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Increase of Retroviral Infection In Vitro by the Binding of Antiretroviral Antibodies

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Monoclonal antiretrovirus antibodies were assayed for their ability to influence retrovirus infection in vitro. Some antibodies increased murine cell infection by an ecotropic virus and both murine and human cell infection by an amphotropic virus. The stability of these viruses was not modified, suggesting that antibody-virus complexes may be more infectious than free virus particles.

It has been known for a long time that the efficiency of in vitro infection by retroviruses may be considerably enhanced (1 or 2 orders of magnitude) by polycations such as DEAE-dextran or Polybrene (9, 24). The mechanism of enhancement is unknown, but it was suggested that polycations could neutralize electrostatic repulsion between virus particles and cellular membrane (24). Polycations affect the early steps of infection, i.e., the adsorption of particles on membranes and perhaps virus penetration (13). In this paper, we present evidence that several monoclonal antibodies directed against murine leukemia retroviruses enhance by 1 order of magnitude the efficiency of infection by retroviruses when they are preincubated with retrovirus particles. Several hypotheses to account for this result are discussed, as well as their potential biological implications.

Enhancement of the efficiency of viral infection by antiretrovirus antibodies. A murine retroviral vector (M-MuLV-SV*tk-neo*) (22) was used to infect murine 3T3 or human HEp2 (19) cells. It was packaged either in the ψ2 cell line, which contains a Moloney ecotropic helper provirus deleted for the packaging signal (16), or in an amphotropic helper cell line (PA12) (17). The former can infect 3T3 cells, and the latter can infect both 3T3 and HEp2 cells. The M-MuLV-SV*tk-neo* vector codes for neomycin resistance, and resistant cells were selected in a medium containing Geneticine (G418; GIBCO Laboratories) (1 mg/ml for 3T3 cells and 1.5 mg/ml for HEp2 cells). Viral stocks (supernatants of ψ2 or PA12 cell cultures producing M-MuLV-SV*tk-neo*) were titrated on 3T3 cells in the presence of Polybrene. Neomycin-resistant (Neo^r) colonies were counted 10 days after infection, and the titers obtained, expressed as the number of colonies per milliliter of viral stock, were comparable for both viruses and were around 2×10^4 to 5×10^4. We used recombinant retroviruses rather than wild-type viruses, since the former allow a very easy quantification of infected cells.

Several anti-murine leukemia virus monoclonal antibodies (MAbs), obtained by Chesebro et al. (5–7), were used. Their characteristics are summarized in Table 1. Some of these antibodies bind to proteins from ecotropic and amphotropic retroviruses while others (273, 372, and 615) do not bind amphotropic proteins. Hybridoma cells were injected in mice and ascitic fluids were collected, precipitated by sodium sulfate (18%), and dialyzed against phosphate-buffered saline (PBS). Immunoglobulin-enriched fractions (immunoglobulin concentrations, 1 to 3 mg/ml) were used for the experiments, as well as purified antibodies eluted from a protein A-Sepharose immunoadsorbent (10) or passed through a DEAE-cellulose column (14) and then dialyzed against PBS.

The cells (5×10^4) were infected with 200 μl of viral stock as follows. Antibodies were incubated with viruses at 4°C for 1 h, and this mixture was then added to cells for 24 h at 37°C. After this, cells were treated with trypsin and seeded in normal culture medium. Selective medium was added 2 days later. The results of several experiments are shown in Table 2. When no antibody or an irrelevant antibody was incubated with the ecotropic virus, we always observed around 2 Neo^r colonies per 10^3 cells. When MAb 615 was preincubated with the virus, up to 35 Neo^r colonies per 10^3 cells were obtained. The effect of MAb 615 was dose dependent. Immunoglobulin-enriched fractions from ascitic fluid or purified antibodies could be used and were active at similar concentrations. To rule out a nonspecific direct effect of MAb 615 on 3T3 cells, we ultracentrifuged the mixture of antibody and virus after the preincubation step to separate antibodies bound to virus from free antibodies (100,000 × *g* for 1 hour on a 30% sucrose cushion). Control experiments had shown that under these conditions, the recovery of the virus particles was nearly complete (data not shown). Under these conditions, we still observed an increased infection when the virus was preincubated with MAb 615 (Table 2). These experiments strongly suggest that complexes formed by the binding of MAb 615 to virus particles are more infectious than are virus particles alone.

Similar experiments were performed on 3T3 cells pretreated with Polybrene (10 μg/ml) for 1 h at 37°C. Under these conditions, MAb 615 did not have an enhancing effect but rather a slight, dose-dependent inhibitory effect (Table 2). The same result was obtained when smaller multiplicities of infection were used, thus excluding the possibility that the number of cells was limiting (data not shown). These data indicate that under the optimal conditions of infection (i.e., with Polybrene-pretreated cells), MAb 615 does not enhance the number of infectious particles but appears to have a neutralizing effect on virus particles.

To evaluate whether the effect of MAb 615 on the infection by the ecotropic virus was a unique phenomenon, we performed similar experiments with other antiretroviral antibodies. Among the six antiretroviral MAbs analyzed, two (615 and R187) clearly increased the infection of 3T3 cells by the ecotropic virus and a third (548) had a less pronounced

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TABLE 1. Main characteristics of antiretrovirus MAbs

MAb	Immunoglobulin class	Antigen recognized on:		Reference
		Moloney ecotropic virus	4070A amphotropic virus	
34	IgG2b	p15	p15	7
273	IgG2a	gp70	None	7
372	IgG3	p15E	None	7
548	IgG2b	p12	p12	5
615[a]	IgG2a	gp70	None	6
R187	IgG (rat)	p30	p30	5

[a] 615 MAb is listed as 715 in reference 6.

effect (Table 3). R187 and 548 are, respectively, anti-p30 and anti-p12 antibodies. This suggests that these proteins are accessible on the surface of virus particles. Although 615 and 273 are both anti-gp70 antibodies, 273 had no effect on the infection of 3T3 cells. The concentrations of immunoglobulins in both ascitic fluids were comparable. This result may therefore be due to the slight difference of specificity observed for these antibodies (6). Similar infection experiments were performed with the amphotropic virus. This virus infects both murine 3T3 cells and human HEp2 cells. Without Polybrene or antibodies, we obtained around 20 and 2 colonies for 10^3 3T3 and HEp2 cells, respectively. R187 binding to the amphotropic virus increased considerably the infection of both 3T3 and HEp2 cells, whereas 548 had a weaker effect on these infections (Table 3) and 615 had no effect at all; this is in agreement with the observation that 615 does not bind the amphotropic gp70 protein (6). Taken together, our results show that several antiretroviral antibodies may increase the infection by retroviruses of both murine and human cells.

TABLE 2. Effect of preincubation of MAb 615 with the ecotropic virus on infection of murine 3T3 cells

Exptl conditions and MAb	Dilution of ascitic fluid fraction	Amt of purified MAb (μg/ml)	No. of Neo^r colonies/10^3 3T3 cells
Preincubation virus + antibody			
PBS[a]			1–2[b]
5E9[c]	1/25		3–3
5E9	1/5		4–1
615	1/25		5–6
615	1/5		31–26
5E9		40	2–2
5E9		100	2–1
5E9		250	2–1
615		40	9–15
615		100	17–21
615		250	34–27
Preincubation virus + antibody with ultracentrifugation			
PBS			2–4
5E9		200	0–2
615		50	9–22
615		200	20–31
Pretreatment of 3T3 cells with Polybrene (10 μg/ml)			
PBS			132–133
615	1/25		107–117
615	1/5		71–96

[a] Identical volumes of PBS or antibodies were added in each case.
[b] Results of two independent infections are reported.
[c] 5E9 is a MAb raised against the human transferrin receptor (12).

TABLE 3. Effect of preincubation of MAbs with retroviruses on infection of murine and human cells

Virus stock	MAb (dilution of ascitic fluid fraction)	No. of Neo^r colonies with:	
		10^3 murine 3T3 cells	2×10^3 human HEp2 cells
Ecotropic	PBS	1–2[a]	0
	548 (1/25)	2–8	ND[b]
	548 (1/5)	15–10	0–0
	615 (1/25)	13–13	0–0
	615 (1/5)	18–25	ND
	R187 (1/25)	11–4	ND
	R187 (1/5)	30–29	ND
	34 (1/25, 1/5)	<4	ND
	273 (1/25, 1/5)	<4	ND
	372 (1/25, 1/5)	<4	ND
	5E9 (1/25, 1/5)	<4	ND
	1DA23[c] (1/25, 1/5)	<4	ND
	IDU 111[c] (1/25, 1/5)	<4	ND
Amphotropic	PBS	18–16	4–4
	548 (1/25)	24–21	4–5
	548 (1/5)	70–74	11–9
	615 (1/5)	12–12	5–3
	R187 (1/25)	36–33	10–6
	R187 (1/5)	131–101	27–24

[a] Results of two independent experiments are reported.
[b] ND, Not done.
[c] IDA23 and IDU 111 are irrelevant MAbs of IgG2b and IgG1 classes, respectively.

Potential biological significance. Several hypotheses could account for the effect of antiretroviral antibody binding on the infection of cells. A simple one is that antibody binding to virus particles modifies the half-life of the virus by preventing the degradation of the particles. To check this hypothesis, we measured the half-life of the ecotropic virus at 37°C, either in the presence or in the absence of antibodies, by two experimental protocols. (i) Viruses were preincubated for 1 h at 4°C with 615 or R187, kept at 37°C for various times, and incubated with 3T3 cells for 24 h at 37°C. (ii) The viruses were incubated for various times at 37°C, the antibodies were then added for 1 h at 4°C, and the mixture was incubated with 3T3 cells for 24 h at 37°C. The results are presented in Fig. 1. In the experiments performed with MAb 615, the half-life of the virus in the presence of the antibody during the preincubation step at 37°C was slightly shorter than the one in the absence of the antibody (5.5 h compared with 9.5 h). In the experiment with MAb R187, the half-lives of the virus in the absence or in the presence of antibodies were similar (9 and 8 h, respectively). These results show that the stability of the virus at 37°C is not increased by the binding of antibodies. Prolonged preincubation of the virus with MAb 615 at 37°C before infection may even cause a slight reduction in the level of infection (Fig. 1).

According to this result, it seems that the complexes formed by antibodies and virus particles have a greater infectivity than do free virus particles. Enhancement of viral replication in macrophage and macrophagelike cells by antiviral antibodies has been previously reported for viruses other than retroviruses (3, 4, 8, 11, 18, 21, 23), but this effect was not observed for cells lacking macrophage characteris-

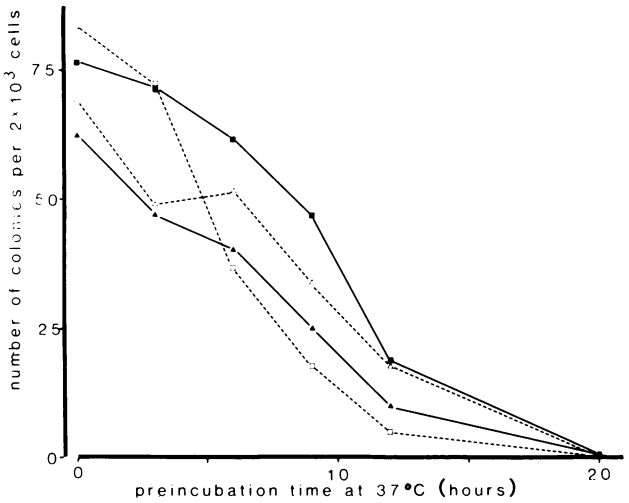

FIG. 1. Stability of virus particles at 37°C in the presence or absence of antiretroviral MAbs. A viral stock produced by ψ2 cells was incubated alone for various times at 37°C, then with 615 or R187 immunoglobulin-enriched fractions from ascitic fluids (1/10) for 1 h at 4°C, and then with 3T3 cells for 24 h at 37°C (continuous lines, solid symbols). In parallel experiments, the same virus stock was first incubated with antibodies for 1 h at 4°C, then for various times at 37°C, and finally with 3T3 cells for 24 h at 37°C (dotted lines, open symbols). Symbols: □, ■, MAb 615; ▲, △, MAb R187.

tics (20). These studies disclosed the important role of the abundant Fc receptors expressed by these cells in the manifestation of the enhancement of viral replication. A possible involvement of Fc receptors in the infection of fibroblastic lines by retroviruses remains to be investigated, but the fact that even in the presence of MAb 615, the ecotropic virus is unable to infect human cells (Table 3) strongly suggests that the specific interaction between virus particles and cellular receptors is not bypassed. The efficiency of adsorption or penetration of the virus into the cytoplasm may be increased by the presence of the antibodies. Similar hypotheses have been postulated to explain the action of Polybrene and DEAE-dextran (13, 24). In the case of antibodies, several comments may be added. First, it may be a convenient way to increase viral infection when Polybrene has no effect (15). Second, some evidence indicates that retroviruses enter cells by receptor-mediated endocytosis (1, 2). It could be possible to follow antibody-virus complexes and determine which step of the infection is modified by the presence of antibodies. Finally, it must be stressed that the phenomenon we observed in vitro may occur in vivo. This could have implications form both a biological and a therapeutic point of view.

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