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Letter to the Editor

Detection of the Epstein–Barr Virus-Associated Antigens EA (Early Antigen) and VCA (Viral Capsid Antigen) by Direct or Indirect Binding of Iodinated Antibodies to Antigen Immobilized in Polyacrylamide Gel*

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THE EBV (Epstein–Barr virus-associated) antigens [1] are detected and defined by the reaction of human sera, containing antibodies to at least two or more EBV-antigens, against defined cells that contain one or more antigens: EBNA (nuclear antigen [2]), EA (early antigen [3]), VCA (viral capsid antigen [4]) and MA (membrane antigen [5]). So far, there are no animal sera available that would detect any EBV-antigens specifically, even less are there any monospecific sera. Therefore, the combination of human sera with cells of known antigen content is the only available material for serological tests. Immunofluorescence has been widely used as a qualitative technique, but this method is quite unsuitable for quantitation in a biochemical study of these antigens. Radioimmunoassays using antigen immobilized in polyacrylamide gel and iodinated antibodies were already developed for MA [6] and EBNA [7]. This paper describes an assay detecting also EA and/or VCA in the presence of EBNA.

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The properties of the cell lines, the preparation of cell extracts, the immobilization of cell homogenates in polyacrylamide gel and the details of the solid-phase radioimmunoassay have been previously reported [6, 7]. Figure 1 shows a comparison of the binding of ^{125}I-IgG prepared from different anti-EBV positive sera, discordant or concordant with regard to their EA/VCA titers. In the case of B95-8, Daudi and Daudi/IUDR treated cells, the highest percentage of radioactivity was bound from "Adala" IgG(A), less from "Katana" IgG(B), "Abdi" IgG(C) and the anti-EBV negative control sera "BA" or "Ingemar" (D). These results are in line with the antibody titers determined by immunofluorescence: "Katana" and "Abdi" are discordant sera with high or medium titers against VCA, but no anti-EA detectable by direct or indirect immunofluorescence, and "Adala" is a serum with high titers against all EBV-associated antigens. The gels prepared with producer lines (EBNA+, EA+, VCA+, MA+) bound 2–3 times more antibody from "Adala" than the non-producer only EBNA positive cell lines [7]. As shown later, this difference is due to the presence of EA and VCA in addition to EBNA in producer lines. In addition, two other observations need mentioning. There is an increased binding of ^{125}I-IgG "Adala" to IUDR treated Daudi compared with untreated Daudi. As "Katana" and "Abdi",

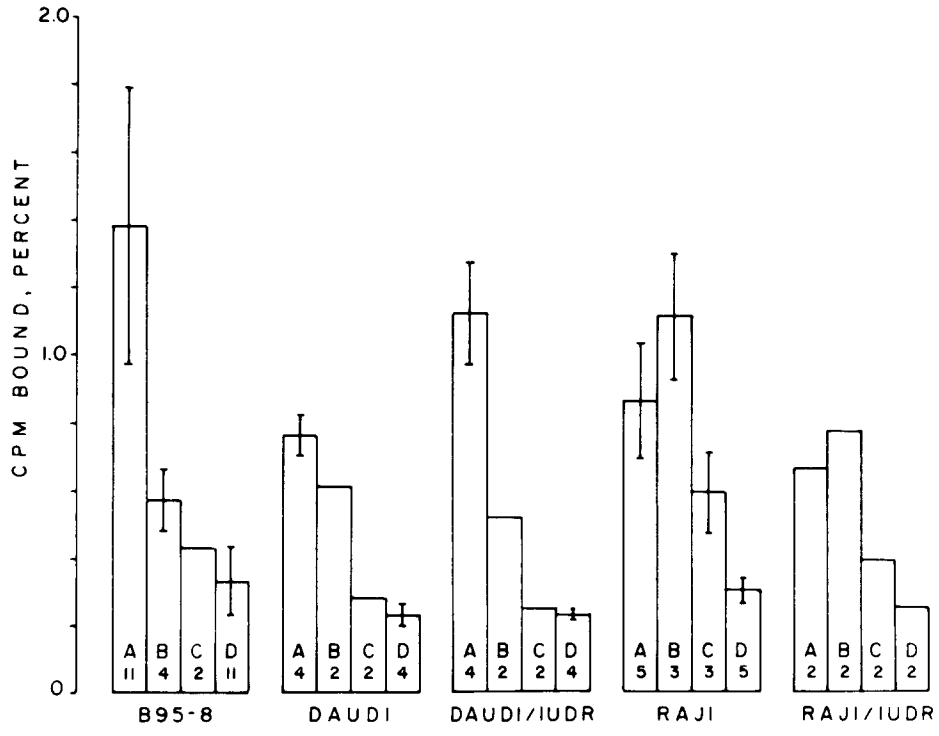

Fig. 1. Binding of ^{125}I-IgG *prepared from anti-EBV positive and negative sera to polyacrylamide gel immobilized cell homogenates prepared from B95-8, Daudi and Raji cells. The standard deviations* (±S.E.) *are indicated. The following sera were used: A="Adala" (anti-VCA 2560; anti-EA 1280; anti-EBNA 80; anti-MA 0.95); B="Katana" (anti-VCA 320; anti-EA <10; anti-EBNA 5; anti-MA not tested); C="Abdi" (anti-VCA 160; anti-EA <10; anti-EBNA 40; anti-MA 0.08); D="Ingemar" or "BA" (anti-VCA <5; anti-EA <5; anti-EBNA <2; anti-MA 0.00). The number below the serum code indicates the number of tests done. Raji and Daudi cells were treated with 60 μg/ml IUDR for two days. IF-positive cells were determined with FITC-Katana (diluted 1:10) and FITC-Chell or FITC-Tabu (anti-EA+, anti-VCA+, diluted 1:40 and 1:80 resp.). Percentage antigen positive cells: B95-8 (3.8% VCA, 5.4% EA); Daudi (0.4% VCA, 1.1% EA); Daudi/IUDR (0.0% VCA, 10% EA); Raji/IUDR (0.0% VCA, 2% EA); Raji (0.0% VCA, 0.1% EA).*

both anti-EA negative, gave less binding to these gels, it can be speculated that IUDR treated Daudi contain an additional antigenic specificity, probably part of the EA complex. Using both Raji and Raji/IUDR immobilized in polyacrylamide gel, "Katana" IgG showed a higher binding compared to "Adala" IgG suggesting an additional EBNA specificity.

Blocking tests (results not shown) carried out with various anti-EBV positive and negative sera [7] showed a good correlation between the anti-EBNA ($r=0.816$; $n=40$), anti-EA ($r=0.779$; $n=48$) and anti-VCA titers ($r=0.815$; $n=49$) of the sera and their blocking indices in the radioimmunoassay, when ^{125}I-IgG "Adala" and B95-8 cells were used. No correlation was found between the blocking indices obtained in the radioimmunoassay and the anti-MA titers of the sera ($r=0.146$; $n=35$). This indicates that the anti-MA present in the "Adala" reagent does not contribute significantly to the total binding of this reagent to the gel made with B95-8 cells. Serum blocking tests with ^{125}I-IgG "Katana" and B95-8 cells immobilized in the gel gave a good correlation between the anti-VCA ($r=0.760$; $n=32$) and anti-EBNA titers ($r=0.795$; $n=32$) of these sera and their blocking indices obtained in the radioimmunoassay, but not with the anti-EA titers ($r=0.560$; $n=32$). This is explained by the fact that "Katana" contains no anti-EA detectable by immunofluorescence.

To gain additional information about the antigenic specificities detected by this assay, inhibition studies were performed with cell extracts. Table 1 shows the results of comparative assays with 100,000 ***g*** supernatants of NP40 (Nonidet P40) extracts from different cell lines tested on B95-8, Daudi and Daudi/IUDR cell homogenates polymerized in polyacrylamide gel. The EBV genome negative BJAB cell line did not significantly inhibit the binding of "Adala" to any of these gels.

Table 1. Inhibition of ^{125}I-IgG "*Adala*" *binding to polyacrylamide gels containing homogenates of different EBV genome positive producer cell lines by preincubation with* 100,000 *g supernatant fractions of NP*40 *treated total homogenates of EBV genome positive cells and the EBV genome negative cell line BJAB*

Extract	Cell homogenate polymerized in polyacrylamide gel		
	B95-8	Daudi	Daudi/IUDR
BJAB	8	0	0
AW-Ramos	24	not tested	57
Raji	25	42	77
Raji/IUDR	40	57	85
Daudi	75	100	83
Daudi/IUDR	70	71	100
B95-8	98	98	88

Supernatant fractions corresponding to 10^7 cells were used per test. The data are given as the percentage of inhibition relative to a control incubation with ^{125}I-IgG "Adala" alone.

In the case of the B95-8 gel the extracts from the EBV genome positive, EBV-antigen-carrying cells inhibited the binding of the reagent in direct relation to their antigen content (see also legend to Fig. 1), i.e., AW-Ramos (only EBNA+) $\leq$ Raji < Raji/IUDR < Daudi < B95-8. Using Daudi and Daudi/IUDR cells as immobilized antigens the various extracts gave the expected inhibition. The cross-inhibition experiments with gels and extracts showed incomplete inhibition since Daudi/IUDR contains much more EA and presumably more EA-specificities than Daudi, and on the other hand Daudi/IUDR no VCA compared with Daudi. As EA is present in all gels, the Raji/IUDR extract inhibited always more than the Raji extract. This is in agreement with the increased EA content of Raji/IUDR compared to untreated Raji. These results show that it is possible to compare the different cell lines for their antigen content with regard to EBNA, EA and VCA. By using ^{125}I-IgG "Adala", all three antigens are detected and contribute significantly to the total binding of the iodinated antibody. Only EBNA and VCA are detected, if ^{125}I-IgG "Katana" is used as the reagent. This was already shown by serum blocking tests, but could be confirmed by the following inhibition assay with B95-8 cells immobilized in polyacrylamide gel (Fig. 2). Non-producer only EBNA positive AW-Ramos and low EA positive Raji cells were used for antigen preparation in addition to B95-8 and the B95-8 virus transformed cord blood cell line cb 37 b4 (M. Dalens, unpublished experiments). EBV-transformed cord blood cell lines are usually non-producers, but the inhibition curve of cb 37 b4 was very similar to the B95-8 extract inhibition

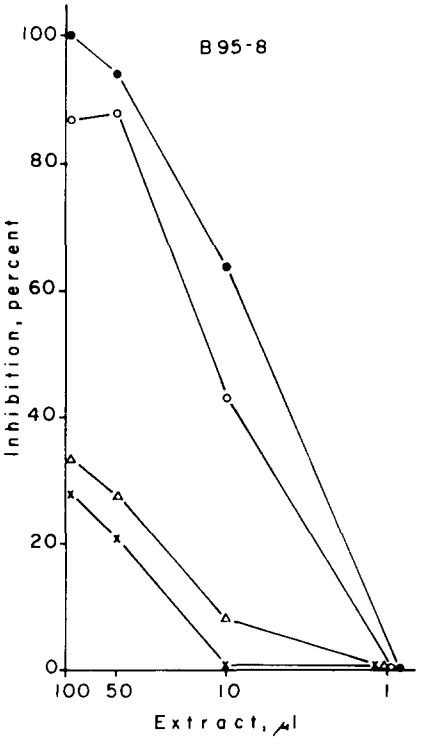

Fig. 2. Inhibition of ^{125}I-IgG "*Katana*" *binding to B*95-8 *cell homogenate immobilized in polyacrylamide gel by preincubation of iodinated antibody with* 100,000 *g supernatant fractions of NP* 40 *treated homogenates of B*95-8 (●—●), *cb* 37 *b*4 (○—○), *AW-Ramos* (×—×) *and Raji cells* (△—△).

curve suggesting that it is a producer line. Parallel staining for EA and VCA by immunofluorescence (IF) and/or autoradiography confirmed this: 7% EA+, 3% VCA+cells. The AW-Ramos and Raji extracts showed no significant difference in their inhibitory capacity. This is again in line with the fact that "Katana" contains no anti-EA.

An alternative way to show that the assay detects EA or VCA than by serum blocking tests or extract inhibition tests is to bind and elute the antibodies of interest from the antigen immobilized in the gel. The specificity of antigen detection can be demonstrated further by autoradiography on fixed cells [8], positive or negative for the antigen. The producer cells, B95-8 or P3HR-1, were polymerized in polyacrylamide gel. ^{125}I-IgG "Adala" or "Katana" were allowed to react with the immobilized antigens. After several washings the bound radioactivity was eluted with 0.2 M glycine pH 2.8 and the eluted antibodies were used in autoradiography on acetone fixed P3HR-1 cells (10% EA+, 4% VCA+by IF) after neutralization of the eluate. With eluted antibodies from "Katana" 4% of the cells were found positive by autoradiography which corresponds to the number of VCA positive cells tested by IF, and with "Adala" IgG 10% positive cells equal to the number of EA/VCA positive cells determined by IF. Control experiments using EA/VCA negative cells for autoradiography with eluted antibodies from "Katana" or "Adala" gave negative results, as expected. However, by autoradiography, we did not observe any nuclear reaction common to all EBV-DNA carrying cells which could be due to an EBNA/anti-EBNA reaction with the eluted antibodies which would be expected to be enriched for anti-EBNA. Nevertheless, since even total ^{125}I-IgG also fail to consistently give a reaction by direct autoradiography, the current detection system may not be sensitive enough, as EBNA is generally detected by anticomplementary IF.

On the basis of the two techniques described it could be possible to prepare monospecific reagents for the various EBV-associated antigens. The specificity of these reagents can be tested by the solid-phase radioimmunoassay at the gross cell level and by autoradiography at the single cell level. Such monospecific reagents will facilitate a comparative analysis of subcomponents within the known EBV-associated antigen complexes [6, 9, 10].

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