Cell-Surface Changes after Infection with Oncogenic Viruses: Requirement for Synthesis of Host DNA

Abstract. *The change in the surface structure of cultured mammalian cells infected with oncogenic DNA viruses was similar to that described for the fully and permanently transformed cell surfaces. The course of appearance of this change was established. Synthesis of host DNA is required for expression of the surface change triggered by infection with the oncogenic virus.*

Cultured animal cells that have undergone transformation by virus are characterized by a variety of changes when compared to the noninfected parent cell (*1*). The changes in cell surface may alter the growth characteristics of infected cells (*2*). Changes in the antigenic (*3*) and chemical (*4*) properties of the transformed cell surface have been reported, and some of them can be correlated with the loss of growth control (*5*).

The agglutinability of almost all permanently transformed cells is increased by pure wheat germ agglutinin (WGA), in comparison with that of noninfected parent cells (*6, 7*). The WGA surface receptor site, which is present in normal cells in a cryptic form, may be exposed by brief treatment with proteolytic enzymes (*7*); it was recently demonstrated that surface receptors for another agglutinin, as well as for tumor antigens, are also components of normal cell surfaces and that they become exposed in transformed cells (*8*).

Other results indicated that the WGA site was exposed approximately 72 hours after the 3T3 cells were infected with SV40 virus (abortive infection). These results were confirmed by data obtained from similar experiments with a permissive virus infection (*9*).

While studying the appearance of a surface change during lytic infection and abortive transformation, we found that the surface structure was changed only when a virus-directed or -induced synthesis of host DNA occurred after infection. Division of 3T3 cells (abortive infection) has been reported to be a requirement for the exposure of an agglutinin site after viral infection (*10*). We now present evidence that virus-directed or -induced synthesis of DNA is required for the cell surface alteration that occurs after viral infection.

Figure 1 shows that in both lytic infection (SV40/CV-1, Ad-5/CV-1, Ad-5/BSC-1) and abortive transformation (SV40/3T3) agglutinability increased five- to tenfold within 24 to 72 hours after infection. The WGA site was exposed in the monkey cell lines BSC-1 and CV-1 24 to 30 hours after infection with adenovirus. These cells, however, showed quite different reactions to SV40 infection: the WGA site in CV-1 cells was exposed as with adenovirus, but at a later time (40 hours); the WGA site was not exposed at all in the BSC-1 cells (*11*), even after 120 hours, at which time a cytopathic effect was evident.

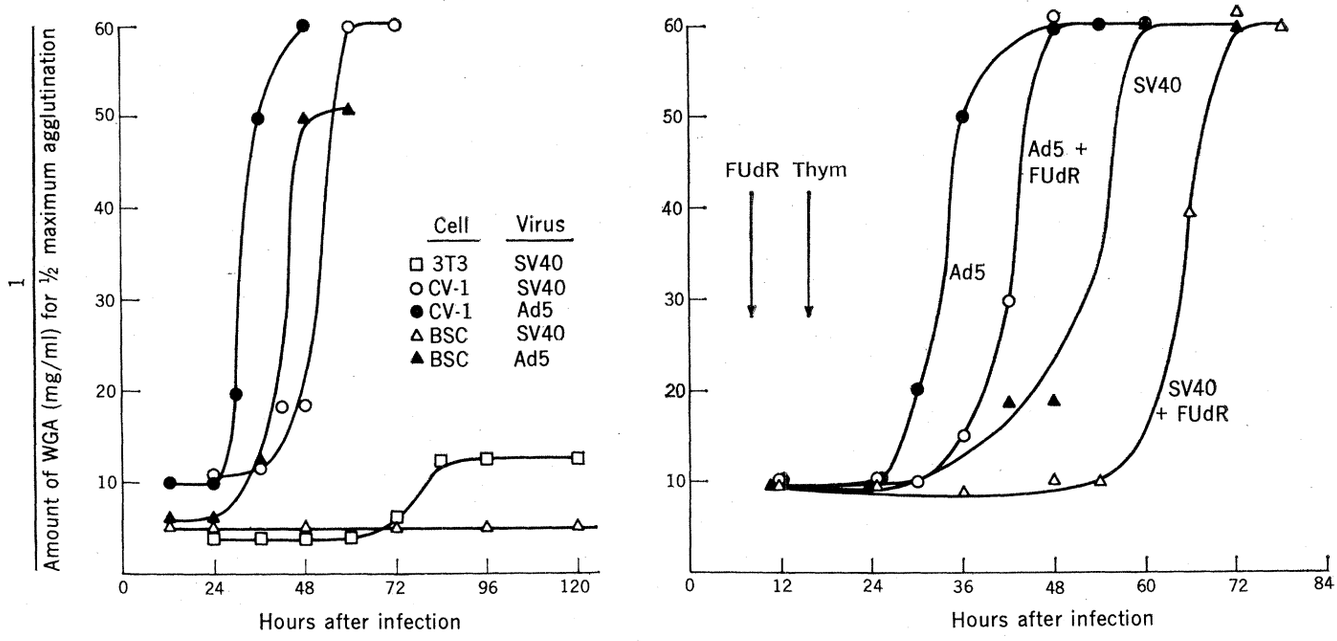

Fig. 1 (left). Time course of agglutinability of cells infected by SV40 or adenovirus. All cell cultures were maintained at 37°C in plastic petri dishes (100 cm^2) containing modified Eagle's medium supplemented with calf serum (10 percent) and penicillin-streptomycin (1 percent) as modified by Smith *et al.* (*17*). Wild-type SV40 and type 5 adenovirus (*Ad*) were used in multiplicities of infection from 50 to 100. After absorption of the virus for 2 hours, the cells were washed twice with medium. In mock infections (controls) the virus was omitted. The agglutination assay has been described (*13*). Half maximum agglutination is the point where 75 percent of the cells have exposed surface sites (= 2 + on a scale from 0 to 4 +); therefore some cells must have undergone this cell surface change before the first increase visible on the graph occurred. Agglutination after infection was not just the result of release of proteolytic enzymes from the damaged surface, since medium collected from BSC-1 cells 48 hours after infection with adenovirus did not cause exposure of WGA sites on uninfected BSC-1 cells. Fig. 2 (right). Time course of agglutinability of CV-1 cells. DNA synthesis was inhibited by FUdR at a final concentration of $2 \times 10^{-6}M$ added 8 hours after infection. The medium was changed 18 hours after infection, and the cells were rinsed once with medium and then with medium supplemented with $10^{-5}M$ thymidine (*Thym*). Similar results were obtained with 5×10^{-3} and $1 \times 10^{-3}M$ hydroxyurea, which is known to specifically inhibit the formation of deoxyribonucleotides from ribonucleotides (*18*) and thereby interfere with DNA synthesis before any other macromolecular synthesis.

Although the WGA site in BSC-1 cells was not exposed after SV40 infection, exposure is possible since the site was exposed after infection with adenovirus. The site can also be exposed by brief treatment of the cell surface with trypsin 60 hours after infection. Agglutination after treatment with trypsin was quantitatively and qualitatively [*N*-acetylglucosamine inhibition (*6*)] identical with agglutination in adenovirus infected cells.

Viral infection usually induces or increases the rate of DNA synthesis in the host cell; however, no such synthesis is induced by infection of BSC-1 cells with SV40 (*12*). Figure 1 shows that the only cell in which the WGA site was not exposed after SV40 infection was also the BSC-1 cell. Since there was potential for exposure of the WGA site after infection of the BSC-1 line with another virus (adenovirus), which also transiently induced host DNA synthesis, we designed experiments to answer the question whether inhibition of DNA synthesis would prevent exposure of the WGA site after infection (Fig. 2).

Infection of CV-1 cells by adenovirus or SV40 would normally lead to exposure of the WGA site after 30 and 40 hours, respectively. An inhibitor of DNA synthesis added 8 hours after infection and maintained for 10 hours delayed exposure of the WGA site (Fig. 2). Similar results have been observed when fluorodeoxyuridine (FUdR) and hydroxyurea were used as inhibitors of DNA synthesis in 3T3 cells infected with polyoma virus (*13, 14*).

These results, together with those obtained when BSC-1 cells were infected with SV40, indicate that synthesis of host DNA is required for the observed change in the cell surface. The alternative interpretation that synthesis of viral DNA is required can be rejected for the following reasons:

1) Little or no SV40 viral DNA is synthesized in 3T3 cells, but the cell surface site is exposed (Fig. 1).

2) A temperature-sensitive mutant of polyoma virus causes exposure of the site under conditions in which viral DNA is not synthesized (Py-ts 616) (*14*).

3) That synthesis of host DNA is required for the cell surface change after infection is also supported by experiments with another BSC-1 cell line (*15*), which was different from the one used in our study. In these other cells in which the synthesis of DNA was induced with SV40, the WGA site was exposed by 36 hours after infection, as predicted. Results of studies with this second cell line, which bears the designation BSC-1 but which appears to be a different line, are in agreement with the results of studies of the inhibition of host-DNA synthesis after viral infection.

Benjamin's host-range mutant of polyoma virus (*16*) has been shown to initiate DNA synthesis in 3T3 cells without the subsequent exposure of the WGA sites. But exposure of the site has not been demonstrated in the absence of previous DNA synthesis. These results indicate that DNA synthesis is necessary for the appearance of the cell surface alteration but that DNA synthesis alone does not ensure exposure of WGA sites after infection with an oncogenic virus.

We conclude that (i) transformation, lytic infection, and abortive transformation lead to the agglutinable state of the cell surface; and, that (ii) synthesis of host DNA appears to be necessary for the alteration in the surface of the membrane brought about by infection with oncogenic viruses.

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Maternal-Fetal Interaction and Immunological Memory

Abstract. *Female rats of the poorly responding, inbred F344 strain were immunized with poly($Glu^{52}Lys^{33}Tyr^{15}$) aggregated with methylated bovine serum albumin, and then they were mated. The first and second litters in the F_1 generation and in the F_2 generation showed an enhanced immune response. When polylysine was used as the aggregating agent, enhancement occurred in only the first litter of the F_1 generation and in the F_2 generation. In both cases, antigen was transmitted from the immunized female to her offspring, where it localized in the bone marrow and, in a few cases, in the thymus and spleen also. The transplacental passage of antigen is probably the basis for the enhanced antibody response, which is a manifestation of immunological memory.*

The major assumption underlying much of the work from this laboratory is that antigen metabolism plays a crucial role in the induction and quantitative regulation of the immune response. This concept has been explored in broad outline by Haurowitz and others (*1*) and by Campbell and Garvey (*2*). We have sought to develop it in such a manner that it might offer a consistent explanation of the various aspects of the immune response as quantitative, antigen-driven phenomena. Studies on the chemistry of immunogenicity (*3*) led to the hypothesis that there is a balance between immunological stimulation and paralysis, which is determined by the structure of the antigen. This structure sets the size of the dose that can be used in immunizing an animal. The rate at which antigen is degraded determines the amount left intact; thus, antigen catabolism acts in concert with the original dose to regulate the amount of antigen available for stimulating antibody formation. Our observations on the enhanced ability of the offspring of immunized rats to form antibody (*4*) suggest an experimental system which can extend these concepts and provide a unique means for investigating the role of antigen in immunological mem-