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A temperature-sensitive mutant of Abelson murine leukemia virus confers inducibility of IgM expression to transformed lymphoid cells

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Lymphoid cell lines were isolated that were inducible for the expression of surface immunoglobulin by shift from 35.5 to 39.5°C after infection of mouse bone marrow cells with a mutagen-treated Abelson murine leukemia virus. Virus produced by one of the cell lines (*ts*49) transmitted the temperature-sensitive phenotype to new lymphoid trnasformants as well as to NIH/3T3 cells. In addition, the tyrosine autophosphorylating activity of the p120$^{gag-abl}$ protein synthesized in *ts*49-transformed cells was found to be temperature-sensitive. Shift experiments using *ts*49-transformed lymphoid cells showed that at 39.5°C they synthesize increased amounts of μ and κ chain RNA and proteins, and that they can be further induced to secrete IgM when treated with lipopolysaccharide.

Key words: temperature-sensitive mutant/Abelson murine leukemia virus/B cell differentiation

Introduction

Abelson murine leukemia virus (A-MuLV) is a replication-defective retrovirus capable of transforming lymphoid cells both *in vivo* and *in vitro* (Abelson and Rabstein, 1970; Rosenberg and Baltimore, 1976). With rare exceptions (Lewis *et al.*, 1982), transformants are blocked at early stages of differentiation, indicating that the v-*abl* oncogene interferes with maturation. The spontaneous release of this block in A-MuLV-transformed clones has been an important tool in studying the process of immunoglobulin rearrangement (Alt *et al.*, 1982; Sugiyama *et al.*, 1983; Ziegler *et al.*, 1984; Reth and Alt, 1984; Reth *et al.*, 1985). However, so far it has not been possible to induce their differentiation in a controlled way, thus limiting the use of this approach for the study of B-cell maturation. Previous experiments with temperature-sensitive (*ts*) mutants of oncogene-containing viruses have shown that erythroid and myelomonocytic cells can be induced to differentiate terminally when shifted to nonpermissive temperatures (Graf *et al.*, 1978; Beug *et al.*, 1984). Here we describe a mutant of A-MuLV that transforms B cells which are arrested at an early stage of differentiation at 35.5°C and which can be induced to express and secrete immunoglobulin molecules when shifted to 39.5°C.

Results

Isolation of A-MuLV-infected lymphoid cell lines that express surface immunoglobulin after temperature shift

In an attempt to isolate a *ts* mutant of A-MuLV, ANN-1 cells were mutagenized with azacytidine and the supernatant used as a stock of mutagenized virus. Bone marrow cells of BALB/c mice were infected with this stock and cells were seeded in Methocel. Transformed colonies appearing 10–14 days later were individually isolated and established in liquid culture in the presence of bone marrow feeders of C57BL/6 origin. These cell lines were then screened by direct or indirect immunofluorescence for the expression of surface immunoglobulin (sIg) and of the B-cell specific B220 antigen. All cell lines tested were B220-antigen positive at both permissive and nonpermissive temperatures. In contrast, essentially all were sIg$^-$ at 35.5°C while among 580 isolated clonal cell lines shifted to 39.5°C, four generated sIg$^+$ cells at high frequencies (Table I, Figure 1). In addition, the volume of these clones decreased and their growth rate was significantly reduced at the higher temperature (data not shown). Control cells transformed by wild-type A-MuLV grown at 35.5 and 39.5°C were indistinguishable for sIg expression. The high frequency of sIg$^+$ cells in line 4-9 at 39.5°C is not due to the selection of pre-existing sIg$^+$ cells, as indicated by the fact that removing sIg$^+$ cells from the initial population before the temperature shift did not reduce the frequency of sIg$^+$ cells obtained after the shift (unpublished results).

Virus-produced by the 4-9 cell line transmits a temperature-sensitive phenotype to newly transformed lymphoid cells

4-9 and subclone 4-9-6 cells were found to contain a single and

Table I. Abelson-transformed lymphoid cell lines exhibiting a *ts* phenotype

Colony no.	Percent of sIg$^+$ cells at	
	35.5°C	39.5°C
3-59	0.2	3.6
4-9	1.9	35.5
5-21	0.5	20.4
5-226	0.1	8.1

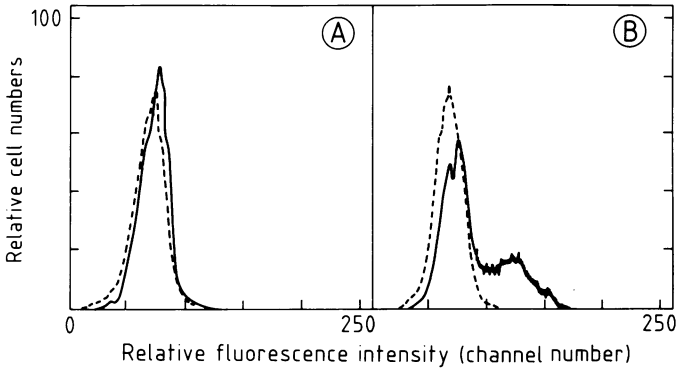

Fig. 1. Analysis of sIg$^+$ wild-type transformed clone 5-49 **(A)** and clone 4-9-6 **(B)** lymphoid cells. Cells were cultured at 35.5°C (- - -) or 39.5°C (——) for 4 days and stained by direct immunofluorescence with purified goat anti-mouse IgM antibodies or anti-κ antibody R33-18-10. The figure shows the result of staining the cells with anti-IgM as analysed by fluorescence-activated cell sorting. Similar results were obtained using the anti-κ antibody.

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Table II. Frequency of temperature-sensitive secondary transformants

Viral source (cell line)	Treated with	No. of colonies tested	No. of sIg^+ colonies	Percent virus-transformed cell clones with *ts* phenotype
4-9	none	28	5 (3.1,3.2,4.8,11,15)[a]	18
4-9-6	none	16	8 (2.2,2.8,3.3,3.5,4.7,5.6,15,20)	50
ANN-1	azacytidine	284	2 (8.1,21)	0.7

[a]In parenthesis: percent of sIg^+ cells per colony.

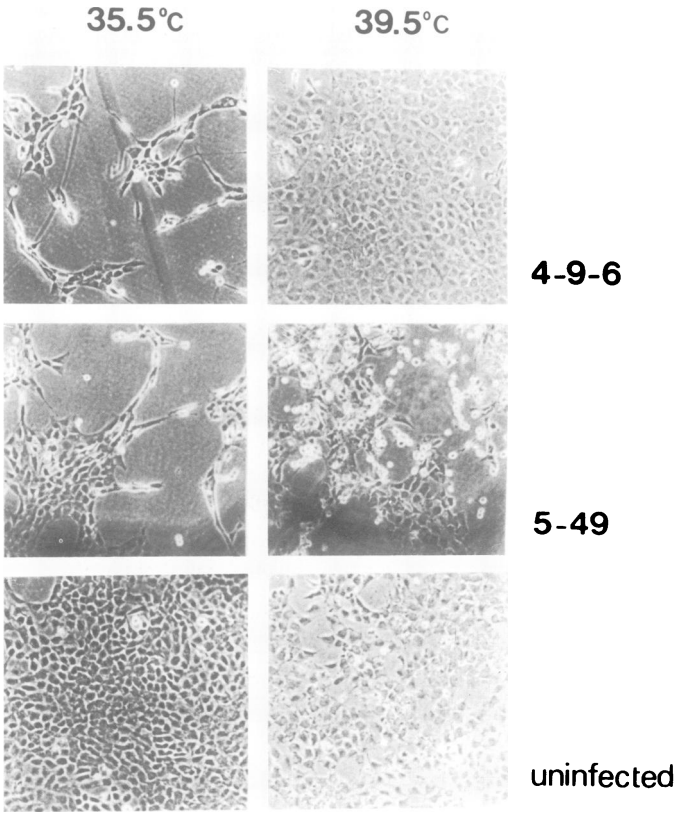

Fig. 2. Temperature-sensitivity of virus-transformed NIH/3T3 cells. The figure shows phase microscopy of cells from a focus obtained with *ts*49 virus (4-9-6 cells) or wild-type A-MuLV (5-49 cells), before and 3 days after shift. Uninfected cells are shown as a control.

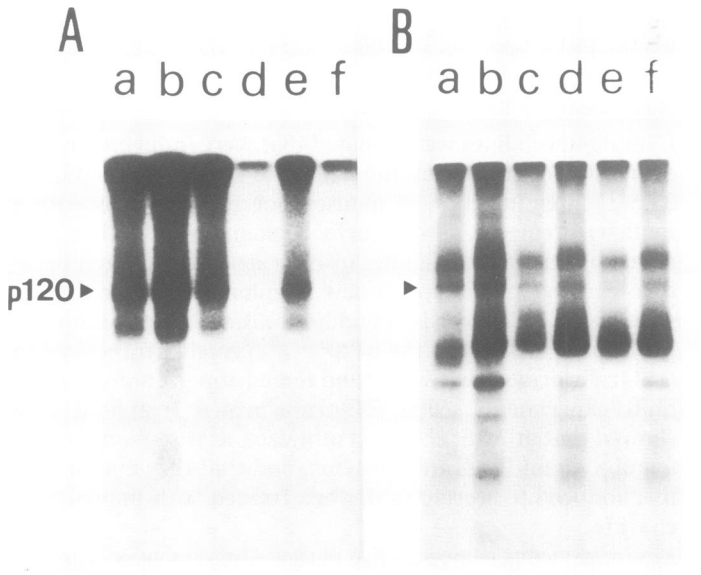

Fig. 3. Temperature-dependence of autophorphorylating activity of mutant and wild-type A-MuLV-transformed lymphoid cells. Immunoprecipitates labelled by ^{32}P **(A)** or [^{3}H]leucine **(B)**. Wild-type control cells (5-49 cells) at 35.5 and 39.5°C **(a,b)**; 4-9-6 cells at 35.5 and 39.5°C **(c,d)**; and 496-80 cells at 35.5 and 39.5°C **(e,f)**. Control experiments performed with labelled cell extracts from NIH/3T3 cells infected with Mo-MuLV and immunoprecipitated with anti-Mo-MuLV serum, as well as A-MuLV extracts incubated with normal goat serum (not shown) were performed to identify the p120 protein bands.

complete copy of the A-MuLV provirus as shown by Southern blotting and to produce $1-3 \times 10^2$ colony-forming units per ml. To establish that the *ts* phenotype of these cell lines was due to the infecting virus and not a cellular property, we determined whether the *ts* phenotype could be transmitted to new targets by virus produced by them. As shown in Table II, infection of bone marrow cells by virus from both cell lines indeed enabled us to establish transformed cell lines that generated sIg^+ cells at the nonpermissive temperature at a much higher incidence than colonies transformed by wild-type A-MuLV. We therefore conclude that the virus produced by the 4-9 cell line harbours a temperature-sensitive lesion and the corresponding mutant will be henceforth designated as *ts*49 A-MuLV.

Morphologically transformed foci induced by ts49 can be reverted to a normal phenotype after shift to 39.5°C

A-MuLV is known to be able to transform not only hematopoietic cells in culture but also established fibroblasts (Scher and Siegler, 1975). We therefore tested whether NIH/3T3 cells infected by *ts*49 would likewise exhibit a temperature-sensitive phenotype. As with wild-type A-MuLV, foci of refractile and spindle-like cells were obtained 6 days after infection with the virus at 35.5°C. As shown in Figure 2, when the cultures were shifted to the non-permissive temperature, the morphology of the cells in 15–30% of the foci became indistinguishable from that of normal NIH/3T3 cells within 2–3 days. In contrast, no morphological changes could be observed in any of the foci induced with wild-type A-MuLV derived from either the 5-49 or ANN-1 cell lines. Thus, virus produced by *ts*49 efficiently transmits the *ts* phenotype to new target cells, regardless of whether these are of lymphoid or non-lymphoid origin. The finding that only a small proportion of the foci shows a temperature-sensitive behaviour might reflect the leakiness of the mutant. It is still possible, however, that changes in a higher percentage of transformed cells could be detected if other more sensitive transformation parameters were tested.

The tyrosine autophosphorylating activity of the p120$^{gag-abl}$ protein encoded by ts49 is temperature sensitive

The oncogne of A-MuLV, v-*abl*, encodes a protein that exhibits a protein tyrosine kinase activity leading to its autophosphorylation on tyrosine (Witte *et al.*, 1980b). Since this enzymatic activity correlates with the biological activity of the virus (Rosenberg *et al.*, 1980; Prywes *et al.*, 1985) we decided to test

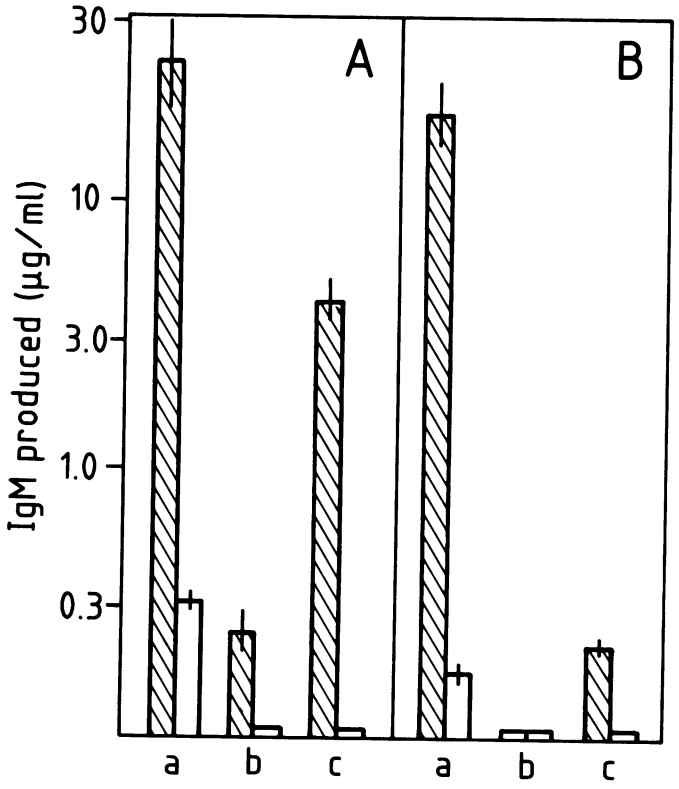

Fig. 4. Induction of IgM secretion by LPS. 4-9-6 cells were precultured at 35.5°C **(b)** or 39.5°C **(c)** for 6 days and stimulated with LPS for 6 days at 37°C **(A)** or 35.5°C **(B)**. As control, freshly prepared spleen cells **(a)** were also stimulated with LPS under the same conditions. Open columns, cells kept without LPS. Shaded columns, cells with LPS. Results represent geometric means with standard deviations of three individual culture supernatants.

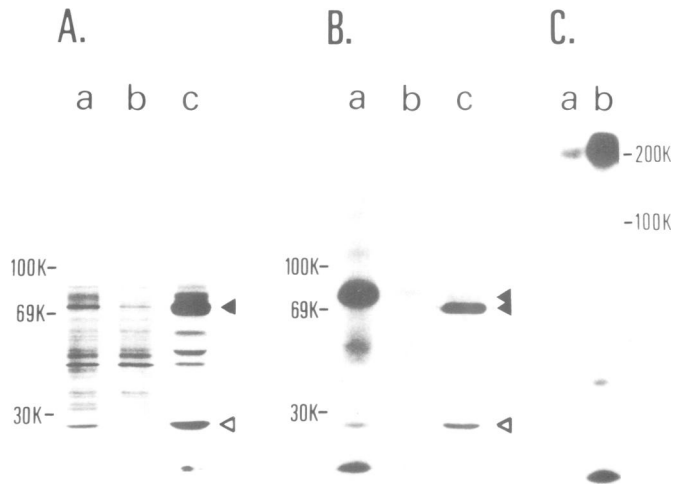

Fig. 5. Immunoglobulin production by 496-80 cells. 496-80 cells labelled with [^{35}S]methionine **(A)** or cell-surface labelled with ^{125}I **(B,C)** were lysed and immunoprecipitated with purified goat anti-IgM antiserum **(A,B)** or antibody 14.8 specific for B220 antigen **(C)**. The lysates were derived from cells cultured at 39.5°C **(a)**, 35.5°C **(b)**, or from B1-8 cells producing IgM antibody as a control **(c)**. The positions of heavy chain (▲) and light chain protein (△) are indicated in the figure.

the autophosphorylating activity of the p120$^{gag-abl}$ protein of the mutant virus. For this purpose we studied *ts*49-transformed lymphoid cell lines, 4-9-6 and 496-80, grown at permissive and non-permissive temperatures. 496-80 is a subclone of 4-9-6 that has been selected to generate sIg$^+$ cells at much higher frequency than the original clone when cultured at nonpermissive temperature (see below). To have an internal standard for the synthesis of the v-*abl* protein, cells were also metabolically labelled and immunoprecipitated with anti-Mo-MuLV virion serum (this serum recognizes the *gag* part of p120$^{gag-abl}$). A comparison of the amount of autophosphorylated p120 (Figure 3A) with the amount of ^{3}H-labelled p120 recovered after immunoprecipitation (Figure 3B) indicates that the kinase activity associated with *ts*49-transformed lymphoid cell lines was 10- to 100-fold reduced at nonpermissive temperature. In contrast, no significant differences were seen with wild-type-transformed cells.

Characterization of the changes induced by temperature shift in lymphoid cells transformed by ts49

The cell line 4-9 and clonal derivatives were used for further characterization of the differentiation-specific changes induced by shift to 39.5°C. As observed in the parental cell line 4-9, a shift of the subclone 4-9-6 from 35.5 to 39.5°C induced the expression of sIg in 30−40% of the cell population (Figure 1). Furthermore, addition of lipopolysaccharide (LPS), a B cell mitogen, to the cultures induced their secretion of IgM molecules, particularly when grown at 39.5°C (Figure 4). This result was obtained by preculturing the cells at 35.5 or 39.5°C followed by stimulation with LPS at 35.5 or 37°C rather than at 39.5°C, since cells treated with LPS at the latter temperature died within 3−4 days of cultivation. Thus, a shift of 4-9-6 cells to the nonpermissive temperature does not merely induce sIg expression but also changes their phenotype into IgM-secreting cells in response to LPS.

That 4-9-6 cells exhibit rearrangements in both heavy and light chain genes, and a minute amount of μ protein in their cytoplasm (unpublished results) suggests that the expression of sIg (μ and $\varkappa$ chains) is reflected by a regulatory mechanism operating at the transcriptional level of Ig gene expression. To analyse this possibility and to facilitate the assay, a cell clone would be desirable which would display the expression of μ and $\varkappa$ chains in almost 100% of the population when shifted to 39.5°C. We therefore screened a number of subclones of 4-9-6 cells and found one, 496-80, which expresses sIg in ~95% of the population at 39.5°C. This clone seems to carry a single $\varkappa$ gene rearranged at the $J_\varkappa$ locus while the second $C_\varkappa$ locus is deleted (data not shown). As shown in Figure 5, it was confirmed by immunoprecipitation experiments that 496-80 cells at 39.5°C produced cytoplasmic μ and $\varkappa$ chains at a high level and were further induced to express both chains as surface-associated forms. The mol. wt difference between the immunoglobulin produced by the control cells and by the *ts*49-transformed cells can be explained by the fact that the former produce the secreted Ig form, known to exhibit a lower mol. wt. Interestingly, in the same experiment we found that the level of B220 antigen decreased in 496-80 cells shifted to 39.5°C.

A Northern blot analysis showed that the increased immunoglobulin synthesis was due to an increase in the amount of μ and $\varkappa$ mRNA produced. As shown in Figure 6, the level of μ and $\varkappa$ chain transcripts increased at least 5- to 10-fold at 39.5°C. A further increase of a factor of 10−20 of μ chain RNA expression was observed after LPS treatment of 496-80 cells precultured at 39.5°C, and a 10-fold increase in $\varkappa$ domain mRNA after the same treatment. Furthermore, the anlaysis by specific oligonucleotide probes of the expression of membrane or secretory forms of the μ chain RNA gene revealed that both types of transcripts were enhanced in the *ts* cells by shift to the nonpermissive

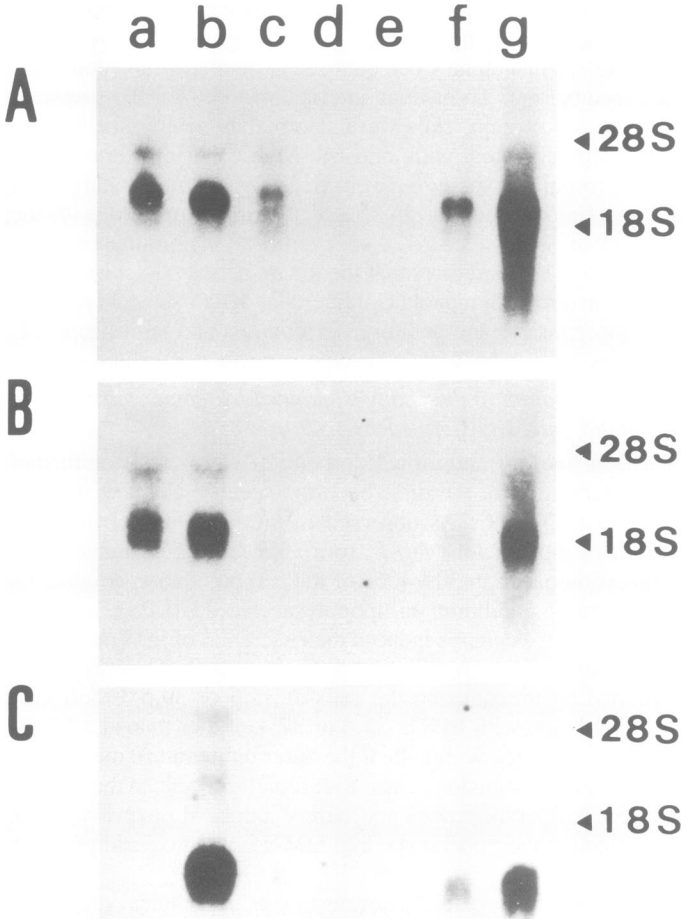

Fig. 6. Expression of immunoglobulin genes in 496-80 cells. Total RNA was isolated from 70Z/3 cells cultured at 37.0°C in the absence of **(a)**, or in the presence of LPS **(b)**; from wild-type A-MuLV-transformed cell line 5-1 cultured at 35.5°C **(c)** or 39.5°C **(d)**; from 496-80 cells cultured at 35.5°C **(e)**, 39.5°C **(f)**, or at 37°C in the presence of LPS **(g)**. The denatured RNAs (10 μg) were electrophoresed on 1% agarose gels, transferred to nitrocellulose membrane, and hybridized with ^{32}P-nick-translated membrane μ probe **(A)**, secreted μ probe **(B)**, or κ probe **(C)**. The same filter was then rehybridized with a ^{32}P-labelled probe to ribosomal DNA to confirm that the same amount of RNA was present in each lane (not shown).

temperature and that a further enhancement could be seen in response to LPS.

In contrast, an augmentation of Ig transcripts at 39.5°C was never observed with wild-type virus transformed cells and in the particular clone shown in Figure 6 we even observed a decrease. As another control, we analysed the level of Ig transcripts in the chemically induced pre-B cell line 70Z/3 which is known to express sIg when stimulated with LPS (Paige *et al.*, 1978; Perry and Kelley, 1979). In this case, the cell line already expresses a high level of μ transcripts without stimulation with LPS, but is induced to produce κ transcripts in response to LPS (Figure 6). This suggests that like 70Z/3 cells, 496-80 cells grown at 39.5°C are at the stage of virgin B cells, but differ from the former in that they do not constitutively express high levels of μ chain RNA.

Discussion

In the present paper we describe the isolation of several transformed lymphoid cell lines that can be induced to synthesize surface immunoglobulins when shifted from 35.5 to 39.5°C, obtained after infection with mutagen-treated A-MuLV. Virus produced from one of these lines, *ts*49 A-MuLV, was found to transmit the temperature-sensitive phenotype to both lymphoid and nonlymphoid target cells. The finding that the tyrosine autophosphorylating activity of p120$^{gag-abl}$ protein synthesized in *ts*49-transformed cells is temperature sensitive, provides biochemical evidence for the nature of the defect in the mutant. This finding is not unexpected since mutants of A-MuLV deficient in their ability to transform lymphoid cells are also deficient in the tyrosine kinase activity of their encoded *abl* proteins (Rosenberg *et al.*, 1980; Prywes *et al.*, 1985). In addition, the tyrosine kinase activity of pp60^{src} synthesized in chicken embryo fibroblasts transformed by *ts* mutants of Rous sarcoma virus shows a 2- to 10-fold decrease in cells grown at the nonpermissive temperatures (for review, see Friis, 1978). We therefore conclude that lymphoid cells transformed by *ts*49 escape at least partially their block in maturation due to the inactivation of the protein kinase activity of p120$^{gag-abl}$ following the shift to 39.5°C.

Similar to the situation observed in erythroid and myelomonocytic cells transformed by temperature-sensitive viral mutants of acute avian leukemia viruses (Graf *et al.*, 1978; Beug *et al.*, 1984) only a proportion of the lymphoid cells transformed by *ts*49 can be induced to differentiate when shifted to the nonpermissive temperature. This suggests that *ts*49 is leaky at the nonpermissive temperature, an interpretation that is also supported by its behaviour in the transformation of NIH/3T3 cells. We cannot rule out, however, that the mutant is unstable, exhibiting a high rate of reversion to the form of virus with wild-type properties. Analysis of virus produced by noninducible clones of *ts* mutant-transformed avian hematopoietic cells (Graf *et al.*, 1978; Beug *et al.*, 1984) suggests that this is not a likely explanation. Another possible reason, particularly for the fraction of cells in responding clones that cannot apparently be induced to synthesize sIg, is that they are formed as a result of nonfunctional κ gene rearrangements which destroy a previously established functional rearrangement. Continuing light-chain gene rearrangements of a single chromosome have frequently been observed in a A-MuLV-transformed cell lines (Lewis *et al.*, 1982; Whitlock *et al.*, 1983b), and we have also seen multiple κ gene rearrangements in 4-9 and 4-9-6 cells at both 35.5 and 39.5°C.

Finally, the high proportion of noninducible clones in cells transformed by *ts*49 might be due to the possibility that expression of the *abl* protein is only needed for the initiation of transformation and that frequent secondary changes occur which obviate the need for v-*abl* expression. We have indeed observed an increase in the proportion of noninducible cells in the 4-9-6 cell line cultured for prolonged periods of time. Subcloning could restore the initial state but noninducible variants gradually appeared again (T.T., unpublished observations). In support of the concept that v-*abl* is mainly needed for the initiation of transformation is the finding by Whitlock and Witte (1981) that A-MuLV-transformed lymphoid cell clones progressively become more feeder (factor) independent and tumorigenic as they are maintained in culture. In addition, certain lymphomas induced by transformation with A-MuLV were observed to lose expression of v-*abl* but to retain their oncogenic phenotype (Grunwald *et al.*, 1982).

The mutant described in this paper represents a potential new tool to probe the late stages of B cell differentiation. Most of the studies which we have performed so far were done with the 4-9-6 cell line and its subclone 496-80, selected for high in-

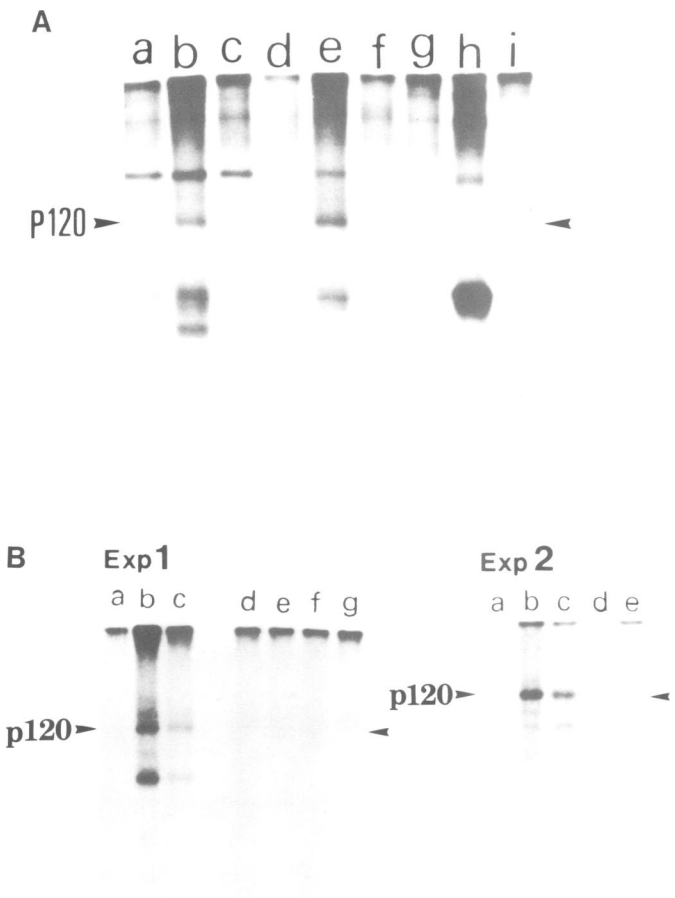

Fig. 7. Demonstration of $p120^{gag-abl}$ protein by immunoprecipitation. **(A)** Methionine-labelled cell extracts. Lymphoma cell clone 5-49 **(lanes a, b, c)** or fibroblast cell line ANN-1 **(lanes d, e, f)** transformed by A-MuLV; and Mo-MuLV-infected NIH/3T3 cells **(lanes g, h, i)** were labelled with [^{35}S]methionine, lysed and immunoprecipitated with anti-Mo-MuLV virion antiserum detecting *gag* determinants **(lanes b, e, h)**; with anti-$p10^{gag}$ antiserum **(lanes c, f, i)** and with normal goat serum **(lanes a, d, g)**. **(B)** Autophorphorylated cell extracts. [^{32}P]ATP-labelled extracts obtained from A-MuLV-transformed ANN-1 cells (Exp 1, **lanes a, b, c)**; Mo-MuLV-infected cells (Exp 1, **lanes d–g**; Exp 2, **lanes d,e**) or A-MuLV-transformed 5-49 cells (Exp 2, **lanes a, b, c**) were immunoprecipitated with anti-Mo- MuLV serum (Exp 1 and 2, **lanes b, e**); with anti-p15 serum (Exp 1, **lanes c, f**; Exp 2, **lane c**) with anti-p10 antiserum (Exp 1, **lane g**) or with normal goat serum (Exp 1 and 2, **lanes a, d**).

ducibility of sIg. Southern blotting analysis indicates that this clone has a single rearranged κ gene that is already present in the parental cell line and that encodes a functional protein. This suggests that the induction of sIg expression observed in 4-9-6 and 496-80 cells at the protein and mRNA level are due to an increase in transcription of immunoglobulin genes that have already undergone functional rearrangements.

Although we realize that the sequence of events of terminal differentiation observed in temperature-shifted *ts* A-MuLV cells may not reflect the precise order of events in normal B cells, we have attempted to draw a comparison between the two. The fact that 496-80 cells express cytoplasmic but not surface Ig and exhibit a rearrangd κ gene indicates that they represent cells at the pre-B stage. Temperature shift induces them to become virgin B cells as indicated by their capacity to express high levels of μ and κ transcripts and to synthesize surface immunoglobulin. These virgin B cells can finally be converted into antibody-producing cells by further stimulation with LPS. However, they may not have reached the terminal stage of maturation since, unlike plasmacytoma cells which express the secreted form of μ chains only (Perry and Kelly, 1979), they still also express the membrane form.

It will be interesting to determine whether the induction of immunoglobulin gene expression by temperature shift is related to the activation of positive regulatory mechanisms, such as the synthesis of *trans*-acting nuclear proteins (Maeda *et al.*, 1986; Staudt *et al.*, 1986), or to the release of the cells from negative regulatory mechanisms peculiar to immature cells or to transformed lymphocytes. In addition, selection of mutant-transformed clones that represent even earlier stages of B cell differentiation than 4-9-6 cells might make it possible to obtain new insights about the events that lead to the recombination of immunoglobulin genes and to the hierarchy of the rearrangement steps that precede the formation of antibody-forming cells.

Materials and methods

Cells

The cell line B1-8 was derived from a fusion of immune spleen cells of C57BL/6 with X63-Ag.8 myeloma cells and expresses μ and λ chains (Reth *et al.*, 1978). 70Z/3 is a chemically induced leukemic cell of murine (C57BL/6 × DBA/2) origin. The cells are essentially sIg^- but become sIg^+ when stimulated with LPS (Paige *et al.*, 1978). ANN-1 is a fibroblast cell line transformed by a A-MuLV (Witte *et al.*, 1980a).

Production of mutagen-treated A-MuLV

ANN-1 cells superinfected with Moloney murine leukemia virus (Mo-MuLV) were cultured at 35.5°C in the presence of 5-azacytidine (Aldrich, 10 μg/ml). After overnight cultivation, culture supernatant was collected, dialysed against PBS using a Pellicon Labocasset (Millipore Co. Ltd, Tokyo, Japan) and frozen at −80°C until use.

Cell transformation, establishment of cell lines and screening for temperature sensitivity

Bone marrow cells were prepared from BALB/c mice at 3–4 weeks of age and infected at 35.5°C according to the method of Rosenberg and Baltimore (1976) using Methocel as a semisolid medium (Graf *et al.*, 1981). Colonies were individually isolated 10–14 days later and cultured in RPMI 1640 medium supplemented with 10% FCS, 2-mercaptoethanol (5×10^{-5} M), and penicillin/streptomycin in the presence of a low density of bone marrow feeders of C57BL/6 (Whitlock *et al.*, 1983a). Each transformant was then divided into two aliquots and cultured in liquid medium at either 35.5 or 39.5°C. 3–5 days later their phenotypes were analysed by direct or indirect immnofluorescence (Takemori and Rajewsky, 1981), using monoclonal antibodies 14.8, R33-18-10, AF-6 or purified goat anti-mouse IgM antiserum. Antibody 14.8 is a monoclonal antibody specific for the B220 antigen expressed on pre-B cell and mature B cells (Kincade *et al.*, 1981); R33-18-10 is a monoclonal rat antibody with specificity for mouse κ chains (Grutzmann, unpublished); and AF-6 is a monoclonal antibody that is specific for the b allotype of IgM antibodies (Stall and Loken, 1984). In all cell lines examined the level of staining with antibody AF-6 was at least 20 times lower than that of cells stained with the goat anti-IgM serum.

LPS stimulation

1×10^6 cells were precultured at 35.5 or 39.5°C for 6 days in conditioned medium as described by Whitlock and Witte (1981). 4×10^5 cells /ml were then treated with 10 μg/ml of LPS (*Escherichia coli* 055:B5, DIFCO, USA) at 35.5 or 37°C. Cultures were fed at 2-day intervals and split when crowded. The amount of IgM antibodies released into the culture supernatant was determined 6 days after the addition of LPS by radioimmunoassay and standardized with the control monoclonal IgM antibody B1-8, as described previously (Takemori and Rajewsky, 1984).

Cell labelling and immunoprecipitation

Metabolic labelling of the cultured cells with [^{35}S]methionine or [^{3}H]leucine was performed as described elsewhere (Witte *et al.*, 1980a). The specificity of the antisera used to demonstrate the $p120^{gag-abl}$ protein is shown in Figure 7A. The cells were also surface labelled with ^{125}I by the lactoperoxidase technique as described previously (Meuer *et al.*, 1983). The labelled cells were washed, lysed and immunoprecipitated with a purified goat antiserum specific for mouse IgM and rabbit Ig. The immunoprecipitates were collected on protein A Sepharose

and the samples were analysed on SDS–polyacrylamide gels. In the case of immunoprecipitation with antibody 14.8, the precipitates were collected with Sepharose coupled to purified rabbit anti-rat Ig.

Kinase assay

Cells were labelled with [^{3}H]leucine (200 μCi/5 × 10^6 cells) for 2 h at the corresponding temperature, lysed in PBS lysis buffer (Konopka *et al.*, 1984), and then adjusted to contain the same counts of [^{3}H]leucine. After immunoprecipitation with anti-Mo-MuLV antiserum, one half of the sample was electrophoresed on an 8% SDS–polyacrylamide gel and fluorographed to determine the relative amount of $p120^{gag-abl}$. The other half was incubated with [γ-^{32}P]ATP at 30°C, as described by Witte *et al.* (1980b) and Konopka *et al.* (1984). This sample was also run on an 8% SDS–polyacrylamide gel but was subsequently autoradiographed without fluorography. Control experiments in Figure 7B show the specificity of the antisera used.

Identification of Ig transcripts

Total RNAs were prepared by the guanidine thiocyanate method as described by Chirgwin *et al.* (1979). They were electrophoresed on 1% agarose gels containing 1 M formaldehyde, transferred to nitrocellulose membranes and hybridized with ^{32}P-nick-translated recombinant DNA probes or synthetic oligonucleotide probes (Schiber *et al.*, 1978). The hybridization probes were Cμ, a 1.2-kb *Hin*dIII fragment containing Cμ3 and Cμ4 exons, isolated from a recombinant plasmid, pSV-Vμ1 (Neuberger, 1983); C_κ, a 279-bp *Hpa*I/*Ava*II fragment containing the C_κ exon isolated from a genomic clone of Ig146 (Sakano *et al.*, 1979). The oligonucleotide probes corresponding to the 5′ end of the first membrane exon and a secretory exon of μ chains (60 mer and 57 mer, respectively) were made by an automated DNA synthesizer (Applied Biosystem Inc.). The relative concentration of Ig sequences in the labelled bands was estimated by scanning with a densitometer (Hoefer Scientific Instruments, San Francisco, USA).

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