Group-Specific Antigens of RNA Tumor Viruses as Markers for Subinfectious Expression of the RNA Virus Genome

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ABSTRACT Antigenic markers associated with the major internal protein of RNA tumor viruses of the C-type have proven extremely useful in natural history studies of these viruses. This protein possesses species-specific antigenic determinants, and, in the case of mammalian C-type viruses, the protein possesses crossreactive determinants as well. These determinants are, thus, useful for species identification and classification of mammalian viruses. A unique distribution of antigens in embryonic tissues of several species (where tests are available) was detected, and in addition, antigen expression in tissues appears to be controlled by a dominant gene. These data have contributed greatly to the theory that RNA tumor-virus infection is inherited as part of the cellular genome.

The ability to detect virus-specific products by immunologic means has proven invaluable for natural history studies of the RNA-containing C-type viruses (1, 2). The most useful antigenic reactivity has been that associated with the major internal virion protein, commonly called the group-specific (gs) antigen. This stable, species-specific antigen is characteristic of C-type viruses regardless of the cellular substrate used to grow the particular virus under study (3–5). Results of tests for this specific marker have shown widespread occurrence of viral gene activity under conditions where infectious virus or even virus particles could not be detected (6, 7). These findings contributed significantly to the theory that viral-genetic information is inherited as a part of the cellular genome (2, 8, 9). This paper will be concerned with a review of the immunochemical properties of the gs antigen of mammalian C-type viruses and the relevance of this specific antigenic marker to studies of the natural biology of C-type viruses.

MATERIALS AND METHODS

The viruses used in the immunochemical studies were obtained from chronically infected tissue cultures. Viruses were purified by isopycnic centrifugation in sucrose gradients and were disrupted by Tween-80-ether or sodium dodecyl sulfate (SDS); subunits were separated either by isoelectric focusing or polyacrylamide gels. Gel diffusion and complement fixation procedures have been described (10). The viruses used were murine leukemia virus (MuLV), hamster leukemia virus, feline leukemia virus, and rat leukemia virus. In many instances, “leukemia” virus is used to indicate C-type viruses in a generic sense and not necessarily a pathological one.

Abreviations: SDS, sodium dodecyl sulfate; MuLV, murine leukemia virus; gs, group specific antigen.

RESULTS

Properties of gs antigens

It is now clear that there are multiple polypeptide chains in the virion, each of which may possess one or more distinct antigenic determinants. For the avian tumor viruses, at least four polypeptides bearing distinct determinants that are group-specific, i.e., shared by all virus isolates within a species, have been clearly described (11, 12). The need for a precise definition of what is being detected (in various experimental situations) is immediately obvious, since the most widely used antisera, which are obtained from tumor-bearing animals in heterologous species, often contain multiple antibodies.

In the case of the four mammalian C-type viruses, it has been possible to isolate a major protein component from each virus (10, 13–15) and to prepare specific antibody against it mainly in guinea pigs. The key isolation procedure has been the isoelectric focusing technique, which is illustrated for MuLV in Fig. 1. While minor components were also present, the separations were characterized by one distinctive peak for each virus. The isoelectric points for the proteins from each of the four mammalian viruses and what we consider to be the homologous protein in avian viruses are given in Table 1. When the purified proteins were analyzed in SDS-polyacrylamide gels, they proved homogeneous.

![Fig. 1. Isoelectric focusing of AKR virus disrupted by Tween-80-ether. Disruption of virus and treatment with enzymes (RNase and DNase) before electrofocusing was performed as described (10, 13). The electrofocusing run was in 1% ampholine in the pH range of 5-8 and 0-40% sucrose gradient at a potential of 700 V for 64 hr at 6°. Absorbance at 280 nm (—) was monitored with an ISCO model UA-2 ultraviolet analyzer. The pH (○—○) was determined at 6° with an Orion model 801 pH meter. In this run, 6.3 mg of purified virus was used; the amount of purified gs protein recovered was 1.5 mg.](attachment:image_url)
were the electrophoresis, fastest migrating pattern (13-18). Antigenic analysis active determinants (gs-3) (20) protein (see gs active protein of specific determinants referred protein, both specific sera antigenic properties 21). (Fig. 3).

determinants precipitation experiments physically (one band) (Fig. 2) and corresponded in mobility to the third fastest migrating band of the three low molecular weight polypeptides usually resolved from mammalian C-type viruses (13-18). Antigenic analysis has revealed that this protein, referred to as the major gs protein, contained species-specific determinants (gs-1) (19) and interspecies cross-active determinants (gs-3) (20) on the same structure (14, 15, 21). Conclusive evidence for this assertion was obtained in precipitation experiments by use of internally labeled gs protein of purified feline leukemia virus. In these experiments, both specific antisera to gs antigen of feline leukemia virus and sera that contain antibodies to gs-3 antigen, prepared against gs protein of MuLV, gave 100% precipitation of the labeled antigen (Fig. 3). This result excludes the possibility of two physically similar molecular populations, one carrying gs-1 antigen determinants and the other carrying gs-3 determinants. The gs-1 antigen determinants are useful in establishing the species origin of viruses, while the gs-3 antigen determinants are useful for establishing the relationship of new C-type viruses to the mammalian viruses (Fig. 4). Both viper and avian C-type viruses possess determinants on their major internal proteins that are distinct from each other and from those on the major proteins of the mammalian viruses (Fig. 4). The specific antisera have proven valuable adjuncts to the antisera to MuLV gs antigen, prepared in rats bearing tumors induced by murine sarcoma virus (1), and to the antisera to gs antigen of feline leukemia virus, prepared in dogs bearing tumors induced by feline sarcoma virus (4, 22). Also, in the case of hamster and rat leukemia viruses, the specific antisera are currently the only antisera to gs antigen available, since tumors in heterologous species have not yet been obtained with these viruses. The need for heterologous species derives from the general inability of the homologous species to produce anti-gs-1 antibodies, presumably based on tolerance to the gs antigen that is present in embryonic tissues (see below) (6, 23).

**Inter-relationships among gs proteins—evolutionary considerations**

The ability to group viruses in families by virtue of morphologic, immunologic, or biologic criteria does not at the same time define precise evolutionary relationships among the

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**Table 1. Isoelectric points of major gs protein from viruses of several species**

<table>
<thead>
<tr>
<th>Species</th>
<th>Isoelectric point*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>6.7</td>
<td>10</td>
</tr>
<tr>
<td>Hamster</td>
<td>6.9</td>
<td>13</td>
</tr>
<tr>
<td>Cat</td>
<td>8.3</td>
<td>14</td>
</tr>
<tr>
<td>Rat</td>
<td>8.6</td>
<td>15</td>
</tr>
<tr>
<td>Chicken</td>
<td>8.9</td>
<td>41</td>
</tr>
</tbody>
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* As determined by isoelectric focusing in all cases.
† Determinations in our laboratory agree with the value reported in ref. 43.

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**Fig. 3.** Precipitation of purified FeLV gs protein by anti-gs-3 antibody. [3H]aminoacid-labeled gs protein—normal serum (A) or gs protein—antibody mixtures (B) were incubated at 37° for 1 hr, cooled to 4°, and layered on 5-20% sucrose gradients prepared in 0.01 M Tris-HCl (pH 7.4), containing 0.15 M NaCl. Centrifugation was done at 4° in a Spinco SW 39 rotor for 18 hr at 30,000 rpm. About 0.25-ml fractions, numbered from the bottom of the tube, were collected, and the pellet (P) was resuspended in 0.5 ml of Tris-HCl buffer. All samples were then counted in a Beckman LS 250 liquid scintillation counter. The gs-3 reactive serum, prepared against MuLV, completely precipitated the labeled FeLV gs protein, as did guinea pig anti-FeLV gs serum that contains mainly FeLV gs-1 antibodies (not shown).
viruses. Is the present distribution a reflection of coevolution of virus and host from some distant period, or did the prototype virus originate recently in one species and sporadically establish itself in new species? If the viral genome is a part of normal inheritance, then we should reasonably expect that the relationship between viral proteins of different species should parallel the known evolutionary relationship among species. The major gs protein of these viruses provides an ideal moiety for such studies.

Viruses from six species from three classes of vertebrates (one snake, one chicken, and four mammals); two mammalian orders (cats, rodents); three distinct species of rodents (hamster, rat, and mouse) are currently under study. Comparison of the primary structure of the major gs protein from these six viruses could be critical in establishment of the coevolution hypothesis. An initial start in this direction has been made by Allen and colleagues with avian gs antigens (24), and by ourselves with murine and feline gs antigens (Oroszlan et al., unpublished data). While the data are still much too fragmentary to permit detailed comparison, we do note that both avian gs antigen (25), and the major murine and feline gs antigens contain proline at the amino terminal.

Current data also show that three of the first four amino acids at the amino terminal are identical in both cat and mouse gs proteins, while, except for the terminal proline, the reported chicken-antigen (24) sequence is distinct. The immunologic data are also consistent with the coevolution hypothesis, since the mammalian virus proteins share a common antigenic determinant (gs-3), while the avian and viper proteins are each antigenically distinct.

**Subinfectious expression of gs antigen**

In recent years, an impressive volume of work has been performed for investigation of the occurrence of C-type virus genome in laboratory and feral mice (1, 2, 6, 26). Normal animals at various developmental stages and animals with viral- or chemically-induced tumors have been included in these surveys. Tests for infectivity have been performed in embryo cells of several animal strains, while complement fixation and gel diffusion assays have been made with tissue extracts. Several interesting points have emerged from these surveys. (a) Sarcomagenic viruses characterized either by in vivo or in vitro activity were never isolated from any of several hundred chemically-induced sarcomas, although non-

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**Fig. 4.** Gel diffusion analyses of C-type viruses from six species. Outer wells contain C-type viral concentrates disrupted by Tween 80-ether treatment. V = viper, A = avian, M = mouse, C = cat, H = hamster, R = rat. Inner wells contain immune serum prepared against purified gs protein in guinea pigs with two exceptions: AS is hamster serum from animals bearing tumors induced by Rous sarcoma virus; I-7 is from rats immunized with rat-tumors induced by and containing murine sarcoma virus. The various guinea pig antisera (VS, MS, CS, HS, RS) are reactive only with the homologous virus giving a single precipitin band. The hamster antiavian serum detects species-specific determinants located on two separable polypeptides (12, 25, 41), while I-7 detects the gs-3 determinant shared by mammalian C-type viruses, in addition to MuLV species-specific determinants.
sarcomagenic "leukemia" viruses could readily be isolated from both chemically-induced and spontaneous tumors in both high- and low-incidence strains (Huebner et al., unpublished data). (b) In most strains, even when infectious virus could not be isolated, gs antigen, as defined by use of specific antisera from tumor-bearing rats and from immunized guinea pigs, could be detected. Confirmation that the anti-
genic determinants detected were those of the major virion polypeptide was obtained in many instances by gel-diffusion analysis (6). An example of the ability to detect gs antigen and not infectious virus was seen in NIH Swiss mice. Despite literally hundreds of attempts over a period of 6 years, infectious virus was never isolated from normal or tumorous tissue of this strain, even though budding C-type particles were visualized and gs antigen was regularly demonstrated by complement fixation and gel diffusion assays at concentrations equivalent to and greater than those found in other strains of mice where infectious virus was demonstrated. (c) Tests of embryonic visceral tissues from most strains of mice revealed gs antigen. The presence of gs antigen in embryonic tissues was not dependent on the presence of infectious virus, although it was always present when infectious virus was detected. Similar findings of gs antigen have been made in chickens and hamsters by the same techniques (6, 23, 27), by use of specific antisera to gs antigen.

**DISCUSSION**

There is now compelling evidence that the major internal structural antigen, gs antigen, of C-type virus can be detected in adult, embryonic, and tumorous tissue of species from which adequate antisera and test materials are available. These findings take on more significance because situations occur in which infectious virus cannot be demonstrated even though gs antigen can readily be detected. The gs antigen, thus, provides a highly useful marker for the study of virus expression under various conditions, e.g., aging, chemical carcinogenesis, and carcinogenesis by DNA tumor-inducing viruses (26). In addition, widely distributed noninfectious expressions imply regular transmission of the genome through natural inheritance. In general, the results of such studies are consistent with the findings that the genome of RNA tumor viruses plays a key role in spontaneous and artificially induced malignancy in the mouse (2, 9, 26).

There are still several key questions to be resolved before attempting to generalize to all species. These include investigation of the evidence that gs antigen can be synthesized in the absence of virus particles, not just in the absence of infectious virus; they also include investigation of the relationships of gs antigen production to host-cell genotype (28). Inherent in the first question is the certainty that the gs antigen is coded by the viral genome.

As emphasized by the results of nucleic acid hybridization experiments, it appears clear that some portion (or all) of the viral RNA exists also in DNA form (29-35). Recently, strong evidence has been provided that, in fact, chicken, mouse, and rat cells contain full viral genomes that can be activated by various treatments (36, 39). Expression of this genome could be controlled in such a way that gs antigen could be synthesized in the absence of full particles (8). Clear evidence of this exists in the chicken and mouse, where inheritance of expression of gs antigen in the absence of particles, as well as in their presence, is under control of a dominant gene (40, 41).

With regard to the coding of gs antigen by the viral genome, it is clear from many studies (3-5) that C-type viruses can multiply in widely divergent cell lines and maintain their species-specific gs reactivities.

The sum of the data on the biology of C-type viruses suggests an intimate association of viral and cellular genomes. Studies of the primary structure of the gs antigen could provide evidence suggesting the existence of gs homologs in all vertebrate species. In the meantime, sensitive assays for the gs-3 determinant common to all mammalian C-type viruses should reveal more extensive occurrence of gs homologs than is now known. The impetus and justification for these studies derives directly from the extensive natural history studies summarized above. Since the gs antigen is a structural component of a cancer-causing agent, it appears justified to consider its detection a strong argument for the presence of the agent (virus or individual genes, including oncogenes) in the species where the antigen is detected.

We emphasize that the viral oncogene theory (2, 8) does not necessitate a complete correlation between oncogenesis and ability to detect gs antigen. Genes controlling these two functions are presumed to be distinct, and thus synthesis of gs antigen can remain repressed while the oncogene is expressed. This situation is found experimentally, for example, in certain hamster and rat tumors produced by murine sarcoma virus, where the sarcoma genome is present but no viral proteins have yet been detected (42). It may well be that a yet to be discovered sarcoma gene product will prove more useful in the search for a viral etiology for human sarcomas than the gs antigen. Studies of the distribution of the gs antigen have, however, until the present time, provided essential information leading to the oncogene theory of cancer.

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