Presence of Chicken Cell Surface Antigen on Rous Virus Activated in Heterokaryons of Transformed Non-permissive Hamster Cells and Chicken Cells

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SUMMARY

Incubation with antiserum to chick embryo (CE) cells, in the presence of complement, inactivates Rous sarcoma virus (RSV) produced by heterokaryons formed by non-permissive RSV-transformed hamster cells and CE cells as well as RSV produced by CE cells. No inactivation of RSV produced by heterokaryons is observed following incubation with antiserum to the transformed hamster cells, plus complement. Hence, the envelope of RSV activated in heterokaryons, as that of RSV produced by CE cells, must contain a surface antigen of the CE cell, and the virus must mature only, or preferentially, at chicken-specific sites of the heterokaryon surface.

We have recently reported (Aupoix & Vigier, 1975) that fowl sarcoma viruses of the A, B, C, D and E subgroups are inactivated markedly, in the presence, but not in the absence, of complement, by the serum of rabbits immunized against normal chick embryo (CE) cells from a RIF-free fowl strain, the same cells being also used for producing the A, B, C and D viruses. This inactivation is paralleled by a characteristic swelling and loss of opacity to electrons of the virus particles which presumably precede virolysis, and it appears to be due to antibodies to some antigen(s) common to the surface of CE cells and to the virus envelope. This host cell surface antigen (HCSA) is also present on the surface of the helper viruses RAV1 and RAV2 of Bryan strain Rous sarcoma virus. Therefore, it may be required for the maturation of all chicken leukaemia and sarcoma viruses (ChiLSV).

If HCSA is necessary for the maturation of ChiLSV, it should also be present on virus which is activated when non-permissive mammalian cells transformed by Rous sarcoma virus (RSV) form heterokaryons with CE cells, in the presence of inactivated Sendai virus (cf. Vigier, 1973). Therefore, we have investigated the presence of HCSA on the envelope of virus produced by heterokaryons formed, in the presence of Sendai virus, by CE cells and cells of a subclone (RS2/3) derived from the RS2 clone obtained by transforming hamster BHK21/C13 cells with SR-RSV-D (Montagnier & Vigier, 1967). As was shown by earlier investigations (Vigier, 1967; Vigier & Bataillon, 1971; Vigier, 1973) RS2 cells all contain the full RSV genome but some subclones, notably RS2/3 and RS2/10 contain significantly more virus group-specific (gs) proteins, as measured by the COFAL test, than others and, also, give rise to a higher number of virus-producing heterokaryons with CE cells. This led us to select the RS2/3 subclone for our investigations.

These investigations required that RSV should originate only from heterokaryons, and be available in sufficient amounts for the inactivation tests. Unfortunately, under optimal conditions of fusion of RS2 cells and CE cells with Sendai virus, only a low proportion of cells (less than 10%) form heterokaryons and, therefore, there remains a large excess of CE cells which can be infected secondarily by the activated RSV (Vigier, 1973). Consequently, only the earliest virus produced in the cultures definitely originates from heterokaryons. However,
Fig. 1. Inactivation by $[^{60}Co]$$\gamma$ rays of the virus-producing capacity of heterokaryons of RS2/3 and CE cells. RS2/3 cells, irradiated at 5000 R with $[^{60}Co]$$\gamma$ rays were plated with a 30-fold excess of CE cells in 5 cm dishes ($3 \times 10^8$ cells/dish) and the mixed cells were fused the next day, with u.v.-inactivated Sendai virus (1 H.A.U./$10^9$ cells). The cultures were subsequently irradiated with various doses of $\gamma$ rays and incubated 2 days at 37 °C. The medium was then harvested and assayed for free virus (▲, ▼). Alternatively, $10^6$ CE cells were added to the irradiated cultures and the cultures were overlaid with agar medium 16 h later. Foci produced by virus-releasing heterokaryons (infective centres: △) were counted 8 days later. △, Expt. 1; ▼, Expt. 2.

the amount of this early virus is generally very low. Hence, to obtain enough virus originating only from heterokaryons it was necessary to prevent secondary infection of non-fused CE cells, so as to be able to use not only the earliest virus produced by heterokaryons (the latent period is generally about 24 h; Vigier, 1973) but also the virus produced later on.

This condition was fulfilled by taking advantage of two earlier observations. First, as was shown by Rubin & Temin (1959), and Temin & Rubin (1959), the capacity of CE cells to support RSV replication following low multiplicity infection is very sensitive to X-rays, when the cells are irradiated before, or immediately after infection. The capacity to support virus replication is then inactivated following low multiplicity infection with the same X-ray dose that is required to halt cell division, i.e. 300 to 600 R for a single lethal hit. Secondly, investigations by one of us (P. Vigier, unpublished results) have shown that when heterokaryons formed by RSV and CE cells are irradiated with $\gamma$ rays immediately after cell fusion, their capacity to subsequently become virus producers is also inactivated following one hit kinetics, but is about 100 times more resistant to photon inactivation than the capacity of CE cells to support virus replication, since about 40000 R are required for a single lethal hit. This is illustrated in Fig. 1, from which it is also seen that inactivation of the capacity of heterokaryons to
become infective centres is accompanied by a parallel decrease of the overall virus production. On the basis of these earlier findings, we decided to expose to γ rays the mixed cultures of RS2/3 and CE cells after the Sendai virus-mediated fusion, so as to reduce to a negligible level the capacity of CE cells to become infected secondarily and replicate the virus without impairing the virus-producing capacity of heterokaryons. The dose of γ rays chosen was 3000 R, which reduces from 100- to 10,000-fold the first capacity, but by less than 10 % the second one.

Two methods of cell fusion, described earlier (Vigier, 1973), were used, in two separate experiments. In the first experiment, 3 × 10⁷ trypsinized RS2/3 cells were irradiated at 5000 R with [⁶⁰Co]-γ rays, in culture medium, then mixed with 15 × 10⁷ CE cells, and plated in fifty 5 cm Falcon Petri dishes (3.6 × 10⁶ cells/dish). The next day, the mixed cells were fused with u.v.-irradiated Sendai virus (1 H.A.U./10⁷ cells) and irradiated immediately afterwards at 3000 R with [⁶⁰Co]-γ rays. The cultures were subsequently incubated at 37 °C, and the medium renewed and harvested 1, 2 and 3 days post fusion. The reason for irradiating RS2/3 cells before plating was to prevent them from overgrowing CE cells before fusion. This treatment has no measurable effect on the virus-producing capacity of the heterokaryons (Vigier, 1973).

In the second experiment, 3 × 10⁷ RS2/3 cells, also irradiated at 5000 R, were mixed with 9 × 10⁷ CE cells, and the mixed cells were pelleted by low speed sedimentation and fused, in suspension, with inactivated Sendai virus. They were subsequently plated in replicate dishes, and irradiated at 3000 R 20 h later. The culture medium was harvested 1, 2 and 3 days post fusion, as in the first experiment. The reason for delaying the irradiation of heterokaryons, in this second experiment, was to reduce the chances of repair of radiolesions in CE cells. On the other hand, the eventuality of secondary infection of CE cells by virus produced by heterokaryons before the irradiation was negligible, since virus production in Sendai-treated cultures is generally not observed before 24 h post fusion (Vigier, 1973), and, moreover, the capacity of CE cells to replicate RSV after low multiplicity infection remains highly radiosensitive in most cells for 7 to 8 h post infection (Temin & Rubin, 1959).

In both experiments, the harvested media were pooled and centrifuged for 1 h at 55,000 g, at 4 °C, to pellet the virus released by the heterokaryons and the pellet was resuspended in 3 ml of culture medium. Samples of the virus thus concentrated were incubated with anti-CE rabbit serum plus complement, and assayed by the focus assay, as described earlier (Aupoix & Vigier, 1975). Since the amount of virus produced by the heterokaryons was low, and the virus samples must be diluted 20-fold before assay, each sample was assayed on 20 replicate cultures of CE cells. In addition, DEAE-dextran (40 µg/ml) was added to the diluted samples, in order to increase the efficiency of infection of the assay cells (Hanafusa, 1969). Samples of the concentrated virus were also incubated, in the presence of complement, with antiseraum to RS2/10 cells, to see whether the virus carried surface antigens of the RS2 cells. Since RS2/3 and RS2/10 cells originate from the same clone, it was assumed that their surface antigens must be the same.

As can be seen from Table 1, the virus produced in both experiments was inactivated only by anti-CE serum plus complement. Therefore, it must carry HCSA on its envelope, but no surface antigen of the RS2 cells. This was confirmed in a control experiment (Expt. 3) in which the mixed cultures were prepared as in Expt. 1, but not irradiated after the cell fusion. Instead, the medium was harvested at 6 h intervals, starting 24 h after the fusion and the different harvests were centrifuged separately, as already described, so as to determine at which time the virus was first produced. Since the latent period for a replication cycle in CE cells in these conditions is at least 14 h (Vigier, 1973), the virus found at that time could
Table 1. Inactivation of RSV from heterokaryons by anti-CE serum and complement*

<table>
<thead>
<tr>
<th>Expt. no.†</th>
<th>Serum</th>
<th>Surviving fraction of virus‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anti-CE 0.05 (20)</td>
<td>0.05 (20)</td>
</tr>
<tr>
<td></td>
<td>Anti-RS2 0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>2</td>
<td>Anti-CE 0.06 (58)</td>
<td>0.06 (58)</td>
</tr>
<tr>
<td></td>
<td>Anti-RS2 0.91</td>
<td>0.91</td>
</tr>
<tr>
<td>3</td>
<td>Anti-CE &lt; 0.25 (4)</td>
<td>&lt; 0.25 (4)</td>
</tr>
<tr>
<td></td>
<td>Anti-RS2 0.75</td>
<td>0.75</td>
</tr>
</tbody>
</table>

* Virus pelleted from the medium of mixed cultures of RS2/3 and CE cells (cf. text) was incubated 1 h at 37 °C with antiserum (anti-CE: anti-chick embryo fibroblasts; anti-RS2: anti-RS2/10 cells) and complement, in the ratio of 9:1:2. The mixture was subsequently assayed by the focus assay.

† In Expt. 1 and 2, the presence of virus originating from CE cells was excluded by irradiating the mixed cultures after the Sendai virus-mediated cell fusion, whereas in Expt. 3, it was excluded by retaining only the harvested medium in which virus was detected for the first time (30 h post infection; cf. text).

‡ In parenthesis, focus count in control (sum of all assay plates).

only originate from heterokaryons. Virus was first found at 30 h, and this virus was incubated with anti-CE or anti-RS2 serum plus complement and assayed as already described. Once again, it was inactivated only by anti-CE serum plus complement. However, the degree of inactivation could not be determined, because of the low titre of the virus. From this last viewpoint, it was further noted that the degree of inactivation by anti-CE serum and complement of the virus produced by heterokaryons in irradiated cultures was lower (5% survival against 1 to 3%) than the degree of inactivation of virus produced by CE cells (Aupoix & Vigier, 1975), although all controllable parameters of the inactivation tests (serum, complement, time of incubation, method of assay) were the same. However, the significance of this difference is doubtful.

To summarize, the results of our experiments show that the envelope of RSV produced by heterokaryons contains HCSA as that of RSV produced by CE cells, and, therefore, the activated RSV must mature and bud at chicken-specific sites of the heterokaryon surface. However, this does not demonstrate that CE HCSA is indispensable for virus maturation and release. Indeed, a low amount of virus could also bud at hamster-specific sites of the heterokaryon surface. On the other hand, some lines of mammalian cells transformed by avian sarcoma viruses can produce low amounts of virus (Svoboda, 1968; Svec et al. 1970). Therefore, the presence of HCSA may account for the high virus production of CE cells, but not be an absolute prerequisite for virus maturation and release.

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REFERENCES


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