Studies of Human Serological Reactivity with Type C Virus and Viral Proteins

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Introduction

The persistent controversy surrounding possible association of type C viruses with human malignancies is the result of a number of not always compatible observations in many laboratories. Thus, while a number of reports of virus-like particles (occasionally with associated infectivity), reverse transcriptase, virus-like RNA, viral proteins, and proviral DNA sequences in human tissues exist [1], the results must be weighed in relation to negative findings by other investigators seeking similar evidence [2].

Our approach to the question of human exposure to type C RNA viruses has been to investigate human sera for naturally occurring antibodies to such agents [3]. Others have followed a similar tactic [4,5]. It is generally accepted that, even if humans are naturally infected with a type C virus, overt virus replication, such as occurs in cats or gibbon apes, is a rare event, if it occurs at all. With this in mind, we have employed a very sensitive radioimmunoprecipitation (RIP) assay in a search for type-C virus-reactive human antibodies, which we assumed might only be detectable in relatively low titers.

We observed that many human sera are in fact reactive with surface antigens of a particular group of type C viruses and capable of precipitating intact radiolabeled virions [3]. By this assay approximately 75% of normal human sera precipitate significant amounts of purified leukemia viruses of woolly monkey (SSV-SSAV), murine (Rauscher-MuLV), and cat (FeLV) origin; in contrast, tests on endogenous type C viruses of the cat (RD114) and baboon (BEV), on an avian leukosis virus (RAV-2) and on two enveloped RNA-containing non-type C viruses (VSV and Sindbis virus) revealed no appreciable titers (for representative data see Fig. 1). Demonstration of activity in the RIP assay with purified IgG and $F(ab')_2$ fragments of IgG indicated that binding of virus is mediated by antibody. The observed reactivity cannot be attributed to the presence of any known heterophil antibody specificity or to the presence of antigens specific to tissue culture cells [3].

Evaluation of Evidence for Viral Specificity in the RIP Assay

The restricted reactivity of the human antibodies for a limited group of type C viruses (Fig. 1) was strongly suggestive of some viral specificity. Comparative RIP titers for a number of human sera with the above viruses have shown that in general, sera react with SSV-SSAV, R-MuLV, and FeLV to roughly

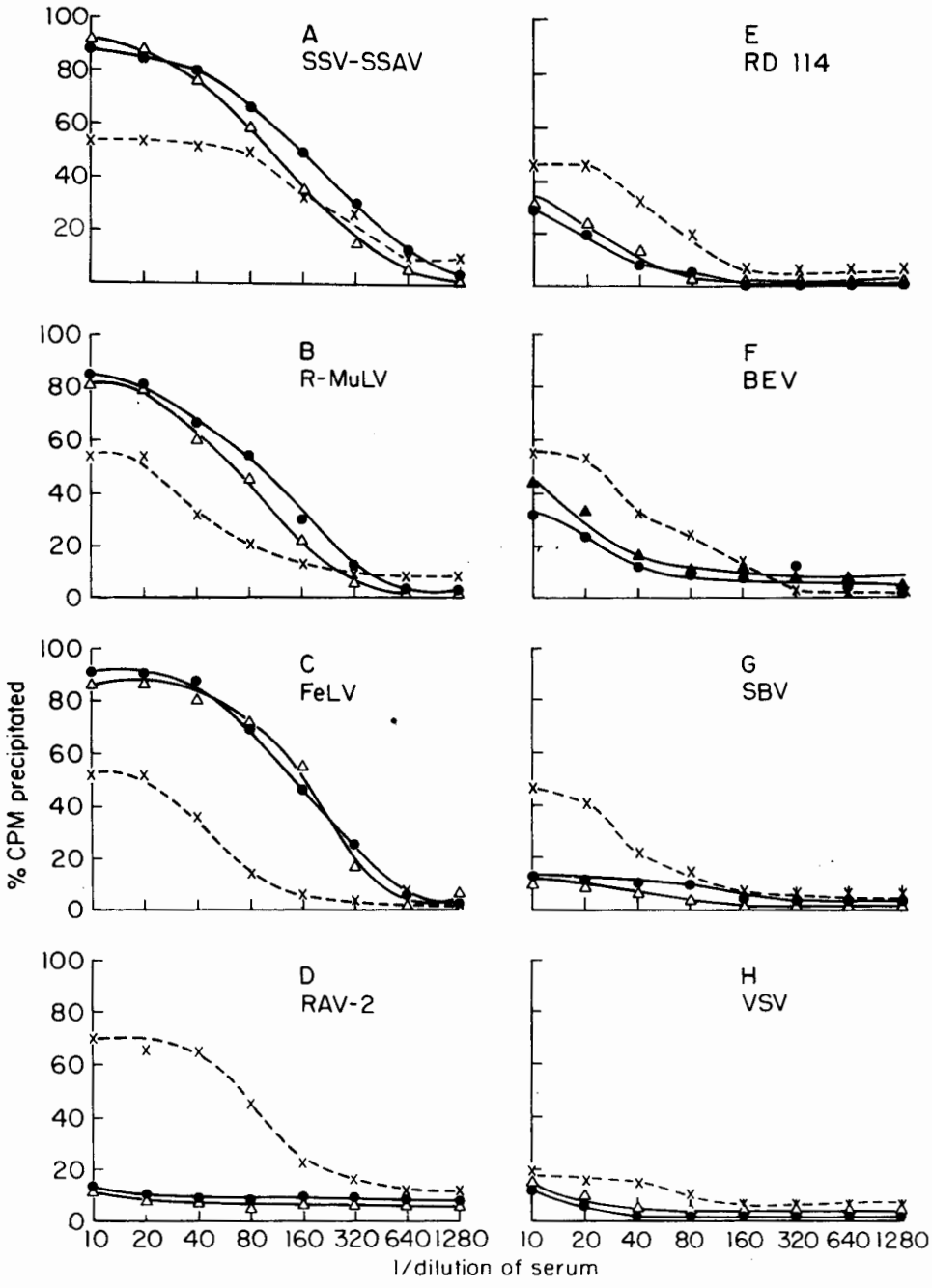

Fig. 1. RIP titrations of two normal human sera (●, △) and rabbit anti-FCS serum (x) with 1 μg intact radiolabelled virus: (A) ^{3}H-amino acid labelled SSV-SSAV, 4000 cpm, (B) ^{3}H-amino acid labelled R-MuLV, 3200 cpm, (C) ^{3}H-leucine labelled FeLV-AB, 4100 cpm, (D) ^{3}H-amino acid labelled RAV-2, 8000 cpm, (E) ^{3}H-amino acid labelled RD114, 2000 cpm, (F) ^{3}H-leucine labelled BEV, 3200 cpm, (G) ^{3}H-leucine labelled SBV, 3500 cpm, (H) ^{3}H-leucine labelled VSV, 3700 cpm. Titrations were performed as described in Snyder et al. [3]

similar titers. The similarity holds whether titers are high or low. The relationship between the reactive antigens in these viruses was studied in quantitative absorption experiments in which increasing amounts of unlabelled virus were tested for the ability to compete with 1 μg of labelled SSV-SSAV (left panel, Fig. 2) and unlabelled R-MuLV (right panel) for binding to antibody in a limiting dilution of a human serum. Unlabelled SSV-SSAV, R-MuLV, and FeLV competed completely in both assays, whereas RD114 competed

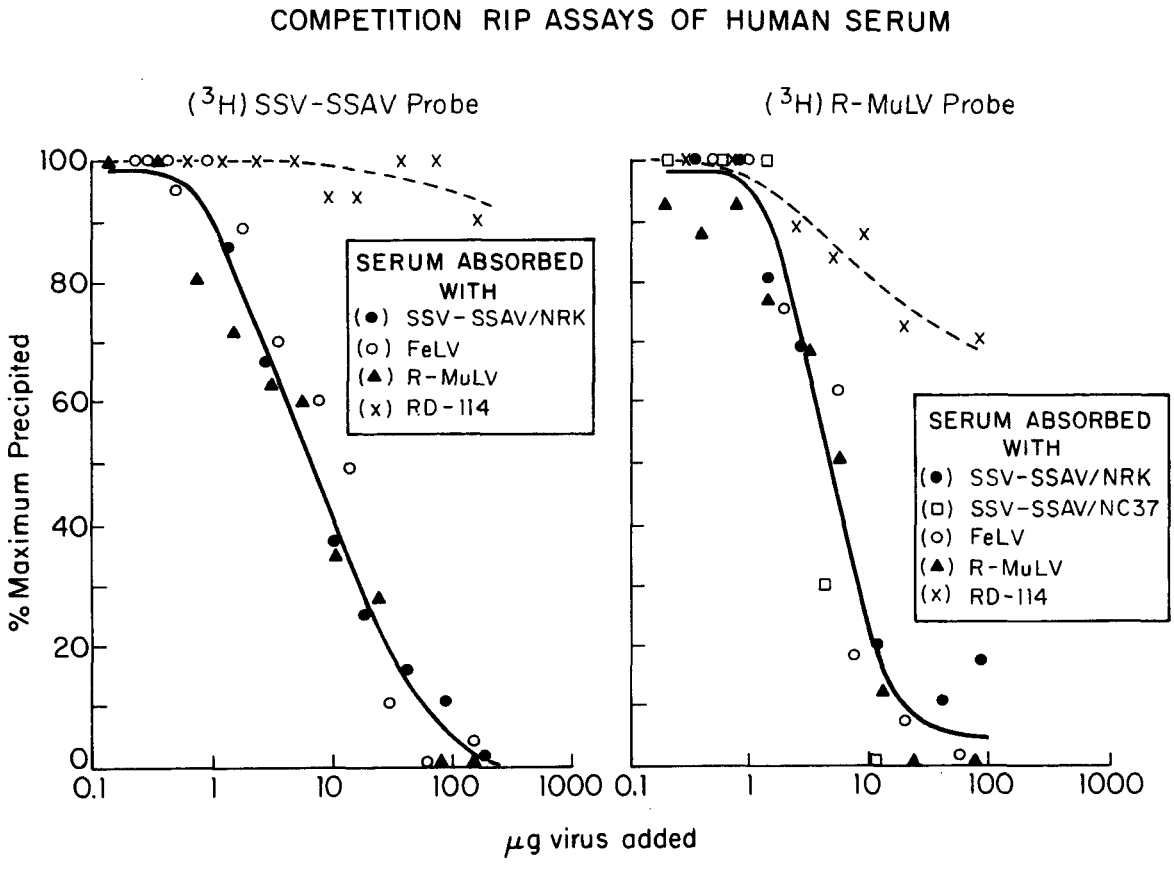

Fig. 2. Competition RIP assay of antibodies in a limiting dilution of a normal human serum for binding 1 μg ^{3}H-amino acid labelled SSV-SSAV (left panel) or R-MuLV (right panel) while in the presence of the amounts of unlabelled virus preparations indicated on the abscissas

only slightly and then only at the highest concentrations tested. The data suggest that the human antibodies being measured are directed against determinants which resemble, however distantly, the broadly cross-reactive viral envelope antigens of the exogenously infectious type C viruses SSV-SSAV, R-MuLV, and FeLV rather than antigens of endogenous viruses belonging to the RD114/BEV class.

Additional evidence of viral specificity was provided by the demonstration that a fraction of SSV-SSAV containing predominantly viral gp70 completely absorbed human serum activity in a RIP assay while fractions containing other viral structural proteins did not [3]. It should be noted also that the competition experiments of Fig. 2 are consistent with the known antigenicities of the respective gp70s [6]. Recently we purified SSV-SSAV which had been radiolabeled in tissue culture to high specific activity with ^{3}H-glucosamine (10^5 cpm/μg), lysed the virus with NP40 and centrifuged it at 100000 g for 1 hr. Labeled viral gp70 in the supernatant served as a probe for analysis of human sera for antibodies. A panel of human sera were analyzed for 1. RIP reactivity against intact SSV-SSAV and 2. precipitating activity against

the glucosamine-labeled gp70 probe. The sera tested were obtained from Dr. R. Kurth (Tübingen) and contained some samples capable of precipitating a purified SSV-SSAV gp70 probe (Kurth, personal communication). While none of these sera reacted with our gp70 probe obtained by gel filtration in 6M guanidine-HCl [3], our new results show a strong correlation between the ability to precipitate intact virus and the ability to precipitate detergent solubilized gp70 (Fig. 3). Partial purification of the gp70 from this supernatant by phosphocellulose chromatography (0,1–0,2M salt elution) did not result in any loss of the antigen detected by the human sera. Further purification is still required to establish unequivocally that viral gp70 is involved in these reactions. Interestingly (and not unexpectedly) observed titers with the intact virus RIP assay were higher than could be observed by using a solubilized gp70 probe, suggesting that the former system can be more sensitive (Fig. 3).

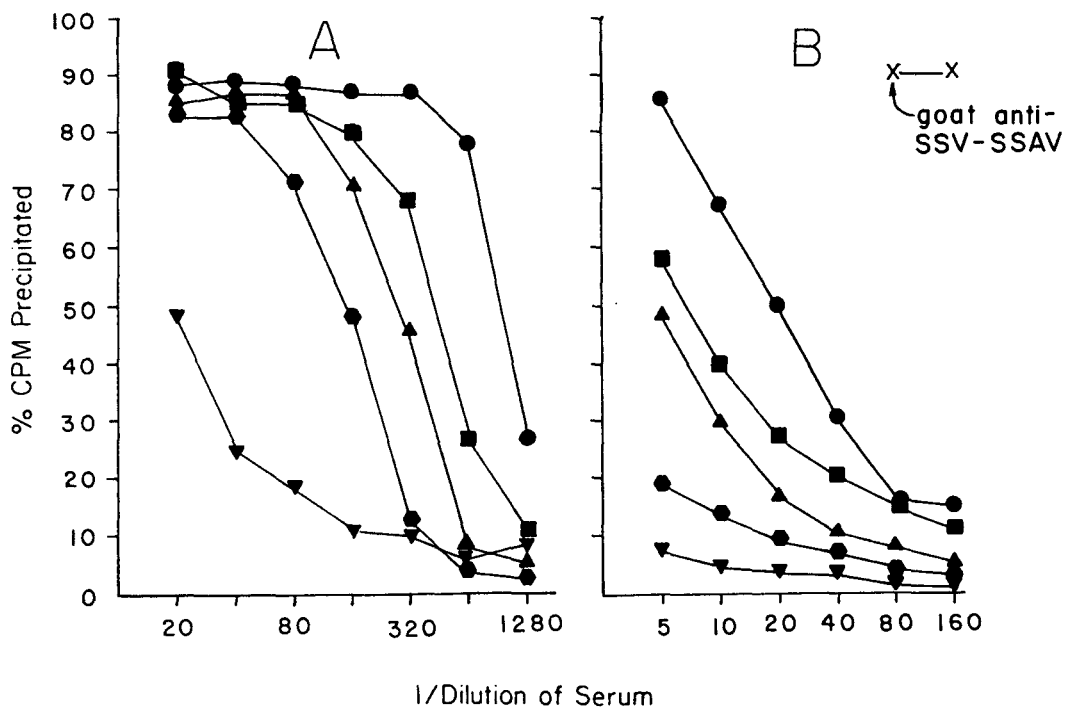

Fig. 3. Left: RIP titrations of 5 normal human sera with 1 μg intact radiolabeled SSV-SSAV. Right: Radioimmunoassay titration of the same sera with a solubilized ^{3}H-glucosamine-labeled gp70 probe from 50 ng SSV-SSAV (approximately 5 ng gp70)

Evidence for a Limited Role of Fetal Calf Serum (FCS) Components in the RIP Reaction

RIP titration experiments with a rabbit anti-FCS serum indicated the presence of FCS components in a subfraction of our virus preparations (Fig. 1). Human serological reactivity with SSV-SSAV was shown to be diminished to a limited extent in quantitative absorption tests with FCS proteins. However

human sera quantitatively absorbed with FCS proteins retained RIP activity with a reduction in titer only averaging approximately 50%. By either 1. alcohol precipitation, followed by concanavalin A chromatography and Sephadex G-150 filtration of FCS protein or 2. chromatography of serum proteins over a human IgG-containing immunoaffinity column, a glycoprotein of approximately 55000 MW has been identified which is a minor constituent of FCS (<0,1% of total protein) [7]. This serum component is thus reactive with a particular class of human antibody, distinguishable by absorption tests from the class of antibody which reacts with type-C virions [3].

Seroepidemiology

In preliminary seroepidemiological studies we have observed that the RIP reactivity in normal sera varies significantly as a function of age: we find that cord sera are barely reactive, maximum titers are reached between 5–10 years of age, a new minimum is reached at about 20 years of age, and there is a slow rise in titer thereafter. In experiments involving small numbers of individuals the sera of patients with neoplastic or autoimmune disease, have, in general, shown no marked differences in titer compared with sera from normal individuals. Studies of natural antibodies to type C viruses, especially in inbred mice, have shown that a complex group of genetic factors can affect immune responses to any particular virus class. Thus further investigations including family studies are planned to identify the basis of human serum reactivity with type-C viruses.

References

1. Gallo, R.C., Saxinger, W.C., Gallagher, R.E., Gillespie, D.H., Ruscetti, F., Reitz, M.S., Aulakh, G.S., Wong-Staal, F.: Some ideas on the origin of leukemia in man and recent evidence for the presence of acquired and endogenous type-C viral related sequences. In: Origins of Human Cancer. Hiatt, H.H., Watson, J.D., Winston, J.A. (eds.), pp. 1253–1286. Cold Spring Harbor: Cold Spring Harbor Laboratory 1977
2. Gardner, M.B., Rasheed, S., Shimizu, S., Rongey, R.W., Henderson, B.E., McAllister, R.M., Klement, V., Charman, H.P., Gilden, R.V., Heberling, R.L., Huebner, R.J.: Search for RNA tumor virus in humans. In: Origins of Human Cancer. Hiatt, H.H., Watson, J.D., Winsten, J.A. (eds.), pp. 1235–1251. Cold Spring Harbor: Cold Spring Harbor Laboratory 1977
3. Snyder, H.W., Jr., Pincus, T., Fleissner, E.: Specificities of human immunoglobulins reactive with antigens in preparations of several mammalian Type-C viruses. Virology **75**, 60–73 (1976)
4. Aoki, T., Walling, M.J., Bushar, G.S., Liu, M., Hsu, K.C.: Natural antibodies in sera from healthy humans to antigens on surfaces of type C RNA viruses and cells from primates. Proc. Natl. Acad. Sci. USA **73**, 2491–2495 (1976)
5. Kurth, R., Teich, N.M., Weiss, R., Oliver, R.T.D.: Natural human antibodies reactive with primate type-C viral antigens. Proc. Natl. Acad. Sci. USA **74**, 1237–1241 (1977)
6. Hino, S., Stephenson, J.R., Aaronson, S.A.: Antigenic determinants of the 70,000 molecular weight glycoprotein of woolly monkey type C RNA virus. J. Immunol. **115**, 922–927 (1975)
7. Snyder, H.W., Jr., Fox, M.: Characterization of a fetal calf serum-derived molecule reactive with human natural antibodies: its occurrence in tissue culture-grown type C RNA viruses. J. Immunol. **120**, 646–651 (1978)