Studies of Simian Sarcoma and Simian Sarcoma-Associated Virus

I. Analysis of Viral Structural Proteins, and Preparation and Characterization of Antiserum Specific for Viral Envelope Components

F. Deinhardt * and C. Bergholz **

Department of Microbiology, Rush-Presbyterian-St. Luke's Medical Center, Chicago

G. Hunsmann and J. Schneider

Lehrstuhl für Immunologie, Universität Freiburg, Freiburg

H.-J. Thiel, H. Beug, and W. Schäfer

Max-Planck-Institut für Virusforschung, Tübingen

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The structural proteins of simian sarcoma virus type 1 and its associated virus (SSV) were analysed; in general they were similar to the corresponding components of murine leukemia viruses (MuLV), *i. e.* glycoproteins of approximately 69,000 and 71,000 Daltons (gp69/71), proteins corresponding to p15(E) and p12(E) which were identified as envelope, and p31, p15, p12 and p10 proteins identified as internal components. As in MuLV, p12 stained reddish with Coomassie blue but a p15(C) distinct from p15(E) was not clearly identified. Using antisera specific for individual components of MuLV cross-reactions were observed between the proteins p15(E), p12(E) and p31 of SSV and MuLV, and to a minor degree also between their respective major glycoproteins. An antiserum reacting strongly, specifically and almost exclusively with the envelope components of SSV gp69/71, p15(E) and p12(E) was prepared in a goat by inoculation of the animal's own cells, previously transformed *in vitro* with SSV and grown in goat serum. Comparative studies with this antiserum in complement fixation and Ouchterlony tests confirmed the strong antigenic similarities between SSV and gibbon ape lymphoma virus but did not identify any interspecies reactivity.

Introduction

Simian sarcoma and its associated virus (SSV), type C oncornaviruses isolated from a spontaneous sarcoma of a woolly monkey [1, 2], are related closely to gibbon ape lymphoma virus (GALV), an agent which is etiologically associated with naturally occurring lymphoma and leukemia in gibbon apes [3]. Identification by several investigators of SSV-related viral components in human tissue heightened interest in known primate oncornaviruses [4–17], although the suggestion that SSV may play a role in the pathogenesis of some neoplastic diseases of man was questioned [18].

Until now a detailed, comparative study of the structural proteins of SSV was lacking: except for the major protein (p31) the structural proteins had been isolated only in minimal amounts [5, 19–25] and potent, component-specific antisera with broad interspecies reactivity spectra were unavailable. Such antisera, prepared in rabbits and goats by inoculation of purified murine Friend leukemia virus (FLV) envelope components, were effective in passive immunization and serotherapy of virus-inoculated or endogenously virus-carrying mice and virus-inocolated kittens [26–32], and it would be interesting to know whether similar antisera were effective in non-human primates and perhaps man.

Requests for reprints should be sent to Prof. F. Deinhardt, Pettenkofer Institute, Pettenkoferstr. 9 a, D-8000 München 2.

* Present address: Pettenkofer Institut, Pettenkoferstr. 9 a, D-8000 München 2.

** Present address: Dept. of Microbiology, University of Illinois College of Medicine, Urbana, Illinois, 61801, USA.

Abbreviations: BSA, bovine serum albumin; CF, complement fixation; FCS, fetal calf serum; FeLV, feline leukemia virus; FLV, murine Friend leukemia virus; FMR, Friend-Moloney-Rauscher leukemia viruses; GALV, gibbon ape lymphoma virus; GLV, Gross leukemia virus; HBSS, Hanks balanced salt solution; HF, normal adult marmoset fibroblast cell line; MFS, normal marmoset fetal skin cells; MuLV, murine leukemia virus; RIP, radioimmunoprecipitation; RLV, Rauscher leukemia virus; SSAV, simian sarcoma associated virus; SSV, complex of simian sarcoma and its associated virus; TE, Tween ether.

The murine FLV structural proteins have been studied in great detail [33, 34]. The major viral envelope component, the glycoprotein gp71, which is arranged in surface protrusions, possesses type-, group- and interspecies antigenic reactivity and seems to play the most important role in immune defense. Additional glycoproteins besides gp71 have been identified in Rauscher leukemia virus (RLV); gp69 has been shown recently to be a degradation product of gp71 [35, 36], and the exact nature of gp45 and gp33 [33, 37–40] remains under discussion. Additional envelope components of FLV are p15(E) [41] and the recently described p12(E) [42, 43]; the latter is biochemically and serologically related to p15(E) and seems to be a cleavage product of it. That they play a role in immune defense is indicated by the finding of natural antibodies against the respective antigens in several mouse strains [44, 45] and by the observation that active immunization with p15(E) confers some protection against murine FLV infection [33]. P15/p12(E) possess mainly interspecies antigenic reactivity and appear to be masked in many murine viruses, probably by gp71 [33]. In SDS-PAGE analysis p12(E) migrates faster than the p15 peak but slower than the p12 component [43] and, in contrast to p12(E), part of the protein p12 is phosphorylated [46]. In addition, p12 binds specifically to viral RNA [47]; however, in the virion it is situated mainly between envelope and core, whereas in the host cell it is exposed on the surface of nonbudding areas of the outer membrane [33]. The antigenic reactivity of p12 is mainly type-specific [33] and appears to represent part of the Friend-Moloney-Rauscher leukemia viruses (FMR) cell surface antigen (see [48]). Also reacting mostly with type specificity is a component which in SDS-PAGE migrates together with p15(E), as demonstrated first in other murine leukemia viruses (MuLV) [49] and recently in FLV [43]. Because it is believed to be located in the viral core this component has been called p15(C). Additional FLV-core components are p31, possessing mainly group- and interspecies reactivity, and p10 possessing preferentially group specificity [33].

Our study was designed to provide a more detailed analysis of SSV, and to explore the possible relationship of SSV to diseases of man and the potential value of antibodies against certain components of SSV in serotherapy. In this first communication an analysis of the structural proteins of SSV is presented (as all preparations of SSV examined in the past and those studied in this report consist of a 10–100 fold excess of SSAV over simian sarcoma virus, the analyzed proteins mirror this relationship), and the preparation and characterization of a virus-specific antiserum which appears to react predominantly with viral envelope components is described. This antiserum permitted rapid serological identification of the respective viral components by Ouchterlony and CF tests and a subsequent, comparative serological study which evaluated whether the serum possessed interspecies reactivity and reacted with C-type viruses of other mammalian species.

Materials and Methods

Viruses. The viruses used in these studies are listed in Table I. Viruses were concentrated and partially purified by sedimenting twice at pi 10 [50]; occasionally they were purified further by banding in a sucrose density gradient. TE degradation was described earlier [51].

Cells. The HF normal marmoset fibroblast cell line was derived from a skin biopsy of a 4-month old, hybrid white-lipped marmoset monkey *(Saguinus nigricollis* × *S. fuscicollis illigeri)*. The minced biopsy tissue was washed with HBSS, and was grown out and maintained by standard techniques [52]. – The SSV-producing, marmoset-HF-SSV/Jü-suspension cell line was developed from the same HF-marmoset line after infection and transformation

Table I. Viruses.

Virus	Origin	Tissue culture in which virus was produced	Refs
Friend leukemia	mouse	Eveline STU mouse	[77]
Gross leukemia	mouse	STU mouse	[62]
Feline leukemia (Rickard)	cat	F-422 cat	[78]
RD-114	cat (endogenous)	human rhabdomyo-sarcoma	[79]
Porcine (PLCP)	pig (endogenous)	V-38A-1 pig	[80]
Simian sarcoma	woolly monkey	marmoset HF-SSV-Jü	[1, 69]
Gibbon lymphoma	gibbon	gibbon ape lymphoma	[3]
Baboon (Bab.-V)	baboon (endogenous)	BKD-dog thymus	[81]

with SSV [52, Thiel, unpublished data]. Cells were cultured in Dulbecco's modified Eagle's medium containing 10% heated (30 min, 56 °C) FCS. The cultures were agitated with a magnetic stirrer and the virus-containing medium was harvested usually 3 to 4 days after starting the cultures. The gibbon ape lymphoma cell line was obtained from Dr. T. Kawakami, University of California, Davis, California and grown on 20% FCS/RPMI-1640.

Cell extracts. Cells for serological studies were extracted as follows: 1 ml of washed, packed cells was suspended in 1.5 ml phosphate-buffered (pH 7.2) saline, the suspension was frozen and thawed three times, homogenized in a Potter homogenizer, centrifuged at low speed and the supernatant used as extract.

Antisera. The antisera used are listed in Table II. The immunization procedure for preparation of the g-SSV-serum was as follows: serum from several bleedings and a skin-muscle biopsy were obtained from a young goat. A fibroblastic cell line was established from the biopsy tissue by standard cell culture techniques. The cells were grown initially in medium 1640 or minimum essential medium (Eagle) supplemented with 5 – 10% autologous goat serum and 100 units of penicillin and 50 μg of streptomycin per ml. Media used for later passages were supplemented with FCS rather than goat serum. Goat fibroblasts were transformed with SSV as described previously [2] and the resulting transformed cell line produced low levels of SSV (10^1 to 10^2 FFU/ml) throughout all passages used for immunization. Cells were always subcultured in medium supplemented with normal goat serum for one week before immunizing the goat from which the biopsy had been obtained originally. Over a period of 5 years the goat received 11 inoculations of cell doses ranging from 8×10^7 to 8×10^8 transformed cells/inoculum of tissue culture passages 11 to 69. Neutralization titers of subsequent bleedings ranged from 1:64 to 1:16284. Serum with a neutralization titer of 1:4000 obtained after the 7th booster inoculation was used throughout these studies.

Table II. Antisera

Sera	Origin	Prepared against	Inter-species reactivity	Refs
g-SSV-serum	goat	SSV-infected and transformed autologous goat cells		see text
FLV-p10-serum	rabbit	Isolated p10 of murine FLV	–	[62]
FLV-p15(E)-serum	rabbit	Isolated p15(E) of murine FLV	+	[41]
FLV-p31-serum	rabbit	Isolated p31 of murine FLV	+	[62]
FLV-gp71-serum	rabbit	Isolated gp71 of murine FLV	+	[74, 82]
FCS-serum	rabbit	Fetal calf serum		[74]

Labelling of viruses with radioactive amino acids or glucosamine. Virus-producing cells were seeded at a concentration of 5×10^5 cells/ml in Dulbecco's modified Eagle's medium supplemented with 10% FCS. Labelling with radioactive precursors began 15 h after seeding and continued for 24 h. Polysaccharides were labelled with 100 μCi/ml of [^{3}H]-glucosamine (20 Ci/mmol) in medium containing 20 mM fructose instead of glucose. Proteins were labelled either with 10 μCi/ml of a [^{14}C]amino acid mixture ([^{14}C]protein hydrolysate, 54 μCi/mAtom) in medium containing 20% of the normal concentration of amino acids or with 100 μCi/ml of [^{3}H]-leucine (50 Ci/mmol) in medium without leucine. Labelled compounds came from the Radiochemical Centre, Amersham, U. K. Radioactive virus was purified by density gradient centrifugation.

Labelling of cells with radioactive amino acids or glucosamine. Cells were labelled metabolically with 300 μCi/ml [^{3}H]glucosamine or 400 μCi/ml of [^{3}H]leucine by the method described for labelling virus, except that cells were preincubated for 30 to 60 min with the respective labelling medium plus 10% dialyzed FCS before addition of the isotope and were labelled for 6 to 8 h only. After labelling, cells were washed four times with ice-cold medium and frozen immediately in dry ice.

Selective labelling of surface proteins of cells by enzymatic iodination with I^{125}. 10^7 cells were labelled for 15 min at 30 °C in 50 μl of buffered (pH 7.2) saline containing 30 mM sodium phosphate, 0.03% H_2O_2, 10 μg Lactoperoxidase (Boehringer, Mannheim) and 1 mCi Na I^{125} (New England Nuclear, Boston) [53].

SDS-polyacrylamide gel electrophoresis (PAGE) techniques: Cylindrical gel PAGE. Analytical SDS-

PAGE was performed according to [54]. To determine the distribution of radioactivity gels were sliced, treated with 3% Protosol (New England Nuclear, Boston) in a toluol scintillation solution containing PPO and POPOP and counted in a Packard scintillation counter (Model 3375, Packard Instrument Company, Inc., La Grange, Ill.). *Slab gel PAGE and autoradiography.* Labelled viruses were analysed after immunoprecipitation was performed by SDS-PAGE on 7.5 – 16% gradient gel slabs using the buffer systems of Laemmli [55], modified after Lugtenberg *et al.* [56]. Gels were fixed with 10% acetic acid in 30% methanol, processed for autoradiography [57, 58], and dried gels were exposed to RP-Royal "X-Omat" films (Kodak) at – 70 °C for 1 to 4 weeks. *Slab gel PAGE and preparation for scintillation counting.* Immunoprecipitates of I^{125} labelled, cell surface polypeptides were analyzed by SDS-PAGE on an 11% acrylamide slab gel using Laemmli's buffer system. Individual lanes were cut out, sliced and counted in a Berthold Gamma counter (Gammascint 5300; Berthold, Wildbad, Germany), and the radioactivity pattern was compared to Coomassie blue-stained [54] FLV polypeptides separated on the same slab.

Radioimmunoprecipitation. RIP of labelled virus and cell extracts was performed in general as described by Gielkens [59]. Cells and viruses were treated with lysis buffer (0.02 M Tris pH 7.4, 0.05 M NaCl, 2 mg/ml BSA, 1% Triton X100, 0.1% sodium desoxycholate, 0.1% SDS) containing the following protease inhibitors: TPCK 2 mM, TLCK 1 mM, PMSF 2 mM, iodoacetate 15 mM.

After treatment of the lysates with DNAse and sonication [43], extracts were centrifuged for 2 h at 150 000 × *g*. In some experiments with cell extracts a similarly prepared extract from unlabelled normal human embryonic lung fibroblasts was added in about 100-fold excess before sonication and centrifugation. Precipitation procedures were generally those described by Montelaro *et al.* [43] and Schneider and Hunsmann [53]. 2 µl of specific antiserum absorbed with a 10 – 20-fold excess of extracts prepared from normal fibroblasts were added to amounts of extracts containing 2×10^6 cpm (cells) or 1×10^5 cpm (viruses); the mixtures were incubated for 2 h at 37 °C or overnight at 4 °C. Immunoprecipitates were formed by addition of appropriate amounts of anti-IgG-antibodies, and incubation for 2 h at 37 °C and overnight at 4 °C; precipitates were washed [59] and dissolved in sample buffer for SDS-PAGE [55].

Cytotoxicity. Cytotoxicity was determined by a micro-^{51}Cr-release test described in detail previously by Hunsmann *et al.* [60].

Neutralization Assays. Twofold dilutions of antiserum in a final volume of 0.6 ml were incubated at 4 °C for 4 h with 0.6 ml virus at a dilution calculated to give 50 – 1000 foci/flask. Dilutions were made with HBSS containing 2% agamma-FCS and neutralization was determined by focus assay on MFS. Cells seeded at 5×10^5 cells/25 cm² were treated on the following day with DEAE-dextran (20 µg/ml) for one h and washed with HBSS before infection with 0.5 ml of virus inoculum. Virus was adsorbed for 90 min before the cultures were refed with maintenance medium. Cultures were incubated at 37 °C, foci were counted on day 10 and the neutralization titer was that serum dilution which inhibited focus induction by 50%, as compared to controls incubated without antiserum.

Complement fixation (CF), Ouchterlony immunodiffusion and protein determination. These techniques followed the descriptions by Schäfer *et al.* [41] and Lowry *et al.* [61]; TE-degraded, purified virus only, or infected or uninfected cell extracts were used.

Results

Analysis of viral structural proteins

SSV proteins were analyzed by gel electrophoresis of virus metabolically labelled with radioactive amino acids or glucosamine and purified by density gradient centrifugation. Virus analyzed by cylindrical gel PAGE yielded a protein pattern very similar to that of MuLV (Fig. 1), although minor differences in mobility of some components were observed. Of the major polypeptide peaks, the two migrating slowest with an approximate mobility of MuLV gp69/71 were associated (although to different degrees) with glucosamine (Fig. 1 a) and therefore apparently represent the analogous major glycoproteins of SSV. Additional proteins migrated in the region of p15 – p12; they were a polypeptide peak with a shoulder at the front and a clearly separated peak (Fig. 1 b), reminiscent of the FLV p15(E) – p15(C) – p12(E) complex and the dis-

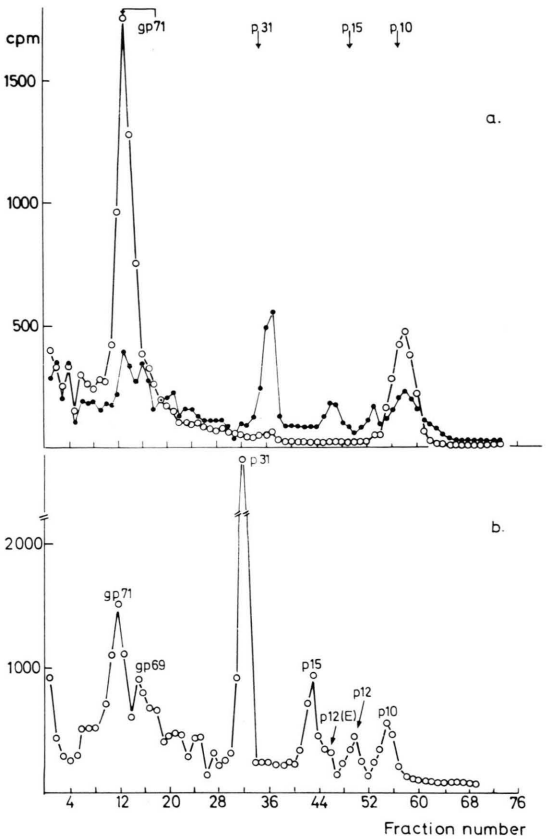

Fig. 1. PAGE analysis of polypeptides of SSV (cylindrical gels). a) Labelling: [C^{14}]amino acid mixture ●—● and [^{3}H]glucosamine ○—○. b) Labelling: [C^{14}]leucine. The shoulder in the p15 peak is more pronounced. Arrows indicate positions of FLV polypeptides which were run in parallel.

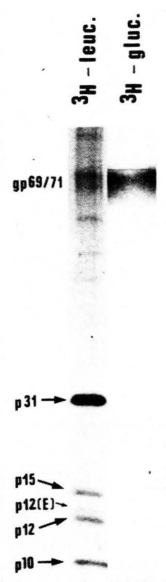

Fig. 2. PAGE analysis of polypeptides of SSV (slab gel, autoradiography). Label: [^{3}H]leucine and [^{3}H]glucosamine. The p15 shoulder material is visible as a separate faint band (p12(E)).

tinct p12 (see Introduction). The fastest migrating SSV-glucosamine labelled component (~FLV p10 mobility) lacked carbohydrate after purification (Thiel, unpublished observations): this suggests, as in the case of MuLV, that the glucosamine peak may result from a comigrating glycolipid.

Results were comparable when the SSV proteins were analyzed by slab gel PAGE autoradiography. Three bands were detected in the p15/p12 region (see Fig. 2): behind the more prominent band, regarded as p12, an intermediate faint band which may be analogous to the p12(E) of MuLV was observed. The two p12 bands were not always resolved by this method although evidence for the regular occurrence of p12(E) was obtained (see below and compare Figs 3, 4 a and 5).

The SSV components, particularly those in the p15/p12 region, were identified more precisely by RIP assays. [^{3}H]leucine-labelled virus was reacted with previously characterized antisera (Table II), which were specific for the isolated viral proteins of FLV [33]. The interspecies-reactig antisera used in these tests included anti-FLV gp71, anti-FLV p31, and anti-FLV p15(E). The latter antiserum reacted with p15(E) and p12(E) but only poorly with the apparent type-specific p15(C) and not at all with the type-specific p12 of the murine FMR viruses [43]. With other MuLV serotypes and other mammalian C-type viruses the FLV p15(E) antiserum reacted only with the p15(E)/p12(E) [49]. FLV p10 antiserum, which had primarily group-specific reactivity, and normal rabbit sera served as negative control sera. The precipitates were analyzed by slab gel PAGE and the autoradiographic patterns are illustrated in Fig. 3. The control sera (p10 and normal serum) did not precipitate significant amounts of label. In contrast, FLP-p31-serum reacted clearly with a corresponding component of SSV, and SSV FLV-p15(E)-serum precipitated two polypeptides which possessed the approximate mobilities of p15 and p12 and thus apparently represent the p15(E)/p12(E) analogues of SSV; some material in the gp71 region was also precipitated regularly by FLV p15(E) serum. Using FLV-gp71-serum only a very small amount of the respective label was precipitated.

P12 proteins of MuLV and FeLV in PAGE gels showed peculiar properties after staining with Co-

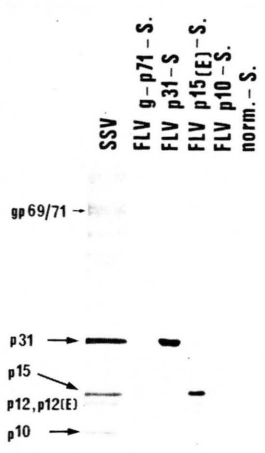

Fig. 3. Immunological identification of several SSV polypeptides by FLV component-specific antisera possessing interspecies activity. RIP assay (slab gel PAGE, autoradiography). SSV labelled with [^{3}H]leucine. Sera: FLV-gp71-S, FLV-p31-S, FLV-p15(E)-S, FLV-p10-S and normal rabbit serum.

omassie blue; instead of the bluish purple stain which characterized the other viral proteins, a reddish color was observed [62]. If larger amounts of purified SSV were assayed on cylindrical or slab gels a similar reddish color was observed.

Thus striking analogies between the structural polypeptides of MuLV and SSV were identified, not only physicochemically but also in part immunologically. So long as the exact molecular weights of the various PAGE components of SSV remain undetermined by more reliable methods, they should be designated in a fashion analogous to MuLV as p10, p12(E), p15(E), p31, gp69 and gp71 (see Fig. 1 b). Whether SSV, like MuLV, contains an additional p15 core protein in the p15 peak remains to be determined.

Characterization of g-SSV-serum

The specificity of the g-SSV-serum prepared in a goat against autologous SSV-transformed cells was assayed by RIP and other serological methods (neutralization, cytotoxicity, Ouchteronly and CF). In a first series of RIP experiments g-SSV-serum was reacted with purified SSV which had been labelled metabolically (radioactive amino acids and glucosamine); PAGE analyses of the precipitates are illustrated in Figs 4 and 5. The major serum reactants were apparently gp69/71 and components behaving electrophoretically like p15(E) and p12(E) (Figs 4 a and 5). Evidence that the latter components are identical with those reacting with FLV-p15(E)-serum is discussed later (see Ouchterlony test, Fig. 9). Minor components, which were observed only occasionally, were 1 or 2 polypeptides migrating somewhat slower than the major glycoproteins (see Fig. 4 a), 2 – 3 polypeptides migrating somewhat faster than the glycoproteins (see Figs 4 a and 5), and a p31-like material (see Fig. 5). Components migrating slower than the major glycoproteins were detected also by normal goat serum.

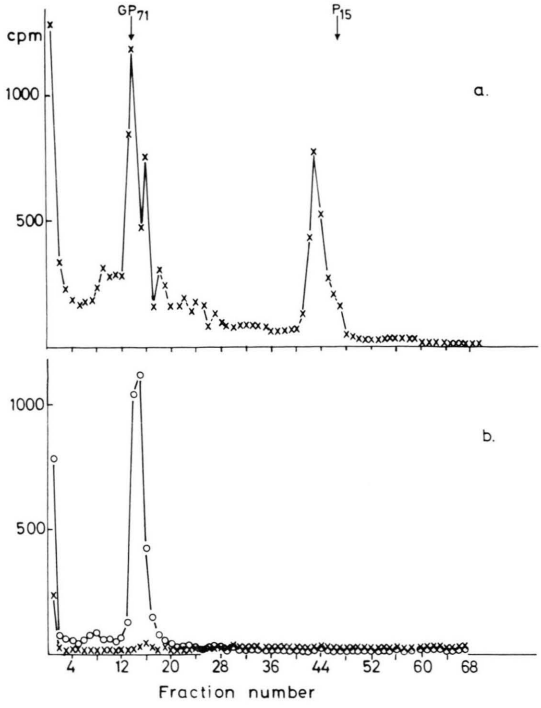

Fig. 4. Evaluation of g-SSV-serum reactivity with [^{3}H]-leucine-(a) and [^{3}H]glucosamine-(b)-labelled SSV respectively. RIP assay (cylindrical gel PAGE).

It was possible that g-SSV-serum would react with an SSV tumor-specific antigen, similar to the "tumor-specific cell surface antigen" (TSSA) [48] or the

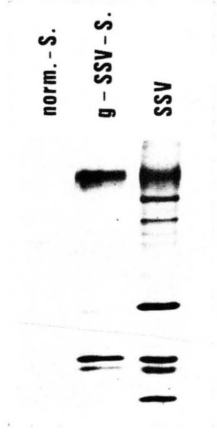

Fig. 5. As Fig. 4. Label: only [^{3}H]leucine. Slab gel PAGE: Autoradiography.

FOCMA-type antigen of feline leukemia or sarcoma virus infected cells [63 – 68]. For this experiment HF-SSV/Jü cells and normal HF-marmoset cells as controls were used; cells were labelled metabolically with [^{3}H]leucine and [^{3}H]glucosamine, lysed by detergents and reacted with g-SSV-serum or normal goat serum. Slab PAGE autoradiographic analysis of the precipitated radioactive components is presented in Fig. 6. As with SSV particles, components behaving like gp69/71, p15(E) and p12(E) were precipitated by g-SSV-serum from the lysates of virus-producing cells, whereas p31 was found only in barely detectable amounts. In addition, two polypeptides migrating slower than gp71 were present but such polypeptides also appeared if normal goat serum instead of g-SSV-serum was used. No antigen reacting specifically with g-SSV-serum was detectable with lysates of normal HF cells.

An RIP assay designed to evaluate which of the cellular components which reacted with g-SSV-serum were situated at the surface of the HF-SSV/Jü cells and were therefore accessible to enzymatic iodination with ^{125}I is presented in Fig. 7. PAGE analysis of the precipitated radioactive components identified a major radioactive component with an approximate PAGE mobility of the major viral structural glycoprotein(s); however p15(E)/p12(E)-like components were apparently only scarcely, if at all, accessible to enzymatic iodination since only a very minor peak was visible in the p15 region.

It appears therefore that RIP identifies gp69/71, p15(E) and p12(E) as the major components reacting with the g-SSV-serum although a very minor

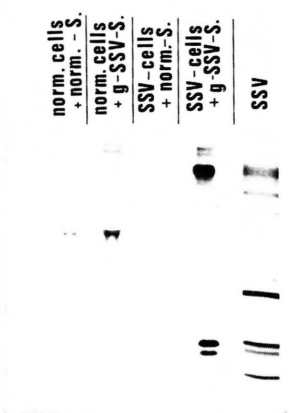

Fig. 6. Evaluation of g-SSV-serum reactivity with HF-SSV/Jü cell antigens. Label: [^{3}H]leucine. RIP assay (slab gel PAGE; autoradiography).

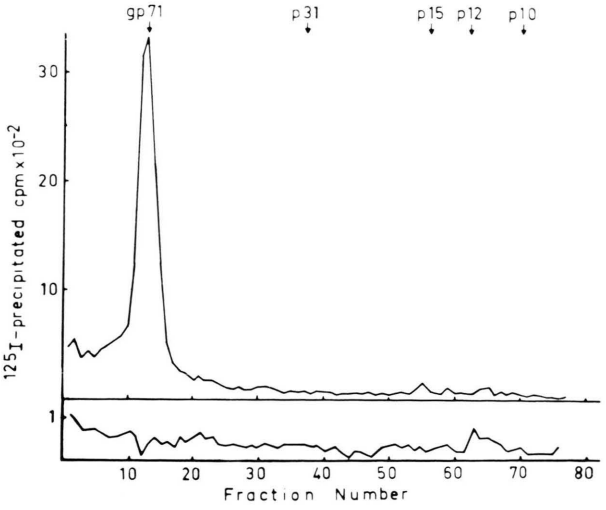

Fig. 7. RIP assay (slab gel scintillation counting) with HF-SSV/Jü cells labelled on the surface by enzymatic iodination with I^{125}. Sera: g-SSV-serum (top) and normal goat-serum (bottom).

reactivity may also exist with p31. No additional reactivity for SSV-transformed cells was demonstrated. Of the components reacting in significant amounts with g-SSV-serum the major viral glycoprotein(s) appeared free for enzymatic iodination at the cell surfaces.

The specificity of the serum in other serological tests agreed with the reactivities demonstrated in RIP assays. Studies of other C-type viruses showed that the main targets for virus neutralization and cytotoxic reaction are the major viral glycoprotein(s) [33]. G-SSV-serum examined in both types of tests possessed both high neutralizing activity (titer for SSV:1/4000) and high cytotoxic activity (titer for HF-SSV/Jü cells: ~ 1/320 (Fig. 8)).

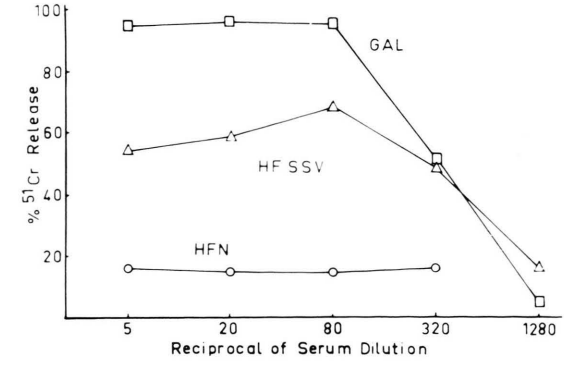

Fig. 8. Cytotoxicity of g-SSV-serum for HF-SSV/Jü (HF-SSV), GAL- and normal HF (HFN) cells. Cr^{51} release test.

The high potency of the anti-serum was demonstrated also by distinct precipitation lines when the serum reacted with TE-degraded SSV in the relatively insensitive Ouchterlony test. As seen n Fig. 9, two prominent lines (lines 1 and 2) were formed when the concentration of the SSV-preparation was high enough (~30 mg/ml); an occasional, very faint line has been observed between them (not visible in Fig. 9). When interspecies-reacting MuLV p15(E) serum was included in the set, it formed a single, clear precipitation line with SSV which was continuous with the line 2 formed by the g-SSV-serum (see Fig. 9 a); line 2 therefore represents p15(E)/p12(E) of SSV. Both seem to be very similar antigenically as otherwise two distinct precipitation lines would be expected to appear. As has been shown in another report [69], line 1 represents the major viral glycoprotein. Normal antigens such as FCS and normal HF marmoset cell extracts formed no visible precipitates with the goat antiserum. These Ouchterlony results provide clear evidence that most of the p15(E)/p12(E) antigen of degraded virus reacts directly with the g-SSV-serum rather than being co-precipitated through association with gp71 [43].

More sensitive (about 10 – 100 times) than the Ouchterlony test is the CF test; this reaction also identified no reactivity of g-SSV-serum with normal antigens (FCS, normal marmoset cell extracts), even when used at a dillution of 1:10. Similarly no reaction of the serum with isolated p10 and p31 of SSV was observed if a serum dilution of 1:10 was used (see Table III). However, clear CF activity was detectable with TE-degraded SSV as antigen; in a checkerboard titration (varying dilutions of antigen against varying dilutions of serum) the serum titer was 1:100.

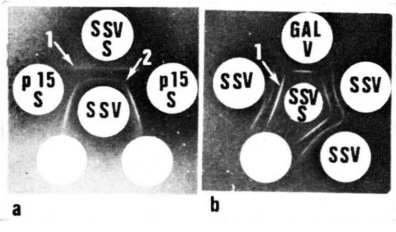

Fig. 9. Ouchterlony tests. a) Reactions of g-SSV- (SSV-S) and FLV-p15(E)-serum (p15-S) with TE-degraded SSV (SSV). b) Reactions of g-SSV-serum with TE-degraded SSV and GALV. Line 1: gp71; line 2: p15(E)/p12(E).

Comparative serological studies

The reactivity of g-SSV-serum with purified preparations of a number of mamalian C-type viruses (TE-degraded) and extracts of uninfected and infected cells was examined by CF (Table III). The antigen p15(E) isolated from FLV was also used [41]. Only SSV and GALV antigen preparations reacted positively and the serum titer was identical (~1:100) when determined with extracts of SSV- or GALV-infected cells. In contrast, the two viruses behaved differently in neutralization tests; the g-SSV-serum titer was about 8 times less for GALV (1:500) than for SSV (1:4000). In Ouchterlony tests (Fig. 9 b) the glycoprotein lines (line 1) of both viruses showed a reaction of identity. The concentration of the GALV preparation was apparently too low to allow demonstration of the p15(E)/p12(E) line (line 2) of this virus. The cytotoxic titer of the serum (that dilution producing 50% of maximum cytotoxicity) was essentially identical for GAL cells and HF-SSV-Jü cells (Fig. 8); for GAL cells its cytolytic effect was stronger (~100% Cr release) than for HF-SSV/Jü cells (~70% Cr release). Although both cell lines are maintained as suspension cell cultures, GAL cells are lymphoblastoid cells whereas HF-SSV/Jü cells were derived from fibroblast monolayer cell cultures; this may account for the difference observed in sensitivity to serum cytolytic activity.

Discussion

The PAGE *protein pattern of SSV* was very similar to that of MuLV; only minor differences in electrophoretic mobilities of some SSV polypeptides were detectable and it is proposed therefore that an analogous nomenclature be used for SSV- and MuLV-components. It was particular interesting, in view of our long term program, that SSV contained, like MULV, gp69/71 and p15(E)/p12(E) components which in analogy with MuLV may all represent envelope constituents.

The p15(E) and p12(E) components of SSV were identified serologically with FLV p15(E) antiserum, the interspecies crossreactivity of which had been shown earlier by CF tests to extend to SSV [41]. In addition this antiserum routinely precipitated some gp69/71-like material from SSV antigen preparations, and previous experience with MuLV suggests that this material may have been trapped, per-

Table III. Comparative CF-reactivity of g-SSV-serum

Viral antigen	Virus Origin	Purified viral material	Infected cell extracts	Protein mg/ml	CF-titer of antigen at serum dilution	
					1:10	1:20
SSV	Woolly monkey	+		4.15	1000 [a]	1000
SSV p31	Woolly monkey	+		0.52	<16 [b]	N. T.
SSV p10	Woolly monkey	+		2.32	<20	<20
HF-SSV/Jü (marmoset cells)	Woolly monkey		+		64	32
GALV	Gibbon	+		0.73	8	4
GAL (gibbon cells)	Gibbon		+		128	64
FLV	Mouse	+		9.7	< 4	N. T.
FLV-p15(E)	Mouse	+		2.32	<20	<20
GLV	Mouse	+		6.00	< 8	N. T.
FeLV	Cat	+		19.2	<32	<32
RD-114	Cat	+		14.2	< 8	< 8
PLCP	Pig	+		1.57	< 8	N. T.
Bab.-V	Baboon	+		1.88	< 2	< 2

Extracts of cells infected with murine, feline, porcine and baboon viruses (see Table I) were also examined. All titered <1:4 at 1:10 and 1:20 serum dilutions respectively.

[a] Reciprocal of antigen dilution giving positive CF.

[b] Antigens listed as < contained at least 4 CF antigen units in the dilution listed as shown by reactions with appropriate homologous antisera.

N. T. = Not tested

haps in some envelope lipid which was not removed by detergent, or by aggregation of p15(E) polypeptides, shown to occur easily in MuLV, or (and most likely) by formation of S-S bonds with p15(E) [43, 70, 71].

P10, p12 and p31 are regarded as possible non-envelope components of SSV; like MuLV p12, which is reported to be phosphorylated [72] the p12 of SSV stained reddish with Coomassie Blue and the SSV p31 was characterized serologically with an antibody against FLV p31 which had been shown earlier to share an interspecies subdeterminant with SSV [73].

Using sensitive RIA techniques, other investigators have found minor cross-reactive determinants of gp71s of FeLV, MuLV, and GALV [23, 70]. However in the less sensitive CF test, an FLV gp71 antiserum had been shown previously to possess a restricted spectrum of interspecies activity, reacting with FeLV and other nonmurine mammalian C-type viruses but not with SSV or GALV [74], so the very low reactivity of this antiserum with SSV in RIP tests reported here is not surprising.

It may be that the identification of two glycoproteins in SSV resulted from a situation similar to that seen in RLV in which gp69 was shown recently to be a degradation product of gp71 [36]; so far, however, no evidence for a serological relationship between the two components of SSV is available and so the possibility that they are serologically different and originate from different viral genes cannot be excluded. However, in this regard it is interesting that even in cloned SSAV, grown in rat kidney cells, two major glycoproteins were detectable gelelectrophoretically (Thiel, unpublished observations). The components, which were observed only occasionally and which possess PAGE mobilities between those of gp69/71 and p31, are reminiscent of the enzymatic degradation products of MuLV gp71 [36]. Recent studies indicate that similar degradation products also occur spontaneously with partially purified SSV glycoproteins [75]. The results do not establish whether a p15, analogous to the p15(C) of MuLV, is present in SSV in addition to the p15(E) envelope component.

The *g-SSV-serum* was prepared to favor the formation of a highly virus-specific serum with antibodies against viral envelope and/or altered host cell surface components. Extensive examination of the serum showed that it reacted almost only with

the gp69/71 and p15(E)/p12(E) envelope components of the virus particle, although the existence of a very low titer against p31 could not be excluded. Reactivity with envelope components, demonstrated by RIP, agreed with the results of the neutralization, cytotoxicity and Ouchterlony tests. In neutralization and cytotoxicity reactions, in which the major viral glycoprotein(s) of C-type oncornaviruses were shown to be the main targets (for references see [33] and [60]), g-SSV-serum demonstrated high activity (titers of ~1/4000 and ~1/320 respectively). In Ouchterlony tests the serum formed two prominent precipitation lines with degraded SSV and by using FLV p15(E) serum, one of these lines was identified as the SSV-p15(E)/p12(E)-line. Recent experiments with purified SSV glycoprotein(s) (gp69/71) showed that the other main line is caused by this viral structural component [69].

In RIP tests using labelled extracts of HF-SSV/Jü cells the g-SSV-serum precipitated components corresponding to those precipitated from purified virus. No reactivity of the serum with component(s) similar to tumor specific surface antigens (TSSA) [48] or FOCMA [63–68] was observed, although the mode of preparation should have favored the induction of such antibodies. From the various components reacting with the g-SSV-serum only gp69/71-like material was demonstrable on the HF-SSV/Jü cell surface in significant amounts when the enzymatic iodination method was used for detecting superficially arranged proteins. This observation agrees with those made in the MuLV system [33] and shows also that in other C-type virus systems the p15(E)/p12(E) component(s) can be masked on the viral and host cell surface. That the virus-producing, transformed goat cells used for the preparation of g-SSV-serum nevertheless induced antibodies to p15(E)/p12(E) indicated that these cells or the virus expressed p15(E)/p12(E) unmasked at the cell or virus surface or that these antigens became unmasked during the process of rejection of the cells or virus by the goat's immune mechanisms. In this regard it is interesting that FLV p15(E) antiserum neutralized MuX and FeLV but not FLV and other MuLV ecotropic viruses in complement-dependent reactions [76].

With the newly prepared g-SSV-serum the CF reaction could also be performed and the titer of the serum in this relatively simple serological system was ~1:100. Nonreactivity of g-SSV-serum with normal control antigens and isolated p10 and p31 proteins of SSV in this system confirmed its specificity for certain viral constituents.

Comparative serological studies with g-SSV-serum confirmed the strong antigenic relationship between SSV and GALV already demonstrated by other authors [14, 19, 20, 22, 23, 25, 70]. Only in neutralization tests were differences in behaviour of these viruses observed; about 5 times more g-SSV-serum was needed for neutralizing 100 infectious units of GALV than for neutralizing corresponding amounts of SSV, perhaps indicating that some antigenic diversity in the surface glycoproteins of the viruses exist. Such a diversity was detected by Aaronson *et al.* [70], using RIA competition assay, but the antigenic difference was apparently too small to be detected in Ouchterlony tests using g-SSV-serum (see Fig. 9 b).

The results of the serological examination of other mammalian C-type viruses with the CF test were somewhat surprising. No reactivity with our g-SSV-serum was demonstrated, even though the serum contained p15(E) antibody and the respective component of SSV particles possesses interspecies antigenicity as demonstrated by its reactivity with FLV p15(E) antiserum (see Fig. 9 a). In addition, as mentioned before, the glycoprotein of SSV was shown by RIA to possess interspecies determinants [70]. A possible explanation for these discrepancies is that under the immunization procedure used for preparing the g-SSV-serum the interspecies portions of SSV-p15(E) and gp69/71 were unable to induce those CF antibodies in detectable amounts. However, some precipitating p15(E)-interspecies antibody must have been induced because otherwise the apparently continuous precipitation line formed when SSV reacted with g-SSV and FLV-p15(E)-serum respectively in Ouchterlony tests (see Fig. 9 a, line 2) could not be explained.

The deficiency of the g-SSV-serum in interspecies reactivity against the envelope components p15(E) and gp69/71 may impair its value for identification and evaluation of new viral isolates or viral components from human material, although g-SSV-serum does react with the SSV-like viruses reportedly isolated from normal and neoplastic human tissues [8, 10, 14]. Other immunization schedules, designed to obtain potent, broadly reacting antibodies, are needed and from our experience, immunization of

animals of a heterologous species with relatively large amounts of purified viral components may be a suitable method [33]. Studies in experimental animal systems (mice and cats) have demonstrated that viral envelope components and antibodies to them play a significant role in host immunological responses and defense mechanisms [26 – 30, 32]. The studies reported here and those in progress have analysed the SSV envelope components and their corresponding antisera for later evaluation of their roles in immunologic responses. For such studies larger quantities of antigens and broadly reacting antibodies will be needed, and the results reported in this paper provide the means for producing such reagents; for example, the major viral glycoprotein of SSV has already been isolated in mg amounts [69, 75].

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