Vol. 151, No. 1, 1988 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 29, 1988 Pages 201-206

INFECTION OF HUMAN HEMATOPOIETIC PROGENITOR CELLS USING A RETROVIRAL VECTOR WITH A XENOTROPIC PSEUDOTYPE

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Received January 4, 1988

In an effort to determine if viral envelope type influences the infectivity of human hematopoietic progenitor cells with retroviral vectors, we have pseudotyped the retroviral vector N2, which confers G418-resistance, in either an amphotropic or xenotropic envelope. Vector titres obtained by the pseudotype procedure were nearly two orders of magnitude lower than the titer obtained when N2 was packaged using the amphotropic PA317 packaging cell line. Despite its low titer, xenotropically pseudotyped N2 generated G418-resistant hematopoietic colonies at levels approaching those observed after bone marrow was infected using vector packaged using PA317 cells. These results suggest that manipulations of vector envelope may lead to improvements in the level of infection of human hematopoietic stem cells.

Several laboratories have reported the successful infection of human hematopoietic progenitors with retroviral vectors (1-4). Transfer of a drug resistance gene was detected by the growth of colonies in vitro in the presence of the appropriate selective drug. In these studies, between 1 and 20% of colonies recovered from vector infection were drug resistant.

To produce infectious vector-derived virions, either a replication-competent helper virus or a "packaging" cell line must be used to provide the structural proteins necessary. The binding of retroviruses to target cells is mediated by the envelope protein which, in murine leukemia viruses, falls into one of three classes depending upon its host-range properties (5). Ecotropic envelopes permit infection of mouse cells and to a lesser extent rat cells, but generally not cells of other mammalian species. Xenotropic envelopes enable viruses to infect cells only of other species, since the receptors which bind these envelopes are not present on the host species' cell surface. The third class, amphotropic envelopes, also has a wide host range, but differs from the

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0006-291X/88 $1.50

xenotropic class of viruses in that amphotropic viruses can infect both homologous and heterologous cells. The different host ranges of the different envelope classes result largely from the envelope glycoproteins interacting with distinct cell surface molecules which act as receptors for viral binding and uptake.

To infect human cells with retroviral vectors derived from murine viruses, packaging systems have been used where the envelope is derived from the 4070A (amphotropic) murine retrovirus (6-7). This virus was isolated from cultured cells obtained from a feral mouse, and was characterized as being amphotropic by cross-neutralization and interference tests (8). Although capable of infecting human cells, it is possible that the limited infectivity of human hematopoietic progenitors reported to date, indeed the low infectivity of several different non-murine hematopoietic progenitors, is a specific quality of the 4070A envelope. In an effort to investigate the role of viral envelope type in potentially improving infectivity of human bone marrow cells, we compared the infectivity of a vector packaged in a xenotropic envelope to the same vector in an amphotropic coat.

MATERIALS AND METHODS

Media and Chemicals All cells were grown in Dulbecco's Modified Eagle's Medium (DMEM, Gibco) containing 10% fetal bovine serum (Hyclone) and supplemented with fresh glutamine (0.03% final concentration). G418 (Gibco) was prepared as a stock solution in 50 mM HEPES at 50 mg (active G418, approximately 50% of dry weight)/ml, and was added to culture medium at a final concentration of 400 ug (active G418)/ml (for fibroblasts) or 1200 ug/ml (for colony assays).

Cell Lines and Viruses The PA317 packaging cell line, capable of packaging vectors in an amphotropic (4070A) coat, was kindly provided by Dr. A. Dusty Miller and has been described previously (9). The Mv 1 Lu (NBL-7) mink lung cell line (10) was the generous gift of Dr. Janet Hartley, who also provided the wild type 4070A amphotropic retrovirus and the 2776X xenotropic retrovirus.

Vector Construction of the N2 retroviral vector, derived from the Moloney murine leukemia virus and containing the bacterial neomycin phosphotransferase (neo^R) gene, has been described previously (11). The N2 retrovirus was introduced as the plasmid pN2 into either PA317 cells or mink cells by calcium phosphate transfection (12). After transfection, cells were allowed to grow for 48 hr, then were selected continuously in 400 ug (active) G418/ml.

Bone Marrow Infection Nucleated bone marrow cells, isolated by centrifugation over Ficoll-hypaque (LSM, Litton Bionetics), were infected as previously described (13) with the N2 vector by plating onto a nearly confluent monolayer of irradiated (24 Gy) vector-producing cells. After co-cultivation for 18 to 21.5 hr, the bone marrow cells were recovered by centrifugation and resuspended for plating into colony assays.

Hematopoietic Colony Assay Hematopoietic progenitors (CFU-E, BFU-E, and CFU-GM) were grown as previously described (14) in Iscove's Modified Dulbecco's Medium (IMDM, Gibco) supplemented with 30% prescreened heat-inactivated bovine fetal serum, 10% phytohemagglutinin-stimulated lymphocyte conditioned medium as a source of colony-stimulating factors, 1% bovine serum albumin, 2.5 units/ml erythropoietin (TCepo, Amgen Biologicals) and 1.2% methylcellulose. CFU-E were counted after 7 days culture at 37^{o} C in 5% CO_2. BFU-E and CFU-GM were counted after 14 days culture.

RESULTS

Mink cells transfected with pN2 (N2-Mink) were infected by incubation with medium obtained from cells producing either the 4070A amphotropic murine retrovirus, or the 2776X xenotropic murine retrovirus. Such infected populations of N2-Mink cells were then grown in culture for over one month before their titre of G418-resistant colony-forming units (cfu) and infection restrictions were determined. As shown in Table 1, G418-resistant N2-Mink cells themselves had no detectable titre, either on murine NIH-3T3 cells or on mink cells, confirming that both the pN2 plasmid preparation, as well as the mink cell line itself, were free of any contaminating helper virus.

To establish that viruses capable of transferring G418 resistance were being produced by N2-Mink cells infected with helper virus, titres were determined on NIH-3T3 cells as well as on mink cells (Table 1A). The titre of N2 vector produced by the amphotropic PA317 packaging cell line (PA317-N2) was greater by more than three orders of magnitude than the titer of N2 vector produced by N2-

Table 1

HOST RANGE AND INTERFERENCE PATTERN OF PSEUDOTYPED N2 VECTOR AS DETERMINED BY TITERING ON VARIOUS TARGET CELLS

	Target Cell[a]				
	A. Host Range		B. Interference Pattern		
Viral Supernatant[b]	NIH-3T3	Mink	PA317 (NIH-3T3)	4070A (Mink)	2776X (Mink)
N2-Mink	<0[c]	<0	<0	<0	<0
PA317-N2	6.70	5.70	3.70	2.60	6.28
4070A-N2 Mink	3.28	4.00	2.00	1.18	4.30
2776X-N2 Mink	<0	4.00	<0	3.60	0.90

[a] Target Cells:
NIH-3T3 = transformed murine fibroblast line
Mink = Mu 1 Lu mink lung cells
PA317 = 4070A amphotropic retroviral packaging line derived from NIH-3T3 cells
4070A Mink = mink cells infected with 4070A virus and passaged for over one month
2776X Mink = mink cells infected with 2776X virus and passaged for over one month

[b] Supernatants:
PA317-N2 = PA317 helper cells transfected with pN2
N2-Mink = mink cells transfected with pN2, G418-selected population
4070A-N2 Mink = N2-Mink cells infected with 4070A virus and passaged for over one month
2776X-N2 Mink = N2-Mink cells infected with 2776X virus and passaged for over one month

[c] Titers expressed as the logarithm base 10. Limit of sensitivity < 1 colony per ml viral supernatant.

Mink cells infected with the 4070A amphotrope (4070A-N2). N2-Mink cells infected with the 2776X xenotrope (2776X-N2), as would be expected, had no titre detectable on the mouse-derived NIH-3T3 cells. On mink cells, vector produced by PA317-N2 cells had a titre 10% that of the same vector on NIH-3T3 cells. In contrast to the absence of detectable titre on mouse cells, 2776X-N2 had a titer on mink cells similar to 4070A-N2.

To confirm that the xenotropically pseudotyped N2 had appropriate restrictions in its range of infection, interference tests were performed using mink cells pre-infected with either the 4070A amphotrope or the 2776X xenotrope, as well as the NIH-3T3 derived PA317 packaging cell line expressing the 4070A envelope on its surface (Table 1B). Viruses with different envelopes do not interfere with one another, so that cells pre-infected with 4070A should still be infectable with 2776X pseudotyped vector.

Specific restriction of infection was found. The infectivity of amphotropically-packaged N2 vector, whether by using PA317 cells or pseudotyped with 4070A mink cells, was suppressed by three orders of magnitude on both PA317 cells and 4070A-producing mink cells compared to the titres on the respective uninfected cells. In contrast, when amphotropically packaged N2 was used to infect 2776X-producing mink cells, the G418-resistant titre is very similar to that measured on uninfected mink cells. Conversely, the xenotropically pseudotyped N2 vector could infect cells expressing the amphotropic envelope; however, infection of mink cells expressing the xenotropic envelope was suppressed by over three orders of magnitude.

Having established that a pseudotyped N2 vector actually had the proper restrictions of infectivity, infections of human bone marrow were performed. Two independant infections were performed, and a summary of the infection efficiencies of the CFU-E compartment, as well as the more primitive BFU-E and CFU-GM compartments, is shown in Table 2. Using PA317-N2, between 0.2 and 0.8% of bone marrow progenitors were infected and expressing neomycin

Table 2

PSEUDOTYPED INFECTIONS OF HUMAN MARROW PROGENITORS

Colony Type	N2 Packaged Using: PA317	2776X	4070A
CFU-E	6/1199 (0.5%) n=1	1/961 (0.1%) n=1	0/2095 (<0.05%) n=2
BFU-E	28/3548 (0.8%) n=2	7/4365 (0.2%) n=2	0/4014 (<0.03%) n=2
CFU-GM	6/4234 (0.2%) n=2	4/3675 (0.1%) n=2	0/3251 (<0.03%) n=2

n, number of separate infections

phosphotransferase at levels sufficient to generate robust colonies when selected in 1200 ug (active) G418/ml. Using the same selection conditions and identical envelope, but with a titer only 2% of PA317-N2, vector produced by pseudotyping with 4070A-N2 mink cells generated no G418 resistant colonies in two independent infections. Even though the titer of 2776X-N2 was as low as that of 4070A-N2, 2776X-N2 vector did generate G418-resistant colonies. These colonies were as robust and were generated at a rate nearly equal to that seen after PA317-N2 infection.

DISCUSSION

We have generated a xenotropically packaged retroviral vector (N2) by pseudotyping in mink cells infected with the 2776X retrovirus, and have used it to investigate whether improvements in infection of human bone marrow cells could be obtained with an envelope derived from other than the 4070A amphotropic retrovirus. The xenotropic nature of the envelope was demonstrated by determining that 2776X-pseudotyped N2 was appropriately restricted in its host range of infection and that it exhibited the proper interference pattern on cells infected with different viruses. This xenotropic N2 was then used to infect human bone marrow cells. G418-resistant colonies were successfully generated, despite the fact that the pseudotyped N2 has a titer reduced by nearly two orders of magnitude in comparison to the same vector produced using the amphotropic PA317 packaging line. Significantly, N2 generated by pseudotyping with the 4070A amphotropic retrovirus produced no G418-resistant human bone marrow colonies, even though its titer was identical to that of xenotropically-pseudotyped N2 when measured on mink cells. It is possible that 2776X-N2 may generate G418-resistant colonies because its xenotropic envelope has an increased affinity to receptors on the surface of the human target cells, which compensates for its lower titer relative to PA317.

Previous studies describing the infection of human hematopoietic progenitor cells with amphotropically packaged retroviral vectors have reported infection of both BFU-E and CFU-GM (1-4). In the studies described here, we have used levels of G418 well above amounts which inhibit survival of uninfected colonies to determine the rate of gene transfer in infected progenitors. However, the proportion of G418-resistant colonies reported here is generally lower than those reported earlier. The stringency of the selection conditions used, the choice of criteria for scoring colonies, or possible variations in lots of G418, could account in part for the lower proportion of G418-resistant colonies obtained in these experiments.

These studies show that alternative packaging systems may be a fruitful avenue for the development of retroviral vectors that have a greater infectivity for human target cells. Although these experiments did not result

in efficiencies greater than already obtained with vectors packaged with amphotropic packaging cell lines, the development of helper-free xenotropic packaging cell lines comparable to PA317 and identification of clones producing higher titres of vector could yield improvements in the infection efficiency of human hematopoietic stem cells. Ultimately, such studies may contribute to the refinement of techniques of gene transfer to permit their clinical application for the treatment of genetic disease.

ACKNOWLEDGEMENTS

We gratefully acknowledge Drs. Robert Bassin, Sandra Ruscetti and Linda Wolff for sharing their insights. We are also appreciative of Dr. Janet Hartley for providing the wild type retroviruses, as well as for her advice. In addition, members of our gene transfer research group provided useful criticisms and suggestions. Sheri Bernstein, Martha Feller and Rinna Connol provided expert technical assistance.

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