Species-Specific and Interspecific Antigenic Determinants Associated with the Structural Protein of Feline C-Type Virus

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ABSTRACT The group-specific protein of feline leukemia viruses has been isolated by isoelectric focusing. This protein has a molecular weight, calculated from sodium dodecyl sulfate-acrylamide gel electrophoresis, of 25,000 and forms a resolved band in such gels when disrupted virions are analyzed. This protein contains both species-specific antigenic determinants, as well as interspecific cross-reactive determinants, as an integral part of its structure.

The mammalian C-type viruses contain, as a major structural component, a protein of about 30,000 molecular weight (1-4). In viruses from two of the three species we have studied (mouse, hamster), this protein has been isolated from Tween-80-ether disrupted virus by isoelectric focusing (3, 4). The isoelectric points were 6.7 (mouse) and 6.9 (hamster). When this protein was electrophoresed in sodium dodecyl sulfate (SDS)-acrylamide gels, it appeared homogeneous; it corresponded to the slowest migrating of three structural polypeptides derived from whole particles by SDS-urea-mercaptoethanol disruption (4). The protein carries the group-specific antigenic determinants, both the species-specific [gs-1 of Gregoriades and Old (1)] and the interspecies cross-reactive determinants [gs-3 of Geering et al. (5, 6); “interspec” of Schafer et al. (7)], as has been clearly shown by gel-diffusion experiments that used sera from guinea pigs immunized with the purified protein and sera from rats immunized with transplant tumors induced by murine sarcoma virus (8). The former sera are highly species-specific; the latter sera are cross-reactive, yet both sera react with the same purified protein.

In contrast to the situation with the hamster and mouse viruses, both Geering et al. (5) and Schafer et al. (7) have concluded that the major protein of cat C-type viruses contained only cross-reactive determinants (gs-3, interspec). This conclusion is based on the development of precipitin lines of identity obtained when cross-reactive anti-mouse gs sera and sera prepared against disrupted cat virus were diffused against cat virus. We report here the isolation of the major protein of feline C-type viruses by isoelectric focusing, and demonstrate the presence of species-specific and interspecific determinants associated with this protein.

MATERIALS AND METHODS

The preparation of amino acid-labeled virus, virus purification, disruption and separation by isoelectric focusing, and SDS-acrylamide gel electrophoresis have been described (3, 4). Tween-ether-treated virus preparations were digested with solid RNase at 0.2 mg/ml (Enzate-RNase, insolubilized enzyme, Miles Laboratories, Inc., Elkhart, Ind.) and 20 μg/ml of bovine pancreatic DNase (Worthington, Freehold, N.J.) before isoelectric focusing.

Gel diffusion was on micro-slides in the LKB apparatus, or on poured immunoplates (Hyland, Los Angeles, Calif.). For absorption studies, a well was first filled with the absorbing antigen, and 20-30 min later, antiserum was added. Complement-fixation was done by the microtiter procedure.

Feline C-type virus was obtained from a suspension cell line derived from a lymphoma case (9).

The preparation of antiserum to electrofocus-purified gs antigens of mouse and hamster (HaLV gs) origin in guinea pigs has been described (3, 4); similar procedures were followed for the cat gs antigen (FeLV gs). Dog antiserum reactive with FeLV gs (10) was obtained from a beagle bearing a progressively growing tumor induced by the Gardner-Arnold strain (11) of feline sarcoma virus. Sera against FeLV disrupted by SDS were prepared in guinea pigs. Sera capable of detecting the cross-reactive antigenic determinants of mammalian C-type viruses (5-7) were prepared in Fisher rats (8) by immunization with a murine sarcoma virus (MSV)-induced transplant tumor (12). Such sera are referred to as MSV-I.

**TABLE 1. Complement-fixation reactions of various immune sera against purified gs antigens**

<table>
<thead>
<tr>
<th>Test antigens*</th>
<th>MuLV gs</th>
<th>HaLV gs</th>
<th>FeLV gs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig anti-murine leukemia virus gs†</td>
<td>128</td>
<td>&lt;4</td>
<td>&lt;4-8</td>
</tr>
<tr>
<td>Guinea pig anti-HaLV gs</td>
<td>&lt;4</td>
<td>64</td>
<td>&lt;4</td>
</tr>
<tr>
<td>Guinea pig anti-FeLV gs</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>64</td>
</tr>
<tr>
<td>Guinea pig anti-SDS-disrupted FeLV</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>128</td>
</tr>
<tr>
<td>Dog anti-feline sarcoma virus</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>64</td>
</tr>
<tr>
<td>Rat anti-MSV-I</td>
<td>128</td>
<td>32</td>
<td>64</td>
</tr>
</tbody>
</table>

* Electrofocus-purified gs antigens at about 50 μg of protein/ml.
† Test sera used at four units, based on titrations with homologous antigens.
‡ Certain of these sera contain interspec antibody in low titer and, when used at high concentration, react with FeLV gs antigen in variable fashion.
**RESULTS**

Isoelectric focusing

Purified virus, labeled with $^3$H amino acid, was disrupted with Tween-80-ether, digested with RNase and DNase, and clarified at 100,000 $\times$ g for 1 hr prior to electrofocusing. When such preparations were analyzed on pH 3-10 gradients, a major radioactive zone with a peak at pH 8.3 was repeatedly obtained. This coincided with a distinct absorption maximum at 280 nm when concentrated virus preparations were analyzed (Fig. 1A). Because of the general similarity of this pattern to that obtained with mouse (3) and hamster (4) viruses, the pH 8.3 fraction was assumed to contain the gs protein.

**Acrylamide gel electrophoresis**

The pH 8.3 fraction derived from isoelectric focusing was electrophoresed in SDS–acrylamide gels, along with a series of protein standards. This material shows a high degree of homogeneity (Fig. 2A), and has a calculated molecular weight of 25,000. In terms of the overall pattern of whole virus disrupted with SDS-urea–mercaptoethanol, this corresponds to the third-fastest migrating polypeptide (Fig. 2B).

**Immunological analysis**

Various antisera prepared against purified antigens, SDS-disrupted virions, and tumor-cell homogenates, and from tumor-bearing dogs, were employed in complement-fixation and gel diffusion tests. Complement-fixation tests revealed the following significant points (Table 1): (a) a high degree of specificity was observed when guinea pig antiserum against electrophoresis-purified antigens were cross-tested. Thus, anti-feline gs serum is specific for feline virus, anti-hamster gs serum is specific for hamster virus, and anti-mouse gs serum is specific.

**Fig. 1.** Isoelectric focusing of Tween–ether-disrupted FeLV. Electrofocusing was in 1% ampholine solution with a pH gradient of 3-10, stabilized with a sucrose gradient (0-40%), at 6°C using a potential of 300 V for 64 hr. After the run, the absorbance at 280 nm (---) was monitored with a Uvicord III absorbometer (LKB, Uppsala, Sweden); the pH was determined at 6°C with an Orion Model 501 pH meter. A. For measuring radioactivity (— — —), a sample (0.1 ml) of each fraction was counted in toluene-based scintillation fluid (3, 4).

B. Fractions were tested for complement-fixing (CF) antigen (— — —) with guinea pig antiserum to purified, SDS-disrupted FeLV. The peak of complement-fixing activity was obtained at pH 8.3 and coincided with the major peak of radioactivity.

**Fig. 2.** Polyacrylamide gel electrophoragrams of FeLV proteins labeled with $^3$H amino acid. The virus, or the purified gs antigen, was treated with SDS-urea–mercaptoethanol, and electrophoresed for 17–18 hr (4). Gels were fractionated on a modified Maizel-type automatic gel divider. Fractions of 0.8 ml, representing about 1.3 mm of gel length, were collected in vials containing 10 ml of toluene-based scintillation fluid (3, 4) and, after overnight standing at room temperature, were counted in a Beckman LS-230 liquid scintillation counter for a sufficient time to obtain a minimum statistical accuracy of 5%.

A. Electrophoresis-purified protein (pH 8.3 fraction). This component migrates to the same position as polypeptide 3 from whole virus.

B. Sucrose density gradient-purified FeLV. The overall pattern is similar to that of mouse and hamster C-type viruses that those three lower molecular weight polypeptides are seen. The average of molecular weight estimates from several runs, calculated from both stained and radioactive gels, was: polypeptide 1-15,000; polypeptide 2-18,000; polypeptide 3-25,000. A higher molecular weight component, polypeptide 4(60,000) is also resolved by this procedure. With other C-type viruses, components in this molecular weight range are generally only clearly resolved when viruses labeled with radioactive glucosamine are analyzed (14, and unpublished results).
C-Type Virus Antigenic Determinants

Fig. 3. Gel-diffusion assays of fractions from the isoelectric focus separation of Fig. 1.
Individual fractions were tested against guinea pig anti-FeLV disrupted by SDS (A), MSV-I (B), and serum from a dog bearing a feline sarcoma virus-induced tumor (C).
Each serum shows a single precipitin reaction, with the strongest activity coinciding with the absorption and complement-fixing maxima at pH 8.3 (fractions 41–43). The activity extending to pH 9.2 (fraction 47) is identical with that seen in the peak fractions. Fractions other than those shown were negative.
Reactions (A) were photographed directly from the micro slides, while (B) and (C) were photographed from polaroid prints.

for mouse viruses. This holds whether test antigens were crude concentrates of disrupted virus or purified antigens. The serum from dogs bearing feline sarcoma virus tumors was also highly species-specific. These anti-FeLV sera all react in superimposable fashion with the fractions obtained from isoelectric focusing, and show a clear peak at pH 8.3 (Fig. 1B).
In gel-diffusion tests, positive reactions were obtained with the fractions corresponding to the small absorption and radioactive peak at pH 9.2 (Fig. 1A). This activity appears immunologically identical to the material in the pH 8.3 fraction.
(b) The MSV-I rat sera, which detect the interspecific antigenic determinant, also react in exactly the same way with electrofocus fractions. When these sera were tested by gel diffusion, the same reaction patterns were obtained (Fig. 3).
There was one apparent paradox, namely that sera specific for cat gs antigen and the cross-reactive MSV-I gave a reaction of identity when diffused against disrupted cat virus or purified gs antigen (Fig. 4A). This paradox was resolved by absorption experiments using the mouse gs antigen. This antigen was able to completely absorb the cross-reacting antibody from MSV-I serum, while it had no effect on the reaction between the anti-cat “specific” serum and cat gs antigen (Fig. 4B). The mouse antigen could be diluted 8-fold (highest dilution tested) and still completely inhibit the reaction of MSV-I with cat gs antigen; no discernable shift in location or intensity of the precipitin line occurred with specific serum, even at the highest concentration of absorbing antigen. The identity reaction clearly results from interactions of antibodies with
Our results and both have SDS-acrylamide gels in distinct 904 Immunology: Oroszlan relative variation weight as an minants gs on cat contained however, the reacting specificities and antigenic composition a component; it appears that the species-specific determinants are “strong” immunogens and may actually inhibit or delay the response to interspec determinants by antigenic competition. The ease in producing interspec antibody of high reactivity in gel diffusion by immunization of rats with MSV tumors (8), and the appearance of complement-fixing interspec antibody in MSV-tumor-stabbed rats (13) may be a special characteristic of rats or, more likely, is based on the nature of the immunizing preparation.

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FIG. 4. Analysis of the cross reaction between murine leukemia virus and FeLV gs antigens by gel diffusion.

A. Identity reaction between MSV-I and guinea pig anti-FeLV gs serum when diffused against FeLV gs antigen.

1. Guinea pig anti-FeLV gs
2. Purified FeLV gs antigen
3. Murine leukemia virus gs antigen (AKR strain)
4. MSV-I

B. Specific absorption of the reaction between MSV-I and FeLV gs by murine leukemia virus gs.

1 and 4. First filled with purified murine leukemia virus gs antigen, then reagents were added as in A.

two distinct specificities against an antigen containing both specificities on the same physical structure.

DISCUSSION

Our results tend to support a unifying hypothesis concerning the structure and antigenic composition of the major gs protein of mammalian C-type viruses. This protein has a calculated molecular weight of 25,000–35,000 (cat, 25,000; mouse, 31,000; hamster, 35,000) in SDS–acrylamide gels, and it carries both species-specific and interspecific cross-reactive determinants as an integral part of its structure. The molecular weight variation for the mammalian virus gs protein appears relatively large for a presumably homologous structural component; however, these estimates are based solely on migration in SDS–acrylamide gels and should be confirmed by other methods. The initial suggestion that the major antigen of the cat contained only interspec determinants is seemingly logical based on identity reactions in gel diffusion; however, as we have shown, this can result from antibodies of two distinct specificities reacting with a single physical structure carrying both specificities. The absorption experiments clearly showed that the cross-reacting anti-murine gs serum is reactive with feline gs determinants other than those detected by guinea pig antiserum to the feline gs antigen. If all gs antigens indeed contain both specific and interspecific determinants, it would seem reasonable to expect immunized animals to produce antibodies of both specificities. In fact, this is the case, as the serum used by Schafer et al. (7) to identify interspec was prepared against purified mouse gs antigen in the rabbit. This serum, and certain of the guinea pig anti-MuLV gs sera, also shows interspec reactions in gel-diffusion assays when concentrated several fold (W. Schafer, personal communication, and our unpublished data). As described here, these sera have also shown positive reactions in complement-fixation tests with purified FeLV gs antigen. It appears that the species-specific determinants are “strong” immunogens and may actually inhibit or delay the response to interspec determinants by antigenic competition. The ease in producing interspec antibody of high reactivity in gel diffusion by immunization of rats with MSV tumors (8), and the appearance of complement-fixing interspec antibody in MSV-tumor-stabbed rats (13) may be a special characteristic of rats or, more likely, is based on the nature of the immunizing preparation.