2 kb of downstream flanking DNA. Experiments are in progress to determine the molecular basis for the differences in the level of expression of the κ_{TNP} gene in the various transformants.

Falkner and Zachau have studied the transient expression of mouse immunobulin κ-chain genes in African green monkey cells (CVl), HeLa cells and mouse L cells[13]. In the case of the monkey CVl cells and the HeLa cells, the κ-chain gene was expressed only when transcription of the gene was placed under the direct control of an SV40 promoter after removal of the putative κ-chain gene promoter region. In the case of the mouse L cells, the κ-chain gene was not expressed. There are many structural differences between the vectors used by Falkner and Zachau and those used here. On the other hand, differences in the cellular environments might underlie the different requirements for κ-chain gene expression. It should be possible to resolve these differences by using the gene transfer system described here on cells of different types.

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Note added in proof: Rice and Baltimore[17] and Oi *et al.*[18] have recently reported the expression of cloned κ light chain genes in transformed lymphoid cells.

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Deduced amino acid sequence from the bovine oxytocin–neurophysin I precursor cDNA

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The nonapeptide hormone oxytocin-like arginine-vasopressin (AVP)[1–4] is synthesized as part of a larger precursor polypeptide. The precursor also includes the neurophysin molecule with which the hormone is associated in the neurosecretory granules of the hypothalamo-pituitary tract. A protein of molecular weight (M_r) ~20,000 has been isolated from supraoptic nuclei of rat hypothalami which, after tryptic cleavage, released a neurophysin-like molecule of M_r ~10,000 and an oligopeptide related to oxytocin[5]. This result was complemented by *in vitro* translation of bovine hypothalamic mRNA[6–8]. Among the primary translation products a single polypeptide of M_r ~16,500 was shown to contain antigenic determinants recognized by specific antisera against bovine neurophysin I and oxytocin. Here we report the amino acid sequence of the bovine oxytocin–neurophysin I (OT-NpI) precursor which was derived from sequence analysis of the cloned cDNA. As is the case for the bovine arginine-vasopressin–neurophysin II (AVP-NpII) precursor[4], the signal sequence of the OT-NpI precursor is immediately followed by the nonapeptide hormone which is connected to neurophysin I by a Gly-Lys-Arg sequence. A striking feature of the nucleic acid sequence is the 197-nucleotide long perfect homology with the AVP-NpII precursor mRNA sequence encoding the conserved middle part of neurophysins I and II.

A cDNA library of bovine hypothalamic mRNA[4] was screened for plasmids containing the mRNA sequence for the OT-NpI precursor. Bovine neurophysins I and II show nearly 80% homology in their amino acid sequences, including a complete homology between amino acids 10 and 74, so considerable cross-hybridization was expected between the mRNA sequences for the AVP-NpII precursor and the OT-NpI precursor.

The cloned cDNA encoding the AVP-NpII precursor[4] was therefore chosen as a hybridization probe for *in situ* colony screening[9]. Out of 5,000 recombinants, 63 clones gave a positive hybridization signal. Restriction analysis of the plasmids revealed two groups of 47 and 16 members containing different types of cDNA inserts (see Fig. 1 for examples). The first group represented cDNA sequences specific for the AVP-NpII precursor[4]. Sequence analysis of members of the second group (Fig. 1) showed that these contained OT-NpI precursor-specific sequences.

Figure 2 shows the nucleotide sequences of the cloned OT-NpI precursor cDNA and of the previously described AVP-NpII precursor cDNA[4] plus the predicted amino acid sequences.

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