Recognition of Simian Sarcoma Virus Antigen by Human Sera

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Abbreviations

RIA: Radioimmunoassay; IEM: Immuno-electron microscopy; SSV: Simian Sarcoma Virus; SSAV: Simian Sarcoma Associated Virus; gp70 SSV (SSAV): The 70000 d virus envelope glycoprotein of SSV (SSAV); NHS: Normal human serum

A. Introductory Remarks

Previous work has indicated the presence of antibodies in human sera, capable of reacting with the envelope antigens of murine and primate C-type viruses [1–5]. These antibodies are present in the majority of all adult sera tested to date, but are absent in cord blood. These, plus related observations (discussed in [3,6]) are compatible with the idea, that horizontally transmissable viruses, which are immunologically related to, but not identical with the currently known C-type tumor viruses, may have elicited the anti-viral antibodies.

Because no human type C tumor virus strains have so far been isolated, it is imperative that the precise nature of the anti-viral immune response is defined and documented beyond any doubt. This is of considerable importance, as two laboratories have been unable to detect anti-tumor virus antibodies in man [7,8]. We have therefore tried to think of ways which, in addition to the sensitive RIAs previously used by us, would indicate the presence of specific anti-viral antibodies in man.

In this communication, we present evidence that the anti-viral human antibodies belong to the immunoglobulin G class, and that they bind with differing affinities to the SSV (SSAV) gp70, the prime target antigen used for the human antibodies. Furthermore, evidence is presented showing that a recently developed immuno-electron microscopical method (IEM) seems suitable to confirm previous positive data, and that sero-epidemiological investigations may enable us to easily detect sera with anti-viral antibody titers.

B. Experimental Approaches

Virus Antigens

Simian sarcoma virus (Simian sarcoma associated virus) SSV (SSAV) is a mixture of two C-type primate tumor viruses first isolated from a woolly monkey fibrosarcoma in 1971. The major envelope glycoprotein gp70 of SSV (SSAV) was purified [9] and kindly made available by Drs. H.-J. Thiel and W. Schäfer, Max-Planck-Institut für Virusforschung, Tübingen.

Sera

The human sera employed in this study originated either from blood donors from the Tübingen area or were obtained through the international services of Behringwerke, Marburg. IgG and IgM were purified according to published procedures. Anti-human IgG and IgM antisera were prepared in goats.

Hyperimmune goat anti-SSV (SSAV) or anti-gp70 SSV (SSAV) control sera were obtained from Dr. F. Deinhardt, Pettenkofer-Institut, Universität München, and from Dr. J. Gruber, Office of Logistics and Resources, National Cancer Institute, Bethesda, respectively.

Radioimmunoassays

A detailed description of our indirect radioimmunoassay procedure has been published previously [3,6]. Anti-immunoglobulin antisera were used to cross-link and sediment antigen-antibody complexes.

Antibody Affinity Determinations

The relative affinity (K_R, litres/mole) were measured by a double antibody precipitation method, as previously described [10], using 10 µl of serum. Briefly, the assay consisted of the determination of free and antibody-bound antigens at equilibrium over a range of antigen concentrations from 2.5 to 20 ng. Total antibody-binding site concentration was obtained by extrapolation to infinite free antigen concentration of a Langmuir plot of the reciprocal of the bound antigen versus the reciprocal of the free antigen.

$$\frac{1}{b} = \frac{1}{k} \cdot \frac{1}{c} \cdot \frac{1}{Abt} + \frac{1}{Abt}$$

where b = bound antigen, c = free antigen, k = affinity and Abt = total antibody binding sites.

Antibody affinity was calculated from this curvilinear plot as the reciprocal of the free antigen concentration when half the antibody-binding sites were bound to antigen.

Immuno-peroxidase (I-POD) Labeling

Besides conventional conjugates of POD and anti-IgG antibody [11], a newly developed indirect labeling technique using protein A-POD was applied in

evaluating the immunoreactivity of human and control animal sera. This conjugate was prepared using glutaraldehyde (GA) in a two-step procedure involving GA-activation of the peroxidase (Boehringer, Mannheim) in the first step, removal of unreacted GA by gel filtration and reaction of the activated POD with protein A (Pharmacia, Uppsala).

A detailed characterization of this conjugate and its potential use in comparison with other types of I-POD and immunoferritin conjugates will be presented elsewhere (H.G. and W.V., manuscript in preparation).

Normal rat kidney cells infected by SSV (SSAV) (NRK-SSV (SSAV) cells) were grown for 24 to 48 hrs in Microtest No. 1 plates (Falcon Plastics, Oxnard, Calif.) and processed for immuno-labeling after GA fixation as published previously [12]. Briefly, human as well as hyperimmune and negative animal control sera where used undiluted, except for the goat anti-SSV (SSAV) which was applied at a 1:20 dilution. These primary sera as well as the anti-immunoglobulin G POD-conjugates were each incubated for 30′ at 37°C with the NRK-SSV (SSAV) cells. The catalytic activity of the bound enzyme was localized according to Graham and Karnovsky [13] using diamino-benzidine and H_2O_2 (Merck, Darmstadt). Following post-fixation in 1% OsO_4 the cultures were dehydrated in ethanol, embedded in situ in Epon and processed as published previously [12].

C. Demonstration of Anti-Viral Human Antibodies

Previous experiments [3] had indicated that the predominant anti-viral antibody activity in exposed animals is directed against so-called "interspecies-specific" determinants on the envelope glycoprotein antigens (gp70s) of the mammalian C-type viruses. These interspecies-specific determinants are shared by viruses originating from different natural host species. A typical titration curve of normal human sera with the gp70 of SSV (SSAV) is shown in Fig. 1. It can be seen that even the titers of sera which react well (e.g. NHS No. 29 and No. 30), are relatively low compared to titers observed after infection with classical horizontally infectious viruses, e.g. rhino- or influenza-viruses. NHS No. 41 (▲ in Fig. 1) is unusual in that it possesses the highest titer by far, and recognizes more than the interspecies-specific determinants on gp70 SSV (SSAV). Since this serum is from a laboratory worker who used to work with SSV (SSAV) we have reason to suspect that the relatively high titer is the result of laboratory exposure to this virus.

In Table 1, the precipitation of gp70 SSV (SSAV) by whole sera is compared to that of immunoglobulins isolated from the sera. The precipitation by isolated immunoglobulin G can account for the entire anti-viral serum reactivity. Only in four out of over one hundred sera, could a slight anti-viral IgM reactivity be observed (R.K. and C. Schmitt, unpublished observation). The association of the anti-viral immunity with the immunoglobulin G class is not only confirmed by the use of isolated IgG, but also by the use in the RIAs of goat anti-human IgG antisera which by themselves have no anti-viral or anti-IgM activities.

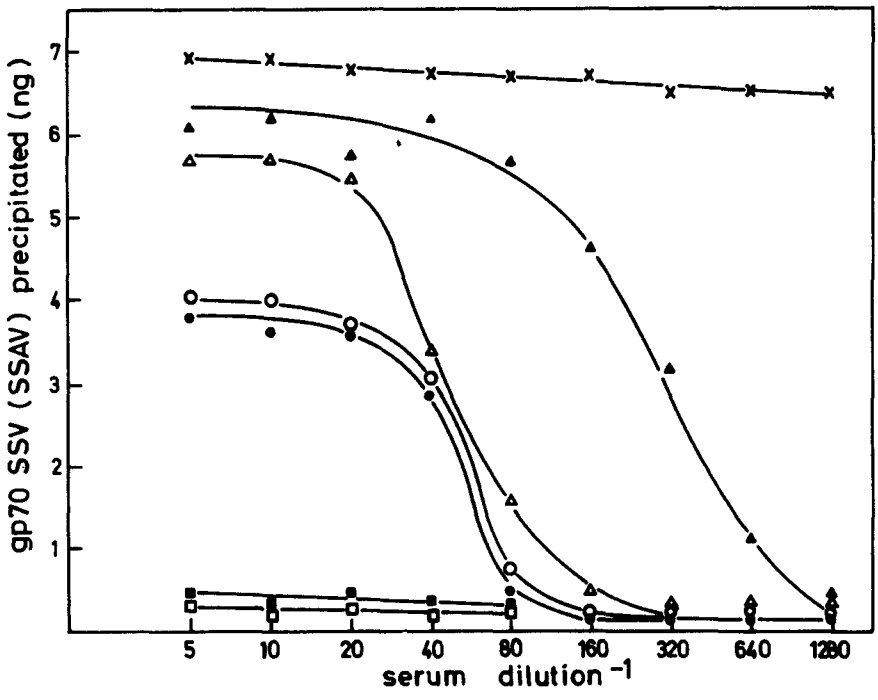

Fig. 1. Titration of human sera with gp70 SSV (SSAV). Goat anti-SSV (SSAV) antiserum: X. Human sera: NHS 41: ▲; NHS 24: △; NHS 29: ○; NHS 30: ●; NHS 63: ■; normal goat serum: □. Input antigen: 7,72 ng gp70 SSV (SSAV)

Preliminary sero-epidemiological investigations yield an interesting pattern of the distribution of positive sera (Table 2). Whereas about 50% of the sera from the Tübingen area react clearly with gp70 SSV (SSAV), the percentage and titers fall drastically when sera from, for example Japan and Egypt are tested. In contrast, sera from South America so far possess unusually high titers.

Table 1. Comparative immunoprecipitation of gp70 SSV (SSAV) by human sera and immunoglobulins

Serum	Serum source	Precipitation by whole serum[a] (ng)	IgG (ng)	IgM (ng)
normal goat serum	goat	<0,5	<0,3	<0,3
goat anti-SSV (SSAV)	goat	4,86	4,42	<0,3
NHS 41	laboratory worker	4,07	3,68	<0,3
NHS 64	laboratory worker	1,10	0,7	<0,3
NHS 24	blood donor	3,22	3,17	<0,3
NHS 29	blood donor	2,66	2,72	<0,3
NHS 30	blood donor	2,22	2,46	<0,3
NHS 63	blood donor	<0,5	<0,3	<0,3

[a] Radioimmunoassay using 10 µl (1:40 final dilution) whole human serum or the equivalent amount of IgG or IgM. Input antigen: 5,15 ng gp70 SSV (SSAV)

Table 2. Immunoprecipitation of gp70 SSV (SSAV) with human sera from different continents

Sera	Serum source	Origin of serum	gp70 SSV (SSAV) precipitated (ng)[a]	number positive[d] sera/ number tested
normal goat serum	goat	Germany	<1,0	
goat anti-SSV (SSAV)	goat	Germany	20,9	
NHS 41	laboratory worker	Germany	17,9[b]	
NHS 42	blood donor	Germany	<1,0[c]	
S1		Japan	1,28	
S2			3,01	
S3			1,35	
S4			1,60	
S5			1,45	
S6			1,01	
S7			1,33	
S8			2,03	
				3/9
S19		Egypt	1,05	
S20			1,30	
S21			0,95	
S22			0,98	
S23			1,76	
S24			1,05	
S25			1,35	
S26			2,48	
S27			1,28	
S28			1,47	
				2/10
S33		South America	5,91	
S34			4,08	
S35			5,07	
S36			11,30	
S37			6,34	
S38			9,57	
S39			3,37	
S40			4,04	
S41			4,20	
S42			6,24	
				10/10

[a] excess gp70 SSV (SSAV) precipitated by 10 µl (1:40 dil) human serum
[b] highest titer normal human serum
[c] negative titer normal human serum
[d] positive sera (10 µl, 1:40 final dilution) precipitate >1,5 ng gp70 SSV (SSAV)

When the affinites of antibodies in sera from blood donors and patients with various diseases were measured, it was found that a proportion reacted as high affinity sera with K_R values between 5×10^8 and 2×10^9 (litres × mole) (Table 3). Scatchard plots of some of the sera listed in Table 3 are shown in Fig. 2.

Table 3. Average antibody affinities of human sera to purified envelope glycoprotein (gp70) of SSV (SSAV)

Serum	Serum source	Average affinity K_R (litres/mole)
normal goat serum	goat	Not calculable
goat anti-SSV (SSAV)	goat	$2{,}4 \times 10^9$
NHS 22	laboratory worker	$1{,}9 \times 10^9$
NHS 24	blood donor	$1{,}0 \times 10^9$
E 5	teratocarcinoma patient	$1{,}3 \times 10^9$
E 33	teratocarcinoma patient	$1{,}0 \times 10^9$
M 102	melanoma patient	$5{,}1 \times 10^8$
L 38	systemic lupus erythematosus patient	$5{,}0 \times 10^8$

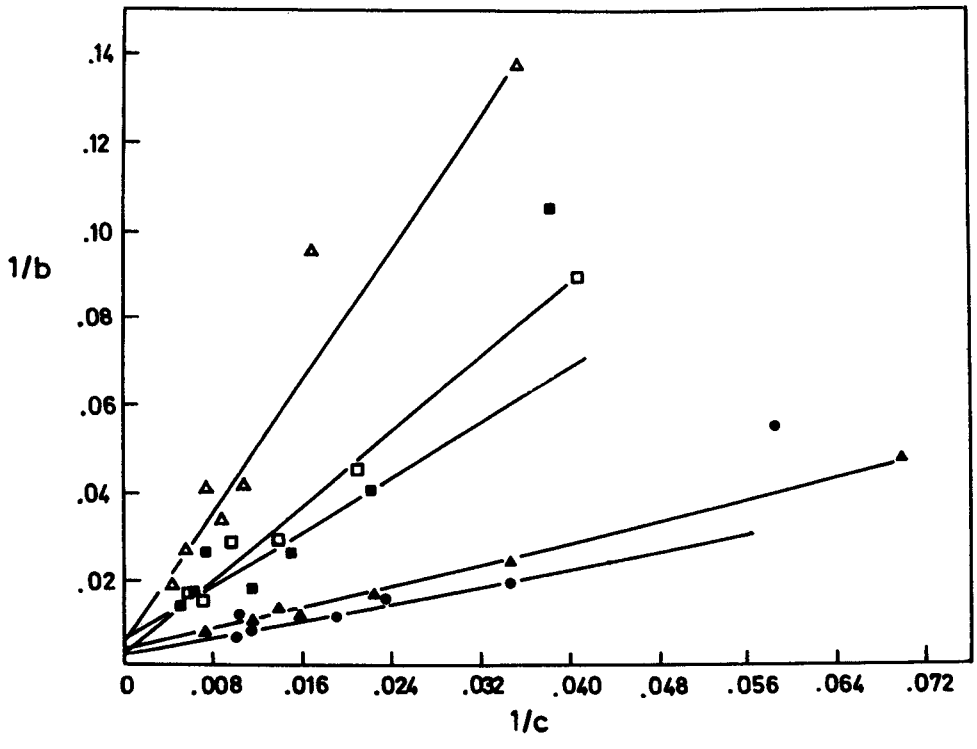

Fig. 2. Relative affinity, (K_R), of anti-gp70 SSV (SSAV) antibody in various human and animal sera. Horizontal axis: 1/c (reciprocal of free antigen), vertical axis: 1/b (reciprocal of bound-antigen). ●: goat anti-SSV (SSAV), 1 : 100 initial dilution. ▲: NHS 41; ■: NHS E5; △: NHS L38; □: NHS M102

In an effort to demonstrate anti-viral immunity by additional techniques, IEM was employed for the detection of anti-viral antibodies. Human sera which reacted well in RIAs also react with the protein A-POD technique (Figs. 3 and 4a). Conversely, RIA-negative human and animal control sera remained negative (Fig. 4b).

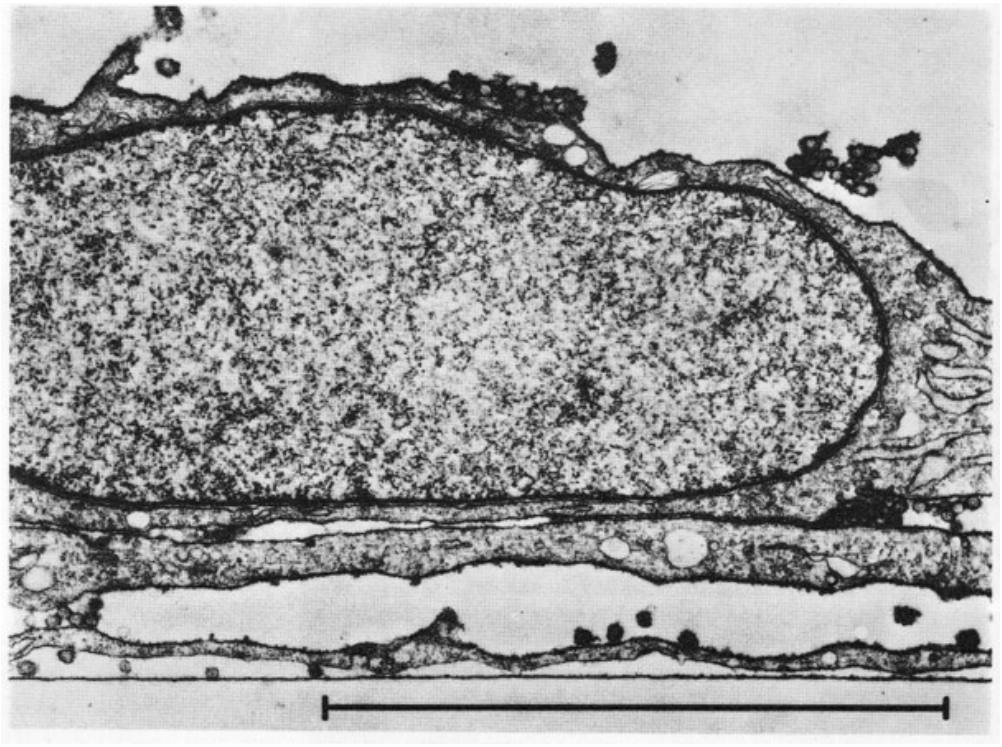

Fig. 3. Low power micrograph showing section of three cells and aggregates of virus particles. Virus and cell surface show electron dense label after incubation with NHS No. 41 and protein A-POD reaction. Bar represents 5 μm; Magnification: × 15000

D. Discussion and Future Prospects

In this communication, additional data are presented which support previous evidence for a widespread human immune reactivity against C-type tumor virus related antigens. The antibodies which react via interspecies-specific determinants with the prototype viral antigen, the gp70 of SSV (SSAV), are here shown to belong to the IgG-class. RIAs, in which purified gp70 SSV (SSAV) was recognized by purified human IgG, and where the cross-linking was achieved with the purified IgG-fraction from goat anti-human IgG (data not shown here) have been performed. IgM reactivity is found only rarely, if at all, and in the few cases where anti-viral IgM activity is observed, the titers are low.

Preliminary sero-epidemiological studies indicate that the percentage and titers of positive human sera may be quite different in different areas of the world. It is obvious, that the limited number of serum samples from overseas (approx. 100), which have been tested so far precludes any premature conclusions. Nevertheless, the differences in activity between sera from e.g. Egypt and South America, are striking. These studies will be extended.

Anti-viral activity has previously been demonstrated for both human antibodies and lymphocytes [5]. Usually, RIAs as well as cytotoxic assays and lymphocyte stimulation techniques have been used. The successful IEM approach with human antibodies made visible by the use of protein A-peroxid-

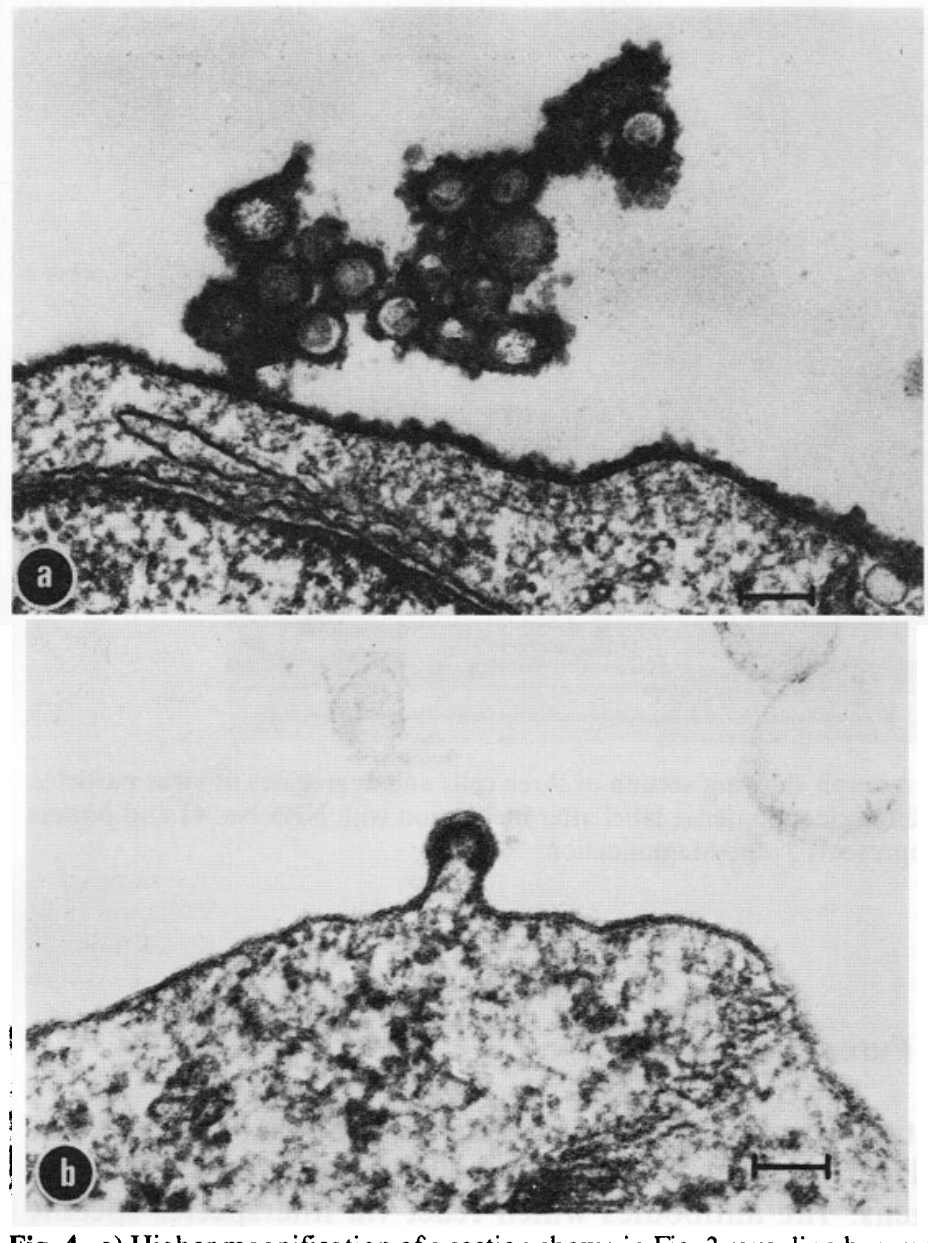

Fig. 4. a) Higher magnification of a section shown in Fig. 3 revealing heavy electron-dense reaction products indicating positive immune reactions. b) Appearance of NRK-SSV (SSAV) cells and budding virion after incubation with normal rabbit serum and protein A-POD reaction. Bar represents 100 nm. Magnification: × 100000

ase conjugates adds another method to the arsenal of refined immunological techniques which detect anti-viral human antibodies. These data confirm earlier results by Aoki and co-workers [1] who used ferritin-tagged antibodies to monitor the absorption of human antibodies to tumor viruses and virus-infected target cells.

One of the strongest pieces of evidence supporting the specificity of the antibody-binding to tumor virus antigens can be derived from the (preliminary in number) affinity data. The high average affinity constants observed with a proportion of human sera indicate that whatever the antigens were that induced the human antibodies, they must have been very similar in antigenicity to the determinants detected on tumor virus envelope antigens.

In closing, however, we would not like to leave the interested but experimentally uninvolved reader with the impression that we are here arguing for the presence of horizontally transmissible C-type tumor viruses which have yet to be identified. Even though this certainly still represents a viable alternative – and there are a number of possibilities why horizontal virus transmission may have been missed so far – other mechanisms are easily conceivable which may have induced an anti-viral immunity in man. Such alternatives have been discussed in some detail previously [6, 14]. Suffice it to say that it cannot be excluded at present that antigens may exist which fortuitously cross-react with tumor virus antigens. To our knowledge, however, no such cross-reacting antigens have yet been demonstrated for any animal C-type tumor virus.

Our present investigations are directed towards elucidating the anti-viral immune status in groups of patients with various diseases, notably tumors. A constant change in immunity in a given group of patients, if observed, would then justify a detailed search for viral foot-prints in the corresponding diseased tissues, e.g. in neoplasms.

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