Immunology 1990 **69** 558–563

A monoclonal antibody to Ly-6 gene product inhibits generation of functionally active T cells and recognizes single antigenic specificity whose expression is up-regulated in virus-transformed rat fibroblast

A. HAQUE, M. CHAMEKH, J. CORNELIS,* A. CAPRON & S. HAQUE *Centre d'Immunologie et de Biologie Parasitaire, Unite mixte, INSERM U167 and *Service d'Oncologie Moleculaire, Institut Pasteur, Lille, France*

Accepted for publication 12 December 1989

SUMMARY

In order to elucidate the relationship between the structure and function of proteins encoded for by the Ly-6 gene complex, a cDNA was constructed for a Ly-6.2 specificity and then monoclonal antibodies (mAb) generated to bacterially synthesized protein. The addition of one of these mAb, designated Pb-19, inhibited the proliferative response of T cells to concanavalin A (Con A) or major histocompatability complex (MHC) alloantigens. Reactivity of Pb-19 to the Ly-6 specificity was blocked by a known anti-Ly-6.A.2 mAb but not by an anti-Ly-6.E.1 mAb. This mAb detected a Ly-6.A.2 specificity (a 33,000 MW antigen) whose expression was increased in a transformed rat fibroblast containing the entire genome of bovine papillomavirus.

INTRODUCTION

A set of surface antigens (Ly-6.A–E, ThB, TAP) expressed variously on cells of bone marrow, lymph node, spleen, and thymus have been mapped to the Ly-6 locus (McKenzie, Cherry & Snell, 1977; Kumura *et al.*, 1984; Hogarth *et al.*, 1984; Houlden, Hogarth & McKenzie, 1986). Panels of mAb have been generated recognizing specificities with distinct tissue distribution patterns controlled by the Ly-6 locus. Recently it has been described that activation signals might be delivered to murine T lymphocytes by antibodies binding to the surface by Ly-6 antigens (Malek *et al.*, 1986; Dumont, 1986; Yeh *et al.*, 1987). Further, *in vivo* administration of Ly-6 mAb resulted in the activation of T-cell dependent functional activities in tumour-bearing mice and up-regulated their anti-tumour immune response (Lu, Palladino & DeLeo, 1989). These observations have generated greater attention on Ly-6 molecules.

The large number of Ly-6 antigens and specificities had raised speculation as to whether the Ly-6 locus contains multiple genes, or whether one gene exists whose protein product undergoes variable post-translational processing. Recently, three Ly-6 genes reported to encode the Ly-6.A.2, Ly-6.C.2 and Ly-6.E.1 specificities have been cloned from cDNA libraries (LeClair *et al.*, 1986; Palfree *et al.*, 1987, 1988), but no direct relationship has been made between a given Ly-6 gene and its protein product. Further, it has been reported that a number of mAb which were previously considered to identify distinct native proteins all reacted with the same gene product following transfection of a cDNA for a Ly-6.2 specificity into COS-7 cells (Korty, Cohen & Shevach, 1988).

It appears that the characterization of mAb reactive to fusion protein from a cDNA encoding a Ly-6 specificity should help establish precisely the relationship between the structure and functions of proteins coded for by the Ly-6 gene complex. To this end, a cDNA was constructed for a Ly-6.2 specificity, by using an insert of 750 bp from a cDNA (pKLy.6.1-2R) encoding the Ly.6.E.1 antigen (LeClair *et al.*, 1986) as a probe, and mAb were generated to bacterially synthesized protein from one of the clones of the cDNA library. The proliferative response of T cells to Con A or MHC alloantigens was suppressed by the anti-Ly-6.2 mAb (Pb-19) thus generated. This mAb detected a Ly-6 specificity (a 33,000 MW antigen) whose expression was enhanced in a transformed rat fibroblast cell line FRBPV8 containing the entire genome of bovine papillomavirus.

MATERIALS AND METHODS

Mice

BALB/c and C57BL/6 (B6) mice, of 6–10 weeks, were purchased from the Centre National de la Recherche Scientifique, Orléans. (BALB/c × C57BL/6) F_1 (CB6F1) mice were bred and maintained at the animal facility in the Institut Pasteur, Lille.

Abbreviations: FACS, fluorescence-activated cell sorter analysis; MLR, mixed lymphocyte reaction.

Correspondence: A. Haque, Centre d'Immunologie et de Biologie Parasitaire, Institut Pasteur, B.P. 245, 59019 Lille Cedex, France.

Cells

The thymic lymphoma BW5147 of AKR mice origin was kindly made available by Dr Christine Mazingue (Institut Pasteur). Meth A fibrosarcomas (AHL-7801) were induced in BALB/c mice by a single subcutaneous injection of 3-methylcholanthrene dissolved in sesame oil, as described earlier (DeLeo *et al.*, 1977). BW5147 cells and Meth A sarcoma were maintained in culture by serial passage in RPMI-1640 medium containing 10% fetal calf serum (FCS), 100 mM L-glutamine, 100 μM non-essential amino acids, 1 mM sodium pyruvate and 2×10^{-5} M 2-mercaptoethanol. The established Fischer rat fibroblast cell line FR3T3 (Seif & Cuzin, 1977) and its transformed derivative FRBPV8, containing the entire genome of bovine papillomavirus type 1 (Grisoni *et al.*, 1984), were grown in Dulbecco's-modified Eagle's medium supplemented with 10% calf serum. FRBPV8 cells, classified as stage 2 cells according to Binétruy *et al.* (1987), display a low level of transformation *in vitro* but are highly tumourigenic, invasive and metastatic in syngeneic animals.

Cell preparation

Spleen cells obtained by mincing the tissues were washed in balanced salt solution, centrifuged, and resuspended in culture medium. T cells were prepared by adding unprimed spleen cells to plastic Petri dishes coated with anti-mouse Ig serum (Sigma, St Louis, MO) and the non-adherent fraction was harvested after a 70-min incubation at 37°. B cells were prepared by treating normal spleen cells with appropriate anti-Thy-1 sera (Cedarlane, Hornby, Ontario, Canada) and complement (C).

Mitogen-induced T- and B-cell proliferation assay

T and B cells (1×10^5 cells/well) were suspended in culture media containing 5 μg/ml Con A and 50 μg/ml lipopolysaccharide (LPS; Gibco Laboratories, Detroit, MI), respectively. With Con A, cells were incubated for 48 hr, with LPS for 72 hr. Cells were pulsed with 0·5 μCi tritiated thymidine ([^{3}H]TdR; CEA, Gif-sur-Yvette, France) overnight and harvested (PH.D. Harvester, Cambridge, MA) by absorption onto glass fiber filters. The radioactivity of the filters was determined by liquid scintillation counting.

Mixed lymphocyte reaction (MLR)

MLR was performed in flat-bottomed microtitre plates by mixing 10^6 stimulator lymphocytes, previously treated with mitomycin C (50 μg/ml; Sigma), and 10^6 responder lymphocytes for 30 min at 37°. After 4 days at 37°, cultures were pulsed for 6 hr with 1 μCi [^{3}H]TdR, harvested, and counted as described above.

The degree of lymphocyte stimulation is expressed by the stimulation index (SI):

$$\text{SI} = \frac{\text{average c.p.m. in stimulated cultures}}{\text{average c.p.m. in unstimulated (control) cultures}}.$$

Construction and screening of a cDNA library for Ly-6.2

Total cellular RNA was isolated from BW5147 lymphoma cells using the guanidium thiocynate technique of Chirgwin *et al.* (1979). The synthesis of double-stranded cDNA was performed from the selected Poly(A)+RNA, exactly as described by Gubler & Hoffman (1983). Subsequent steps in processing of the double-strand cDNA are discussed in detail by Watson & Jackson (1985), and the significant modifications made are described by LeClair *et al.* (1986). Fractions containing cDNA were collected, concentrated and inserted via EcoRI linkers into the γgt10 vector arms, packaged and titered as described elsewhere (Huynh, Young & Davis, 1985).

Production of mAb to bacterially synthesized protein from a Ly-6.2 cDNA

BALB/c mice were immunized by a single injection directly into the spleen with the fusion proteins (50 μg) or with BW5147 cells (250,000 cells). Six days later, spleen cells from the immunized mice were fused with SP2-0 myeloma cells. Details of fusion procedures and hypoxanthine-aminopterin-thymidine (HAT) selection have been described elsewhere (Galfre *et al.*, 1977). From 2–3 weeks after fusion, culture supernatants were screened in an enzyme-linked immunosorbent assay (ELISA), as described by des Moutis *et al.* (1983). Polyvinyl microtitre plates were coated either with different concentrations of fusion proteins or of extracted cell proteins from BW5147. As controls, plates were also coated with *Escherichia coli* (Y1089 strain) cell extracts. Only the hybrids whose supernatants gave a positive reaction to the fusion proteins and BW5147 cell proteins but not to *E. coli* cell extracts were selected for induction of ascitic tumour. Class-specific anti-mouse Ig (Nordic, Tilburg, The Netherlands) antibodies were used to determine Ig isotype of 10-fold concentrated hybridoma supernatants. This mAb was IgM and designated as Pb-19. Ascites fluid from one of the positive hybridoma-bearing mice was used in these studies. This mAb has been shown to recognize a Ly-6.A.2 determinant (see the Results) and is referred to as anti-Ly-6.A.2.

Fluorescence-activated cell sorter (FACS) analysis

Cells from triplicate cultures were pooled, washed and were aliquoted at $0{\cdot}5 \times 10^6$ cells/sample for staining. Samples were incubated with optimal concentrations of mAb. Single parameter fluorescein analysis consisted of incubation with a previously titrated quantity of fluorescein-conjugated rabbit anti-mouse Ig (Nordic, Tilburg, The Netherlands). In blocking experiments, cells were preincubated with anti-Ly-6.A.2 or Ly-6.E.1-specific mAb prior to the addition of biotinylated Pb-19 mAb. Bound biotinylated antibody was stained with fluorescein-conjugated avidin. All incubation steps were carried out on ice for 30 min, followed by two washes with PBS containing 2% FCS and 0·1% azide. The histograms of fluorescence distribution shown in results are always plotted as number of cells (y-axis) versus fluorescence intensity (x-axis). Fluorescence intensity is expressed as log relative fluorescence unless otherwise specified. The FACS analysis was performed by using an Ortho Instruments fluorescence-activated cell sorter (Ortho Cytoflorograph, Westwood, MA).

Cell labelling, immunoprecipitation and SDS–PAGE analysis

Cells showing exponential growth were obtained and washed twice with calcium- and magnesium-free PBS. Then they were resuspended in 0·5 ml of extraction buffer. The extraction buffer contains 10 mM Tris-HCl, pH 7·2, 0·15 M NaCl, 0·02% NaN3 and 0·5% (w/v) Nonidet P-40. The cells in the extraction buffer was vortexed and left on ice for 15 min. Then it was centrifuged at high speed and the supernatant was collected. The protein content in the sample was determined. Proteins were labelled with Na^{125}I using the chloramine T method. Free iodine was

removed on a Sephadex PD-10 column (Pharmacia, Uppsala, Sweden). Extracts containing cell proteins were then incubated with mAb, and immune precipitates were absorbed on protein A–Sepharose (Pharmacia) beads previously incubated with 10 μl of rabbit anti-mouse IgG serum (Nordic). Immune precipitates eluted from protein A–Sepharose beads were electrophoresed in 13% polyacrylamide gels according to the procedure of Laemmli (1970) under reducing conditions. The gels were then dried and autoradiographed using Kodak XAR-5 film (Eastman Kodak Company, Rochester, NY) at −70°.

RESULTS

Ly-6 cDNA cloning

In order to isolate cDNA encoding the Ly-6.2 protein, poly(A)+ RNA was isolated from BW5147, an AKR thymoma cell line. The BW5147 cell line was chosen for its constitutive expression of high levels of Ly-6.A.2 antigen (Palfree *et al.*, 1987). Double-stranded cDNA was prepared and fractions containing cDNA were ligated into EcoRI-digested, dephosphorylated γgt10 vector arms. Eleven clones had been isolated from the primary plating on the basis of hybridization with a 750 bp fragment of Ly-6.E.1 cDNA, that was provided by Drs Ken LeClair and Al Bothwell (Howard Hughes Medical Institute at Yale University Medical School, New Haven, CT). These were subsequently cloned in the expression vector γgt11. The fusion proteins were obtained from three of these clones as described earlier (Collins *et al.*, 1986). The characteristics of constructed cDNA clones will be a subject of a separate publication.

Binding of mAb generated to fusion protein from a Ly-6.2 cDNA (FACS analysis)

mAb to fusion protein from a Ly-6 cDNA were produced as described in the Materials and Methods. One of these mAb (Pb-19) has been analysed for its reactivity to Ly-6 antigen present on the surface of different cell lines. Representative results of immunofluorescence analysis by FACS are shown in Fig. 1. The mAb Pb-19 identified a cell-surface antigen that was expressed at a relatively high density on BW5147 cells (Fig. 1b). The presence of this antigen detected by Pb-19 was minimal on BALB/c Meth A sarcoma (AHL-7801) or SP 2-0 myeloma cells (A. Haque, unpublished results). Furthermore, it was consistently noted that a greater percentage of spleen cells from C57BL/6 mice reacted with Pb-19 than spleen cells from BALB/c mice (data not shown). Thus, it could be inferred that mAb Pb-19 recognizes an alloantigenic determinant which is linked to Ly-6.2. This anti-Ly-6.2 mAb also detected a determinant expressed on a Fischer rat fibroblast cell line FRT3T3 (Fig. 1c), as well as on its transformed derivative FRBPV8 (Fig. 1d, f) containing the entire genome of bovine papillomavirus type 1. However, the level of fluorescent staining was more than two-fold greater on the transformed (Fig. 1d) than on the non-transformed cells (Fig. 1c).

Binding of PB-19 mAb was blocked by a known anti-Ly-6.A.2 but not by an anti-Ly-6.E.1 mAb (FACS analysis)

Blocking studies were performed with other known anti-Ly-6 mAb to establish if the Pb-19 mAb really reacts with the Ly-6.A.2 specificity on the cell surface. Preincubation of cells with

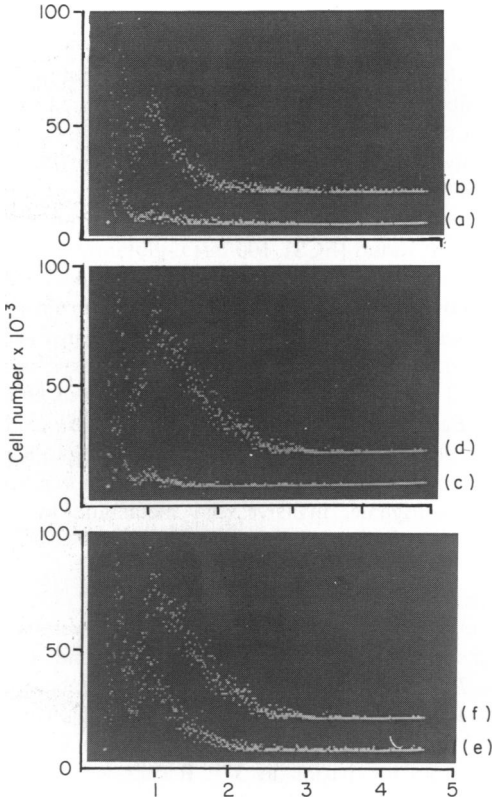

Figure 1. Expression of Ly-6.A.2 antigens on various cell lines. (b) and (f) represent detection by Pb-19 mAb on BW5147 murine tumour cells and rat fibroblast FRBPV8 (transformed by integration of genome of papillomavirus), respectively. (a) and (e) indicate expression on BW5147 and FRBPV8 cells, respectively, incubated with SP 2-0 ascites (negative controls). Expression on transformed FRBPV8 cells (d) was compared with non-transformed FR3T3 (c) cells by superimposition. Fluorescence intensity (*x*-axis) is expressed as log relative fluorescence.

S8-106 mAb, an anti-Ly-6.A.2 (Kimura *et al.*, 1984; Palfree, Dumont & Hammerling, 1986) antibody, inhibited significantly the binding of Pb-19 to the surface of BW5147 (Fig. 2 a versus b) or of FRBPV8 (Fig. 2 e versus f) cells. On the other hand, pretreatment of these cells with Pb-19 mAb markedly blocked the binding of a known anti-Ly-6.A.2 mAb (i.e. S8-106 mAb) (data not shown). Preincubation with SK-7094 mAb, an anti-Ly-6.E.1 (Kimura *et al.*, 1984; Palfree *et al.*, 1986), did not result in a significant reduction in the reactivity of Pb-19 mAb to the surface molecules of BW5147 (Fig. 2 a versus c) or of FRBPV8 (Fig. 2e versus g) cells. When these cells were first incubated with Pb-19 mAb and then with SK-7094 mAb, there was no competition in the binding between these two mAb (data not shown).

Immunoprecipitation of radiolabelled Ly-6.A. antigen

The autoradiogram of SDS–PAGE in Fig. 2 shows material immunoprecipitated from lysates of cells radiolabelled through Chloramine-T-mediated iodination. When anti-Ly-6.2 mAb Pb-19 was used to immunoprecipitate from lysates of BW5147, a 33,000 MW band was detected (Fig. 3h). An identical band was recognized when lysates from FR3T3 (Fig. 3d) or FRBPV8 (Fig. 3f) were reacted with mAb Pb-19. Radiolabelled antigen of 33,000 MW was precipitated specifically from bacterially syn-

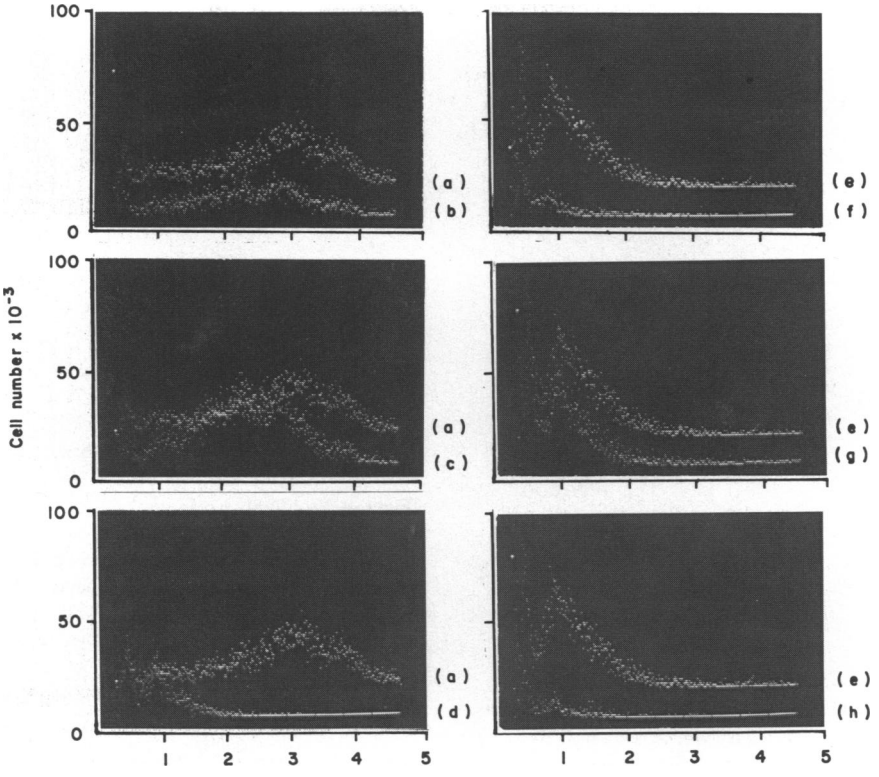

Figure 2. Effect of preincubation with antibodies recognizing Ly-6.A.2 or Ly-6.E.1 molecules on the staining of BW5147 or FRBPV8 cells with Pb-19 mAb. (a) and (d) represent binding to BW5147 cells of Pb-19 mAb and negative control, respectively. (b) and (c) indicate staining of BW5147 cells by Pb-19 mAb when cells were preincubated with S8-106 mAb and SK-7094 mAb, respectively. (e) and (h) demonstrate binding to FRBPV8 cells of Pb-19 mAb and negative control, respectively. (f) and (g) indicate staining of FRBPV8 cells by Pb-19 mAb when cells were preincubated with S8-106 mAb and SK-7094 mAb, respectively (see the Materials and Methods).

thesized material (Fig. 3b) but not from lysates of *E. coli* (Fig. 3a) with mAb Pb-19. This mAb failed to detect any iodinated material when lysates from Meth A sarcoma or SP 2-0 myeloma were used (data not shown).

Proliferative responses of T cells to Con A or MHC alloantigens were suppressed by anti-Ly-6.A.2 mAb

Con A-induced T-cell proliferation was abrogated when anti-Ly-6.A.2 mAb (Pb-19) was added at the beginning to the culture of spleen cells from B6 mice (Fig. 4c, d). The inhibitory effect was dose-dependent of mAb Pb-19. The antibody had no effect on LPS activation on any spleen cells (A. Haque, unpublished results).

In another series of experiments, B6 ($H\text{-}2^b$) or CB6F1 ($H\text{-}2^{d/b}$) responder cells were mixed with equal numbers of mitomycin C-treated BALB/c ($H\text{-}2^d$) stimulator cells, and the proliferative response was measured in the presence or absence of anti-Ly-6.A.2 mAb. Mixed lymphocyte reaction (MLR) was suppressed significantly when mAb Pb-19 was added at the beginning of culture (Fig. 4g, h). The addition of control SP 2-0 ascite at the same dilution did not display any inhibitory effect (Fig. 4e, f).

DISCUSSION

This study describes the isolation of a Ly-6.2 cDNA clone and the production of a new anti-Ly-6 monoclonal antibody (designated as Pb-19) to this cDNA's encode products. The aim of the present study was to clarify the molecular and functional heterogeneity of the Ly-6 family. The characterization of mAb Pb-19 should be an aid in achieving this objective.

The phenotype of the staining profile in FACS, i.e. high or low percentage of Pb-19-positive cells, mapped to the Ly-6.2 as assayed by strain distribution, tumour cell lines. The results of blocking experiments with already known anti-Ly-6 mAb clearly demonstrated reactivity of Pb-19 mAb to a Ly-6.A.2 specificity, since binding of Pb-19 could be inhibited by an anti-Ly-6.A.2 mAb but not by an anti-Ly-6E.1 mAb. Pb-19 mAb recognized an antigenic determinant expressed on a Fischer rat fibroblast FR3T3 as well as on its transformed derivative FRBPV8 containing the entire genome of bovine papilloma-virus type 1. Interestingly, the expression of this determinant was markedly higher on the transformed than on the non-transformed cells. Although the role of anti-Ly-6 mAb in the inhibition of tumour growth has been suggested (Lu *et al.*, 1989; Flood *et al.*, 1985), this is the first report to show an increased expression of these molecules on virus-transformed cells compared to non-transformed cells. It has not yet been possible to show an increased expression of mRNA in transformed cells, since hybridization of DNA fragments from cDNA clone (AH-3) with RNA from transformed and non-transformed cells in Northern blot analysis was negative (A. Haque, unpublished data). Similar failure in Northern blot hybridization with BALB3T3 RNA with nick-translated Ly-6.E.1 has been de-

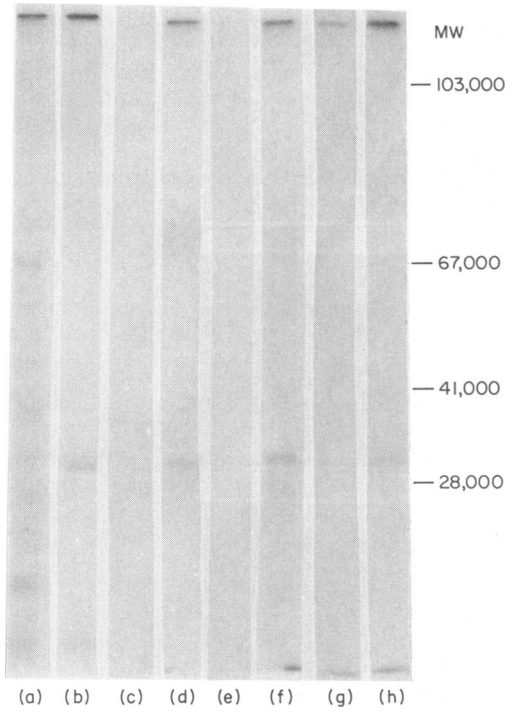

Figure 3. Radioimmunoprecipitation and SDS–PAGE analysis of proteins in the cell extracts recognized by anti-Ly-6.A.2 mAb. Cell extracts from (a) *E. coli*-Y 1089, (b) Fusion protein, (d) FR3T3 (non-transformed), (f) FRBPV8 (transformed by integration of genome of Papilloma virus), (h) BW5147. Columns (c), (e) and (g) represent negative controls (i.e. cell extracts from FR3T3, FRBPV8 and BW5147 failed to show reactivity with SP 2-0 ascites).

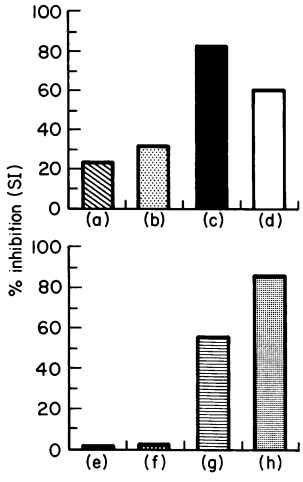

Figure 4. Proliferative response of T cells to Con A or MHC alloantigens was inhibited by anti-Ly-6.A.2 mAb. (a) 10%, (b) 20% of SP 2-0 ascite; (c) 10%, (d) 20% of anti-Ly-6.A.2 mAb. The ascites were added at the beginning to the culture of T lymphocytes with Con A. (e) 10%, (f) 20% of SP 2-0 ascite; (g) 10%, (h) 20% of anti-Ly-6.A.2 mAb (ascite) added at the beginning to the mixed lymphocyte reaction (see the Materials and Methods). Percentage inhibition was determined by the formula:

$$100 - \frac{\text{SI with addition of mAb}}{\text{SI with medium only (maximum response)}} \times 100.$$

See the Materials and Methods for SI determination. The results are representative of five different experiments.

scribed previously (LeClair *et al.*, 1986). This failure may reflect post-translational modification of Ly-6.2 protein at least in these cells. Clearly further studies are necessary in this regard.

Most investigators have found it difficult to radiolabel and immunoprecipitate Ly-6 antigens. Thus, a large disparity in molecular weights has been reported for the Ly-6-encoded proteins. This study describes how mAb Pb-19 precipitated radiolabelled antigen of 33,000 MW from the lysates of FR3T3, FRBPV8 and BW5147 cells under reducing conditions. This observation is consistent with the earlier reports in which antibodies to Ly-6.A.2 reacted to a 33,000 MW protein (Matossian-Rogers *et al.*, 1982; Sutton *et al.*, 1985). However, this is not in accordance with other reports of their molecular masses; their relative masses range between 15,000 and 18,000 (Palfree *et al.*, 1986; Ortega *et al.*, 1986). These differences in the biochemical properties of the Ly-6.2 products may be due to differences in the cell types studied or in the labelling conditions used. It should be emphasized that mAb Pb-19 reacted to both native antigens and Ly-6.2 cDNA's encode products with the same molecular mass. Recently it has been shown that a number of mAb (D7, anti-Ly-6.A.2 and Ly-6.E.1; 34-11-3, anti-Ly-6A and anti-TAPa; HD-42, anti-Ly-6.E.1; 3E7.1, anti-TAP) all reacted with the same gene product following transfection with a cDNA for Ly-6.2 into COS-7 cells (Korty *et al.*, 1988). The molecules recognized by these mAb were approximately 14,000 MW in size, while the molecule detected by Pb-19 was approximately 33,000 MW. This has led to the conclusion that mAb Pb-19 is distinct from above mentioned mAb. It would be interesting to test Pb-19 mAb for their reactivity in transfection studies to various cDNAs' encoded products.

Although mAb to both Ly-6.A and Ly-6.C can induce T-cell activation (Malek *et al.*, 1986; Dumont, 1986; Yeh *et al.*, 1987), the physiological ligand for these cell-surface antigens remains unknown. The results of this study suggest that the ability of activated T cells to clonally expand in response to stimulation by either a mitogen or an antigen is inhibited by mAb Pb-19. Similar functional activities of an anti-Ly-6.E.1 mAb, HD42, were described previously (Flood *et al.*, 1985). Thus, the Ly-6 antigens may represent an important set of cell-surface molecules involved in the control of signal transduction across the plasma molecule.

In the present study it was attempted to establish a correlation between a given gene and its products via characterization of a given mAb that is produced to the gene products. The approach used in the present study should be useful to further elucidate the complexities of the Ly-6 alloantigen system.

ACKNOWLEDGMENTS

We are grateful to Dr Roger Palfree, Sloan-Kettering Centre, New York for providing us with various anti-Ly-6 mAb. We thank Joelle Dewulf, Anne-Marie Schacht and Nadine Duponchel for their technical assistance and are grateful to Dr Jean-Pierre Kusnierz for his help in FACS analysis. Work concerning cloning of cDNA was initiated while A. Haque was spending his sabbatical at Dr Al Bothwell's lab at Yale University, New Haven, CT.

This work was supported by CNRS (624) and INSERM (U167).

REFERENCES

BINÉTRUY B., MENEGUZZI G., CUZIN C. & CUZIN F. (1987) Papillomaviruses. In: *Cancer Cells* (eds B. M. Steinberg, J. L. Brandsma and

L. B. Taichman), Vol. 5, p. 223. Cold Spring Harbor Laboratory, Cold Spring Harbor.

CHIRGWIN J.M., PRZYBYLA A.E., MACDONALD R.J. & RUTTER W.J. (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry*, **18,** 5294.

COLLINS W.E., ANDERS R.F., PAPPAIOANOU M., CAMPBELL G.H., BROWN G.V., KEMP D.J. *et al.* (1986) Immunization of Aotus monkeys with recombinant proteins of an erythrocyte surface antigen of *Plasmodium falciparum*. *Nature (Lond.)*, **323,** 259.

DELEO A.B., SHIKU H., TAKAHASHI T., JOHN M. & OLD L.J. (1977) Cell surface antigens on chemically-induced sarcomas of the mouse. I. Murine leukemia virus-induced antigens and alloantigens on culture fibroblasts and sarcoma cells. Description of a unique antigen on Balb/c Meth A sarcoma. *J. exp. Med.* **146,** 720.

DUMONT F.J. (1986) Treatment of resting T lymphocytes with interferon γ augments their proliferative response to activation signals delivered through their surface Ly-6 antigen. *Cell. Immunol.* **101,** 625.

FLOOD P.M., MURPHY D., HOROWITZ M., LECLAIR K.P., SMITH F., STOCKERT E., PALLADINO M.A. & DELEO A.B. (1985) A monoclonal antibody that recognizes an Ly-6-linked antigen inhibits the generation of functionally active T cell subsets. *J. Immunol.* **135,** 63.

GALFRE G., HOWE S.C., MILSTEIN C., BUTCHER G.W. & HOWARD J.C. (1977) Antibodies to major histocompatibility antigens produced by hybrid cell lines. *Nature (Lond.)*, **266,** 550.

GRISONI M., MENEGUZZI G., DE LAPEYSIERE O., BINÉTRUY B., RASSOULZADEGAN M. & CUZIN F. (1984) The transformed phenotype in culture and tumorigenicity of Fischer rat fibroblast (FR3T3) transformed with bovine papillomavirus type 1. *Virology*, **135,** 406.

GUBLER U. & HOFFMAN B.J. (1983) A simple and very efficient method for generating cDNA libraries. *Gene*, **25,** 263.

HOGARTH P.M., HOULDEN B.A., LATHAM S.E., SUTTON, V.R. & MCKENZIE I.F.C. (1984) Definition of new alloantigens encoded by genes in the Ly-6 complex. *Immunogenetics*, **20,** 57.

HOULDEN B.A., HOGARTH P.M. & MCKENZIE I.F.C. (1986) Interrelationships of the Ly-6 complex regions. *Immunogenetics*, **23,** 226.

HUYNH T., YOUNG R.A. & DAVIS R.W. (1985) Constructing and screening cDNA libraries in gt10 and gt11. In: *DNA Cloning. A Practical Approach* (ed. D.M. Glover), Vol. 1, pp. 49–78. IRL Press, Oxford.

KIMURA S., TADA N., LIU-LAM Y. & HAMMERLING, U. (1984) Studies of the mouse Ly-6 alloantigenic system. II. Complexities of the Ly-6 region. *Immunogenetics*, **20,** 47.

KORTY P.E., COHEN D.I. & SHEVACH E.M. (1988) A single cDNA encodes multiple Ly-6 antigenic specificities. *Eur. J. Immunol.* **18,** 1631.

LAEMMLI U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)*, **227,** 680.

LECLAIR K.P., PALFREE R.G.E., FLOOD P.M., HAMMERLING U. & BOTHWELL A. (1986) Isolation of a murine Ly-6 cDNA reveals a new multigene family. *EMBO J.* **5,** 3227.

LU L., PALLADINO M.A. & DELEO A.B. (1989) The immunotherapeutic effects in tumor-bearing mice of Ly-6 monoclonal antibodies. *J. Immunol.* **142,** 719.

MCKENZIE I.F.C., CHERRY M. & SNELL G.D. (1977) Ly-6.2: a new lymphocyte specificity of peripheral T cells. *Immunogenetics*, **5,** 25.

MALEK T.R., ORTEGA G., CHAN C., KROCZEK R.A. & SHEVACH E.M. (1986) Role of Ly-6 in lymphocyte activation. II. Induction of T cell activation by monoclonal anti-Ly-6 antibodies. *J. exp. Med.* **164,** 709.

MATOSSIAN-ROGERS A., ROGERS P., LEDBETTER J.A. & HERZENBERG L.A. (1982) Molecular weight determination of two genetically linked cell surface murine antigens: ThB and Ly-6. *Immunogenetics*, **15,** 591.

DES MOUTIS I., OUAISSI A., GRZYCH J.M., YARZABAL L., HAQUE A. & CAPRON A. *Onchocerca volvulus*: detectlon of circulating antigen by monoclonal antibodies in human onchocerciasis. *Am. J. Trop. Med. Hyg.* **32,** 533.

ORTEGA G., KORTY P.E., SHEVACH E.M. & MALEK T.R. (1986) Role of Ly-6 in lymphocyte activation. I. Characterization of a monoclonal antibody to nonpolymorphic Ly-6 specificity. *J. Immunol.* **137,** 3240.

PALFREE R.G.E., DUMONT F.J. & HAMMERLING U. (1986) Ly-6A.2 and Ly-6E.1 molecules are antithetical and identical to MALA-1. *Immunogenetics*, **23,** 197.

PALFREE R.G.E., LECLAIR K.P., BOTHWELL A. & HAMMERLING U. (1987) cDNA characterization of an Ly-6.2 gene expressed in BW5147 tumor cells. *Immunogenetics*, **26,** 389.

PALFREE R.G.E., SIRLIN S., DUMONT F.J. & HAMMERLING U. (1988) N-terminal and cDNA characterization of murine lymphocyte antigen Ly-6C.2. *J. Immunol.* **140,** 305.

SEIF R. & CUZIN F. (1977) Temperature-sensitive growth regulation in one type of transformed rat cells induced by the tsa mutant of Polyoma virus. *J. Virol.* **24,** 721.

SUTTON V.R., MIKELSON C.A., TOBIAS G.H., HOGARTH P.M. & MCKENZIE I.F.C. (1985) Identification of two forms of the Ly-6.2 antigen showing differential expression. *Immunogenetics*, **21,** 539.

WATSON C.J. & JACKSON J.F. (1985) An alternative procedure for the synthesis of double-stranded cDNA for cloning in phage and plasmid vectors. In: *DNA Cloning. A Practical Approach* (ed. D. M. Glover), Vol. 1, pp. 79–88. IRL Press, Oxford.

YEH E.T.H., REISER H., DALEY J. & ROCK K.L. (1987) Stimulation of T cells via the TAP molecule, a member in a family of activating proteins encoded in the Ly-6 locus. *J. Immunol.* **138,** 91.