A Search for Type-C Virus Expression in Man

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Introduction

Two immunological approaches can be used to search for type-C virus expression in man. Firstly, viral proteins present on intact cell membranes can be detected by using specific anti viral sera in cytotoxic or immunofluorescence assays. Secondly, an immune response to the viral antigens can be detected by looking for antibody specific for type C viral proteins. Both types of studies have yielded positive and negative reports.

Leukemic lymphocytes (Metzgar et al., 1976), a human fibrosarcoma cell line in early passage (Smith et al., 1977) and kidney sections from systemic lupus erythematosus (SLE) patients (Panem et al., 1976; Mellors and Mellors, 1976) are reported to possess antigens cross reactive with either SiSV or BaEV/RD114.

Assays for human antibodies against whole or detergent disrupted primate viral particles such as SiSV, GALV or BaEV, have yielded positive results (Snyder et al., 1976; Aoki et al., 1976; Kurth et al., 1977); whereas, other studies using purified proteins such as SiSV p30 and gp70 (Stephenson and Aaronson, 1976), NZBp30 (Charman et al., 1975), Mason Pfizer monkey virus p30 (Charman et al., 1977) or FeLV gp70 and p30 (Krakower and Aaronson, 1978) have produced completely negative results.

We have surveyed fresh peripheral blood lymphocytes from normal persons and a selection of leukemic patients using a panel of anti type C sera including high titer rabbit antisera raised against the SiSV component of HL23 V-1 (Teich et al., 1975) and BaEV.

We have also used a sensitive cell monolayer radioimmunoassay to look for differential binding of sera from normal individuals to pairs of virus infected and uninfected cell lines (Hogg, 1976). The major advantages of this latter approach are that viral antigens may be presented on cell membranes in a manner analogous to the way they are presented "in vivo" and that sera can easily be assayed for binding affinities on many types of virus infected/uninfected cells.

Materials and Methods

Anti Type C Viral Sera

Rabbit antisera specific for the SiSV component of HL23 V-1 and BaEV were raised by inoculating rabbits intramuscularly with sucrose gradient

banded virus (1 mg × 6 months). Other sera were obtained from sources listed in Table 1. Each antiserum was titrated by immunofluorescence on the appropriate virus infected and uninfected cell lines and was adsorbed with the uninfected cell line until the uninfected cell was fluorescence negative. This usually required 2 to 4 adsorptions at a ratio of one part cells to four parts serum.

Table 1. Leukemia screening sera (fluorescence)

	Adsorbed with
Rab anti-BEV (Pepsin)	NC37, 8155
Rab anti-HL23-SSV (Pepsin)	KNRK
NRS (Pepsin)	NC37, 8155
	KNRK
Rab anti-disrupted Mol-MuSV/MuLV	TO EF
Goat anti-Rauscher p30 (NCI 2S-658)	MEF
Goat anti-FeLV (Jarrett)	FEA
Goat anti-SSAV (NCI 5S-295)	NC37, Tonsil
NGS	Appropriate cells

Immunofluorescence Assay

Antisera were used at dilutions of 1:20 to 1:80. $(Fab^1)_2$ fragments of fluorescent goat anti-rabbit immunoglobulin (f-GaRIg) were obtained from Robert Sutherland (I.C.R.F.) and used at a dilution of 1:20. Fluorescent rabbit anti-sheep immunoglobulin (Miles-Yeda Ltd.) which was cross reactive with goat immunoglobulin was also used at a dilution of 1:20.

Cell Monolayer Radio Immunoassay

These assays were done as described by Hogg (1976). The cell monolayers were fixed for 10 minutes with 0.25% glutaraldehyde in phosphate buffered saline (PBS), then washed with 2% foetal calf serum (FCS) in PBS before use. This fixing procedure preserves the antigenicity of Moloney-MuLV gp70 and p30 (N. Hogg, unpublished).

Human Peripheral Blood Lymphocytes (PBL) and Sera

Blood samples were obtained from laboratory workers and from leukemic patients at St. Bartholemew's Hospital, London. Upon arrival the PBL were prepared by Ficoll-Hypaque density gradient centrifugation and used either immediately or after overnight storage at 4°C in an immunofluorescence assay. Sera were obtained from four selected individuals and stored at −20°C.

Results

Fresh human peripheral blood lymphocytes (PBL) were tested by immunofluorescence for the presence of type C viral proteins using rabbit antisera to HL23 V-1 and BaEV viruses. In most cases, the PBL were also examined with

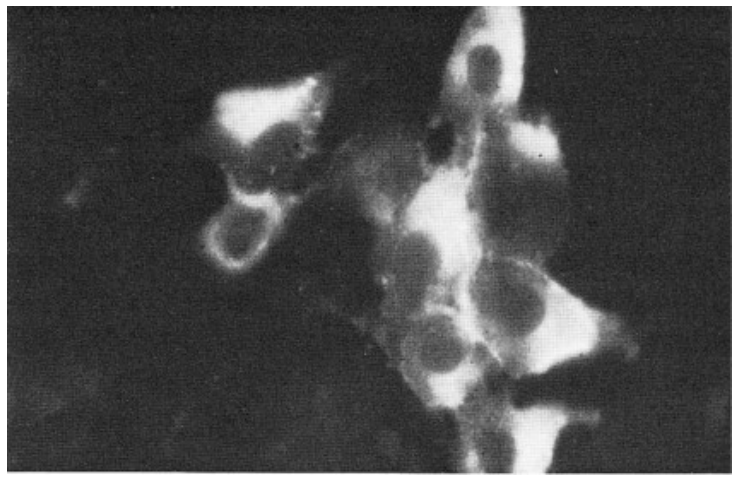

Fig. 1. Reaction of rabbit anti HL23 V-1 (1:40 serum dilution) with KNRK-HL23 V-1 cells (immunofluorescence). The same antiserum was negative when reacted with uninfected KNRK cells

antisera specific for feline leukemia virus (FeLV), subgroup A and B several murine leukemia viruses (MuLV) and other sera shown in Table 1. To date, 30 samples from a variety of leukemic patients (ALL, AMC, CML and others) plus 19 normal controls have been examined. No virus positive cells have been detected. The activity of each antiserum was tested with laboratory cell lines infected with the appropriate virus. For example, the reaction of rabbit anti HL23 V-1 with KNRK cells infected with HL23 V-1 is shown in Fig. 1. Uninfected KNRK cells show no fluorescence with this antiserum.

The second approach has been to look for antibody to type C viral proteins using a sensitive cell monolayer radioimmunoassay to detect differential binding to pairs of virus infected and uninfected cell lines in the sera of normal individuals. One of the selected individuals has been shown to possess anti-primate virus antibodies in a conventional radioimmunoassay (Kurth et al., 1977). A titration of rabbit anti HL23 V-1 and rabbit anti BaEV on appropriate infected and uninfected cell monolayers is shown in Fig. 2, demonstrating that this type of assay is as sensitive as a conventional radioimmunoassay. Antibody binding to BaEV, HL23 V-1, FeLV (A and B) and AKR MuLV infected cell lines was assessed. There were dramatic differences in antibody titers between individuals (1:10 to $>$ 1:270). However, the titers were similar on both infected and uninfected cell lines (Figs. 3.1 and 3.2). Adsorption of sera with uninfected cells (KNRK and 8155) reduced antibody titers to virus infected cells to background levels (Figs. 3.1 and 3.2). If the sera were titrated in the presence of increasing quantities of FCS (5, 10, 25, 50 percentage) the titers also dropped to background levels (Fig. 3.3).

Discussion

PBL samples from 30 leukemic patients and 19 healthy persons were examined by immunofluorescence for antigens related to HL23 V-1, BaEV and other type C viruses and *no* positive cells were detected. In another study, PBL from 20 SLE patients were also negative when examined with the same panel of sera (N. Hogg and N. Zvaifler, unpublished). Therefore, either viral antigens were not present in sufficient quantity to be detected or our antisera were not specific for the relevant antigens.

Human sera were tested for differential binding to pairs of virus infected and uninfected cell monolayers. Although antibody titers varied dramatically between individuals, each serum had an equivalent titer on both types of cells and this binding could be eliminated by adsorption of the serum with uninfected cells or by doing the assay in 50% FCS. Therefore, the only type of antibody that we are at present able to detect has specificity for a FCS component which is adsorbed to tissue culture cells similar to that described by Irie et al. (1974) and Snyder and Fox (1978).

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