Introduction of Human Genomic Sequences Renders CHO-K1 Cells Susceptible to Infection by Amphotropic Retroviruses

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To learn more about the nature of the block to infection by amphotropic retroviruses exhibited by Chinese hamster cells (CHO-K1), CHO-K1 cells were made susceptible to amphotropic retrovirus infection by introducing genomic DNA from infectable human cells. A clone, designated CHO18, was obtained and shown to be infected as efficiently as NIH 3T3 fibroblasts. Susceptibility of CHO18 cells to infection was specific to retroviruses and vectors bearing an amphotropic envelope. By comparison to CHO-K1 cells, CHO18 cells may provide a useful model for analysis of the molecular events involved in the retrovirus-receptor interaction.

Retroviruses may be classified by the range of species that they are able to infect. Mouse type C retroviruses fall into five general classes: amphotropic, which exhibit the broadest host range, encompassing a diversity of mammalian species including humans and rodents; polytropic, which use a receptor distinct from that for amphotropic viruses and also exhibit a more restricted host range; 10A1, which uses both the amphotropic receptor as well as a second, widely expressed, receptor (13); xenotropic, which also have a wide mammalian species host range but cannot infect mouse cells; and ecotropic, which infect only rodent species. Of these five mouse retrovirus receptor classes, only the receptor for the ecotropic Moloney murine leukemia virus (MLV) has been cloned (1).

Human cells have been characterized as having at least eight distinct receptors for retroviruses (16). Of these, two have now been cloned: the receptor for human immunodeficiency virus, a lentivirus (8, 9), and the receptor for gibbon ape leukemia virus, a type C retrovirus (12). The receptor used by human immunodeficiency virus, the CD4 antigen, is similar to receptors used by a number of other viruses in that it is a member of the immunoglobulin superfamily of cell surface proteins. In contrast, the gibbon ape leukemia virus receptor is not an immunoglobulin-like protein (12). Both it and the receptor for MLV have multiple membrane-spanning domains characteristic of transporter proteins. Indeed, the MLV receptor has recently been shown to be a transporter of cationic amino acids (7, 18).

Although amphotropic mouse retroviruses have a broad tropism, there are restrictions to their infection, both phylogenetic and in terms of tissue distribution. Cell lines derived from certain mammalian species or tissues, for example, the bovine kidney cell line MDBK and the Chinese hamster ovary cell line CHO-K1, are not infectable by mouse amphotropic retroviruses (4, 14). Other tissues, such as human lymphoid cells, are poorly infectable and express relatively low amounts of the receptor on their surfaces (6). In an effort to learn more about the nature of the block to infection, we have attempted to render the uninfected CHO-K1 cells

susceptible to amphotropic retrovirus infection through the introduction of human genomic DNA.

The scheme for the generation of an amphotropically infectable CHO-K1 derivative is outlined in Fig. 1. Ten 100-mm-diameter tissue culture dishes of CHO-K1 cells were transfected (20), each with 20 µg of DNA (average size, 25 to 50 kb as judged on a 0.7% agarose gel) prepared from K562 cells (CCL 243; American Type Culture Collection) together with 1 µg of a hygromycin resistance plasmid (pHyg) (17). After selection for the cotransfected hygromycin-selectable marker, a stably transfected population was obtained. These selected cells were then incubated overnight in medium containing the N2 retroviral vector (2) packaged in the amphotropic PA317 (CRL 9078; American Type Culture Collection) packaging cell. The N2 vector carries the neomycin resistance marker and confers resistance to G418. The vector titer in the PA317/N2-conditioned medium was 5×10^6 CFU/ml. Infected cells were identified by selection in medium containing 800 µg of active G418 per ml. Twenty plates, each seeded with 5×10^5 hygromycin-resistant CHO-K1 transfectants, yielded several hundred G418-resistant clones after transduction with PA317/N2-conditioned medium. Twenty-four of the transfected clones were further evaluated for susceptibility to infection by amphotropic retroviral vectors by transduction with PA317-packaged LBgSN (5). The proportion of cells which expressed β-galactosidase (β-gal) were determined by using the method of Sanes et al. (15). Twelve of these clones could be transduced at efficiencies substantially above that seen with nontransfected CHO-K1 cells. One of the 12 clones, CHO18, transduced at a level very similar to that observed in NIH 3T3 cells with the same vector-conditioned medium (Fig. 2). This clone was therefore selected for further evaluation. Less than one in 10^4 nontransfected CHO-K1 cells transduced with the PA317/LBgSN vector exhibited β-gal expression (Fig. 2d).

The envelope specificity of the acquired susceptibility to infection observed in the CHO18 clone was tested by exposing the cells to an ecotropically packaged Ψ2/BAG vector (CRL 9560; American Type Culture Collection). The β-gal titer of this ecotropic vector was similar to that of the PA317/LBgSN-conditioned medium when tested on NIH

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FIG. 1. Scheme for generating CHO-K1 cells infectable with amphotropically packaged retroviruses.

3T3 cells. As illustrated in Fig. 3, CHO18 exhibited specific susceptibility to infection with amphotropically packaged vector, with less than 1 in 10^4 cells exhibiting β -gal activity after transduction with Ψ 2/BAG-conditioned medium. Less than 1 in 10^4 normal CHO-K1 cells exhibited β -gal activity after exposure to Ψ 2/BAG.

Transduction efficiency of CHO18 was then quantitatively compared with that of other amphotropically infectable cells lines. NIH 3T3, HeLa, CHO18, and CHO-K1 cells were plated at a density of 5×10^5 cells per 100-mm-diameter tissue culture dish and grown for 18 h. They were then incubated overnight in 10 ml of PA317/LBgSN-conditioned medium containing a total of 5×10^5 CFU. The next day, the proportion of cells expressing β -gal was determined. Under these conditions, 50% of NIH 3T3 cells acquired β -gal, compared with 5% transduction of HeLa cells. CHO18 cells exposed to the same conditioned medium exhibited 50% positive β -gal activity, similar to NIH 3T3 cells and 10 times greater than the level exhibited in HeLa cells. Less than 0.01% of CHO-K1 cells demonstrated staining with 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside.

Two major pieces of data support the conclusion that the acquisition by CHO18 cells of susceptibility to infection by amphotropically packaged retroviral vectors is the result of the introduction of human DNA, rather than an artifact of the transfection procedure. First, compared with native CHO-K1 cells, CHO18 cells are infected 5,000 times more efficiently by amphotropic retroviral vectors. This level of efficiency is equivalent to the level observed in NIH 3T3 cells transduced with the same vector. Second, the sensitivity to infection by amphotropically packaged retroviral vectors is a stable quality of CHO18 cells. As shown in the figures, CHO18 could be reinfected by PA317/LBgSN (Fig. 2 and 3) in an envelope-specific fashion. Furthermore, we have been able to infect CHO18 with other amphotropic vectors, such as PA317/BABE-puro (11), but not with vectors bearing ecotropic envelopes (data not shown). We have also been able to productively infect CHO18 with wild-type amphoto-

pic retroviruses, as evidenced by reverse transcriptase activity detectable in medium conditioned by cells 1 week after infection (data not shown). This susceptibility to infection persists for more than 20 passages. In contrast, when rare G418-resistant clones of CHO-K1 were isolated after exposure to PA317/N2-conditioned medium and then evaluated for the stability of this sensitivity to infection, such clones could not be reinfected. In addition, the presence of human DNA in CHO18 has been confirmed in Southern blots of CHO18 DNA hybridized with probes to human *Alu* and *LINE* repeat sequences, in which hybridizing bands could be detected in CHO18 but not in CHO-K1 DNA (data not shown).

These data lend support to the hypothesis that the block to infection by amphotropic retroviruses in CHO-K1 cells is receptor mediated. Recent studies have demonstrated that the block to ecotropic (21) and amphotropic (10) retroviral infection can be overcome by treating cells with tunicamycin. This effect has suggested that the block to infection of CHO-K1 cells is glycosylation dependent. Our data indicate that the glycosylation-dependent block to infection of CHO-K1 cells is specific to the endogenous hamster protein(s). Since expression of a human protein permits infection, the ability of the human protein to permit infection must not be restricted by glycosylation. Thus, the inactivation of the amphotropic receptor in CHO-K1 must be due to properties inherent to the hamster protein and does not reflect an inherent quality of the cell. It should be noted, however, that the introduced gene may not encode a specific cell surface receptor for amphotropic retrovirus. While CHO18 cells clearly have changed in their ability to be infected by amphotropically packaged vectors, this change could be caused by the acquired expression of a gene for an accessory protein. Such an accessory protein could permit use of a naturally occurring CHO-K1 cell surface protein as a receptor for amphotropic retroviruses. An accessory factor has been postulated for the ecotropic receptor of MLV (19). Alternatively, tunicamycin may act to interfere with formation of an endogenous inhibitor-receptor complex. This inhibitor could block interaction of the endogenous CHO-K1 receptor with the retroviral envelope. It is unlikely that the behavior of CHO18 cells reflects some gene dosage effect, wherein the introduced human gene provides a "sink" for an endogenous blocking agent, thereby leaving some of the endogenous receptor free to bind to amphotropic retroviruses. In a similar model system, overexpression of the murine ecotropic receptor in virus-infected cells did not overcome superinfection interference (3).

Amphotropically packaged recombinant retroviral vectors are currently being evaluated for the ability to introduce therapeutic genes into individuals. Because of their potential clinical utility, it is important to understand the molecular events involved in the amphotropic retrovirus-receptor interaction. The CHO18 cells described here provide a useful infection model which can be compared with noninfectable CHO-K1 cells. Because CHO18 and CHO-K1 cells should differ only in the capacity to be infected by amphotropic retroviruses, these cells permit an analysis of the molecular events involved in retrovirus infection focused on the specific interaction of the virus with its receptor. Furthermore, while CHO-K1 cells have been a popular cell line in which to express exogenous proteins, the ability to express multiple genes has been limited by the inefficiencies of DNA transfection. CHO18 cells provide a target cell essentially identical to the well-characterized CHO-K1 cells, but with the

FIG. 2. Comparison of level of infection of cells with PA317/LBgSN. Cells were transduced with vector and then stained for expression of bacterial β -gal. (a) Uninfected CHO18 cells. (b) CHO18 cells transduced with PA317/LBgSN. (c) NIH 3T3 cells transduced with PA317/LBgSN. (d) CHO-K1 cells transduced with PA317/LBgSN.

FIG. 3. Comparison of transduction of CHO18 cells with ecotropic Ψ 2/BAG and amphotropic PA317/LBgSN. CHO18 cells were exposed to either Ψ 2/BAG-conditioned medium (A) or PA317/LBgSN-conditioned medium (B). Only the cells exposed to PA317/LBgSN exhibited blue staining, indicating that CHO18 cells had become specifically infectable by amphotropically packaged retroviruses.

advantage of being able to efficiently introduce genes by amphotropic vector transduction.

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