

Characterization of Epstein-Barr Virus Antigens

I. Biochemical Analysis of the Complement-Fixing Soluble Antigen and Relationship with Epstein-Barr Virus-Associated Nuclear Antigen

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The Epstein-Barr virus-soluble (S) antigen extracted from RAJI cells was characterized by sucrose gradient centrifugation, gel filtration, and ion-exchange chromatography. The sedimentation coefficient was estimated to be 8.5S corresponding to a molecular weight of 180,000. The S antigen binds to DEAE-A25 ion exchanger from which it can be eluted with 0.3 M NaCl in Tris buffer (pH 7.2). All fractions which contained complement-fixing S antigen also inhibited the anticomplement immunofluorescence reaction as used to detect the Epstein-Barr virus-associated nuclear antigen. These results are consistent with the hypothesis that the S and Epstein-Barr virus-associated nuclear antigens are either a single antigen or that both activities are present on the same molecule.

Virus-specific nuclear antigens, detectable by complement fixation (CF), are regularly found in cells transformed by DNA viruses such as polyoma virus, simian virus 40, certain adenoviruses, and Epstein-Barr virus (EBV).

In all EBV nonproducing but genome-carrying cell lines, only the EBV-determined nuclear antigen (EBNA) (8, 9) and the soluble (S) CF antigen (CF/S) (3, 7) can be detected (5). Whether or not both antigens are identical has not been settled. However, they should be closely related since tests to detect both antigens are based on CF, and comparison of EBV serological reactivities, on a large number of sera, between viral capsid antigen, early antigen, EBNA, and CF/S showed that only EBNA and CF/S reactivities were well correlated (2, 6). In this paper we report on the biochemical comparison of CF/S and EBNA antigens.

The EBV-soluble CF/S antigen was prepared as previously described (11). Briefly, RAJI cells (a nonproducer cell line), at a cell density of 1.5×10^6 to 2.0×10^6 /ml, were pelleted and washed three times in veronal-buffered saline. Cell suspensions (10^8 cells/ml) in veronal-buffered saline were frozen and thawed three times, sonicated for four 30-s periods, and clarified at $5,000 \times g$ for 30 min. The supernatant from a 90-min centrifugation at $100,000 \times g$ was used as source of S antigen. The CF test was conducted after the microtiter technique of Sever

(10) using 2 U of guinea pig complement. Each dilution of antigen was tested for anticomplementary activity. Sera, used at 1:10 dilution for the CF test, were obtained from normal donors with CF titers of 1:80, EBNA titers of 1:1,024, and no anticomplementary activity. Anticomplement immunofluorescence (ACIF) absorption tests were carried out as described by Reedman et al. (9), except that the antigen extract was incubated with the serum two dilutions below titer for 1 h at room temperature; the titers corresponded to the final dilution of antigen that extinguishes the ACIF reaction. The conditions of centrifugation and column chromatography are described in the legend of the figures.

As seen in Fig. 1, the S antigen sedimented as a single peak in sucrose gradient with a sedimentation coefficient estimated at 8.5S, using bovine serum albumine (4.4S) and *Micrococcus* catalase (11S) as markers. This value would correspond to a molecular weight of 180,000 for a globular protein. This was confirmed by gel filtration (Fig. 2) where the S antigen eluted immediately after the void volume in Sephadex G200 and in front of human immunoglobulin G in 6B Sepharose. The S antigen also bound to DEAE Sephadex A25 in 0.01 Tris buffer (pH 7.2), and could be eluted with 0.3 M NaCl as a single peak of activity (Fig. 3). In each of the above experiments the recovery of the CF activ-

ity was at least 75%.

All fractions obtained from gradients or columns were tested in parallel for both CF activity and for inhibition of the ACIF reaction by ACIF absorption tests done on RAJI cells. As seen in Fig. 1 to 3, both activities were found in

the same fractions. Antigen titers obtained by CF tests were twofold higher than those obtained by the ACIF absorption test: for example when CF titers would reach 1:8, ACIF inhibition titers would be 1:4. This indicates that, in our hands, the CF test was more sensitive than

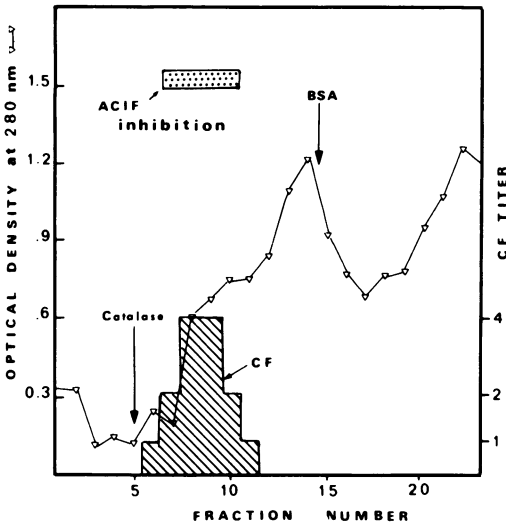


FIG. 1. Sucrose gradient sedimentation analysis of EBV CF/S antigen. A 0.7-ml sample of EBV S antigen, containing 20- μ l of catalase as a marker, was layered on a 5 to 20% (wt/vol) linear sucrose gradient (in veronal-buffered saline) and centrifuged for 36 h at 30,000 rpm in a SW41 rotor at 4 C. A 0.5-ml sample of bovine serum albumine (10 mg/ml) was run in parallel. Fractions (0.5 ml) were collected by bottom puncture and analyzed for absorbance at 280 nm and for biological activity.

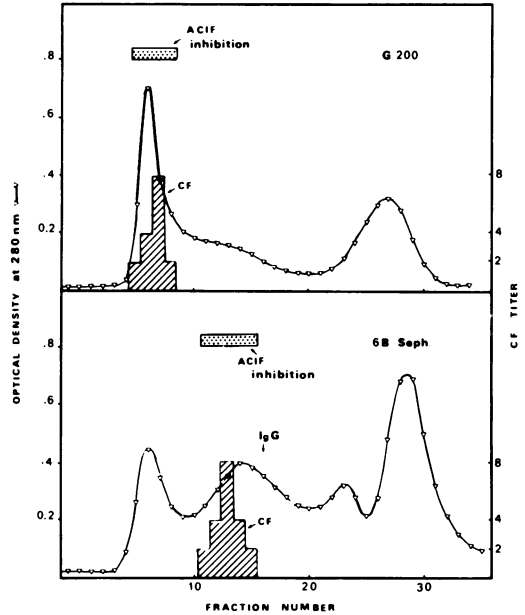


FIG. 2. Gel filtration chromatographic analysis of EBV CF/S antigen. A 1-ml sample of S antigen was applied to columns (1.6 by 40 cm) of G200 Sephadex and 6B Sepharose in 0.01 M Tris buffer (pH 7.2). Fractions (2 ml) were collected, lyophilized, resuspended in distilled water (0.2-ml), and assayed for biological activity.

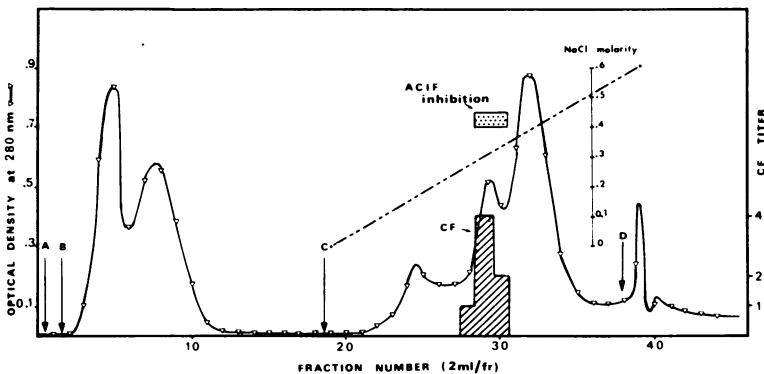


FIG. 3. Ion exchange chromatographic analysis of EBV CF/S antigen. A 3-ml sample of S antigen was applied to a column (1 by 15 cm) of DEAE Sephadex A25 equilibrated with 0.01 M Tris buffer (pH 7.2). The application and elution involved four steps starting at the points indicated by letters as follows: (A) sample application; (B) washing with 0.01 M Tris (pH 7.2); (C) elution with a 40-ml linear gradient (0 to 0.6 M NaCl in 0.01 M Tris, pH 7.2); (D) elution with 1.0 M NaCl in 0.01 M Tris (pH 7.2). Fractions (2 ml) were collected, dialyzed against 0.001 M Tris (pH 7.2), lyophilized, resuspended in 0.2 ml of distilled water, and assayed for biological activity.

the ACIF absorption test.

These results indicate that RAJI extracts, when used as the source of CF/S antigen, contain a molecule of about 180,000 daltons, which carries both CF/S and EBNA activities. Preliminary experiments using high salt concentration or dissociating agents such as urea failed to recover CF/S or EBNA activity on a smaller molecule.

This 180,000-molecular-weight molecule supporting both EBNA and CF/S activities may represent the EBV counterpart of the high-molecular-weight proteins described as early products in infection by other herpesvirus (1, 4), since EBNA is the first antigen detectable after *in vitro* infection by EBV (12).

The biochemical data presented here and those obtained by Baron et al. (personal communication; D. Baron, W. C. Benz, and J. L. Strominger, *Fed. Proc.* **34**:527, 1975) should allow to proceed to the next step, i.e., the purification of this antigenic molecule and the production of specific antisera.

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