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Modulation of Lentivirus Replication by Antibodies: Fc Portion of Immunoglobulin Molecule Is Essential for Enhancement of Binding, Internalization, and Neutralization of Visna Virus in Macrophages

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Antibodies to visna virus neutralized the virus in fibroblasts and macrophages but specifically enhanced the binding, penetration, and uncoating of the virus in the latter cells. $F(ab')_2$ fragments of the immune antibody neutralized the virus in fibroblasts but did not enhance the early stages of the virus life cycle in macrophages. Furthermore, these fragments did not neutralize infectivity in macrophages but delayed the appearance of infectious virus in cells after the inoculation of preincubated virus-$F(ab')_2$ complexes.

Visna-maedi virus is a prototype member of the lentivirus family (6) that includes the human immunodeficiency viruses. Among the cardinal properties of these viruses are their tropism for cells of the macrophage lineage (1, 2, 7) and their ability to cause a persistent infection which is unaffected by the later development of neutralizing antibodies (3, 4, 9). In previous reports on the evaluation of neutralizing antibodies to lentiviruses of sheep and goats, we showed that in non-macrophage cell types the antibodies reduced binding and internalization of the virus, whereas in macrophages the opposite effects were seen (5). In this report we repeated this experiment as a background study for further investigation of neutralization with $F(ab')_2$ fragments of the neutralizing antibody molecules. The new data showed that these fragments were not as effective in neutralization as whole immunoglobulin molecules. Moreover, they caused retardation of binding and penetration of the virus in both fibroblasts and macrophages. Both whole immunoglobulin molecules and $F(ab')_2$ fragments were much more efficient at neutralization in fibroblasts. In macrophages, effective neutralization titers were lower because of virus breakthrough after relatively long periods of quiescence within the cells.

Visna virus strain 1514 (10) was used in this study. It was cultivated in sheep choroid plexus fibroblasts (9) and concentrated by membrane filtration in a pellicon system (11). A goat was immunized with the virus by repeated injections, first by intradermal inoculation of virus emulsified in Freund complete adjuvant and then by three sequential injections of virus in Freund incomplete adjuvant. Final boosting was performed by intravenous inoculation of an aqueous virus suspension. The same animal was given a weekly course of four injections of 10^8 chicken erythrocytes (RBC). Serum was then collected for the studies.

Both whole immunoglobulin molecules and $F(ab')_2$ fragments were evaluated for their effect on the binding, penetration, uncoating, and neutralization of visna virus in fibroblasts and macrophages. The same two reagents [immunoglobulin and $F(ab')_2$] were used to study the interaction between chicken RBC and the macrophages and provided a means to visualize the function of the molecules. The effects of the reagents on the binding, penetration, and uncoating of the virus were investigated with [^{35}S]methionine-labeled virus purified from cultures of inoculated sheep choroid plexus fibroblasts (5). Binding of the virus to the cells was analyzed by evaluating the number of radioactive counts that became irreversibly associated with the cells after an incubation period of 14 h at 4°C. The appearance of acid-soluble counts in the supernatant fluids after the inoculated cultures had been shifted to 37°C was used as an indication of uncoating of the virions as previously described (5). Failure to obtain progeny virus after preincubated virus-serum or virus-$F(ab')_2$ mixtures were inoculated into cultures was indicative of neutralization (8). Crude immunoglobulin G was precipitated from serum in 33% saturated ammonium sulfate. The immunoglobulin was dialyzed first against 0.1 M sodium formate buffer (pH 2.8) and then against 0.1 M sodium acetate buffer (pH 4.5) (12, 13). Immunoglobulin G (10 mg) was digested with 2,250 U of immobilized pepsin (Pierce Chemical Co., Rockford, Ill.) in 0.1 M sodium acetate buffer. A sample of the digested material was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The immunoglobulin G was completely cut to yield $F(ab')_2$ fragments with molecular weights of approximately 100,000.

In Fc receptor-binding assays chicken RBC were preincubated with immune serum, control serum, or immune $F(ab')_2$ fragments for 60 min at 37°C and added to cultures of macrophages. The cultures were incubated for 30 min at 37°C, washed, and examined for binding of the RBC. RBC that were preincubated with immune serum bound tightly and in great numbers to macrophages (Fig. 1A), while RBC preincubated with normal serum or $F(ab')_2$ fragments did not bind (Fig. 1B). The addition of rabbit anti-goat IgG or rabbit anti-goat Fab to $F(ab')_2$-chicken RBC mixtures restored the binding of the RBC (Fig. 1C), suggesting that $F(ab')_2$ fragments had bound to the RBC and that anti-Fab antibodies had contributed effective Fab and Fc functions.

The greatest binding of ^{35}S-labeled strain 1514 virus to fibroblasts was seen with virus that was incubated with the control serum, which lacked antibodies to the virus. When virus was preincubated with neutralizing serum and $F(ab')_2$ fragments from the neutralizing serum, there was 32 and 29% less binding ($P < 0.01$ and $P < 0.05$, respectively) than when virus was preincubated with control serum (Fig. 2). In macrophages, there was 36% less binding of ^{35}S-labeled strain 1514 virus preincubated with $F(ab')_2$ fragments from

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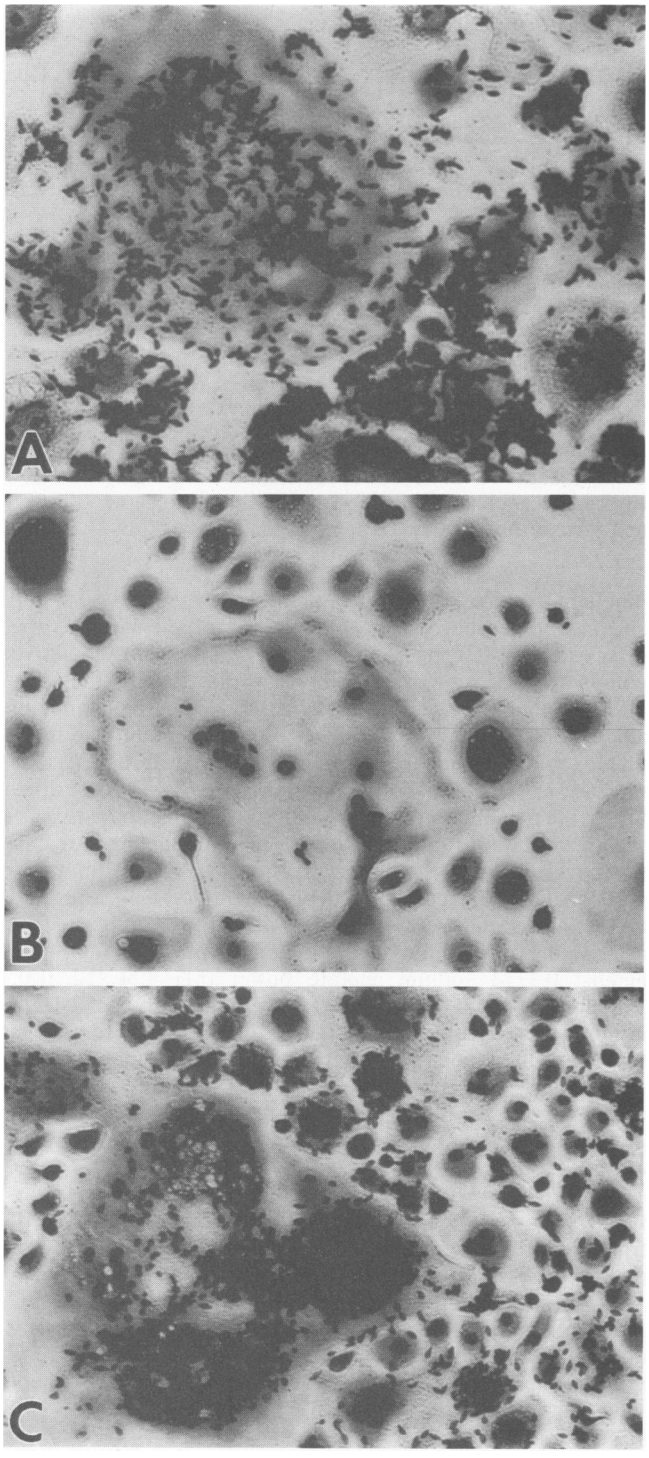

FIG. 1. Fc receptor binding in macrophages. (A) Binding of antibody-chicken RBC complexes. (B) Lack of binding of $F(ab')_2$-chicken RBC complexes. (C) Binding restored by the addition of rabbit anti-goat antibody to $F(ab')_2$-chicken RBC complexes.

neutralizing serum ($P < 0.005$) compared with the control; however, binding of radiolabeled virus preincubated with neutralizing serum was increased by 53% ($P < 0.005$) over binding of radiolabeled virus preincubated with control serum (Fig. 2).

In fibroblasts, the greatest amount of uncoating of ^{35}S-labeled strain 1514 virus occurred with virus preincubated with control serum (Fig. 3A). Using this value as 100%, we found that only 42 and 35% ($P < 0.025$ and $P < 0.05$, respectively) of viruses preincubated with immune serum and immune $F(ab')_2$ fragments, respectively, were uncoated. In macrophages, the greatest amount and fastest rate of viral uncoating occurred with virus preincubated with immune serum. There was a 104% increase ($P < 0.0025$) in uncoating with this serum as compared with control serum. In contrast, the amount and rate of uncoating with $F(ab')_2$ fragments in macrophages were similar to those in the control (Fig. 3B).

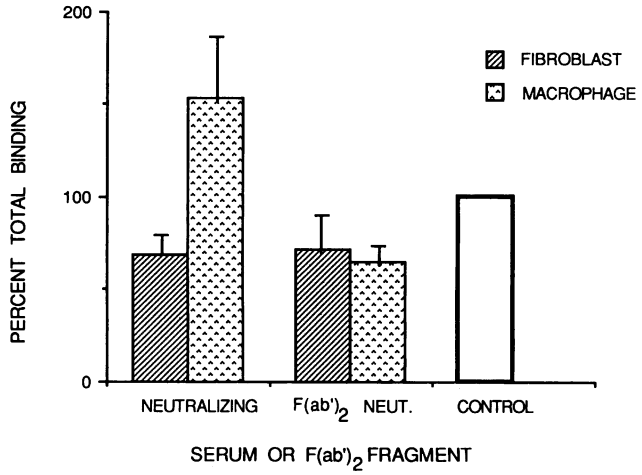

FIG. 2. Binding of ^{35}S-labeled strain 1514 virus in fibroblasts and macrophages. Labeled virus was incubated with serum or $F(ab')_2$ fragments and inoculated into cell cultures kept overnight at 4°C. Virus inocula were removed, the cells were washed and lysed, and the radioactive counts were obtained. Neutralizing serum reduced the binding of virus to fibroblasts 32% but increased the binding of virus to macrophages 53% as compared with the control. $F(ab')_2$ fragments decreased binding to fibroblasts and macrophages 29 and 36%, respectively. The bar graph shows the treatment/control ratios of virus bound ± the standard errors of the means. Means represent an average of two replicates for fibroblasts and an average of four replicates for macrophages. NEUT., Neutralizing.

Immune serum neutralized visna virus in fibroblast cultures at a titer of 1:5,000 and in macrophage cultures at a titer of 1:320 (Table 1). $F(ab')_2$ fragments neutralized the virus at a titer of 1:800 in fibroblasts. In macrophages, $F(ab')_2$ fragments delayed the production of infectious virus for 5 days. This delay was determined by a lack of virus-induced cytopathic effects when supernatant fluids from macrophage cultures were transferred to indicator fibroblast cultures.

These data provide a strong suggestion that the differences in the kinetics of binding, uncoating, and neutralization of visna virus in macrophages and fibroblasts mediated by antibodies were caused mainly by the interaction between the Fc portion of the whole immunoglobulin molecule and Fc receptors on the macrophages. Proof that the $F(ab')_2$ fragments were functional was indicated visually in experiments with chicken RBC and also by the ability of the fragments to neutralize virus in fibroblasts. It was evident that these fragments, when severed from Fc, bound to virus and delayed binding and internalization of virus in both fibroblasts and macrophages. However, they were less effective than whole immunoglobulin molecules in neutralizing virus, suggesting that Fc may provide a more stabilizing bond between $F(ab')_2$ fragments and virus particles. However, the reduced efficiency of both whole immunoglobulin molecules and $F(ab')_2$ fragments in neutralizing virus in macrophages

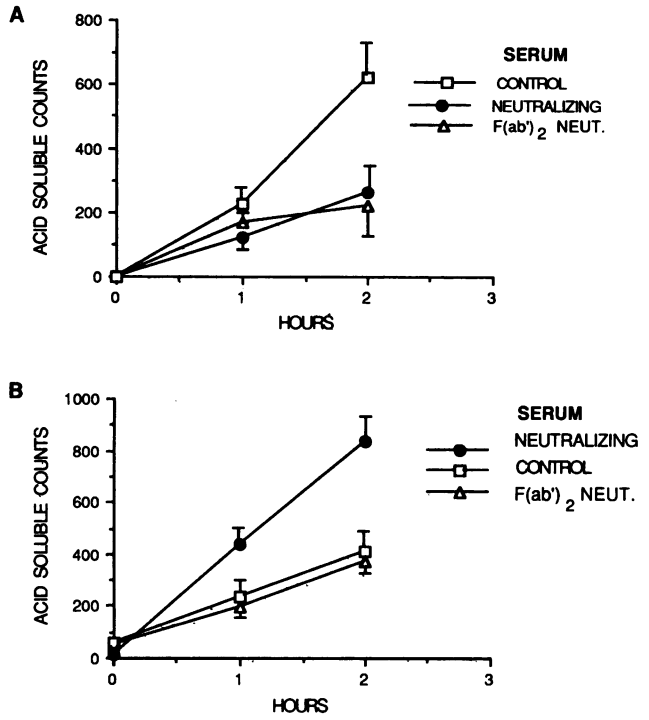

FIG. 3. (A) Uncoating of ^{35}S-labeled strain 1514 virus in fibroblasts. Labeled virus was incubated with serum or F(ab')$_2$ fragments and inoculated into cell cultures kept overnight at 4°C. Virus inocula were removed, the cells were washed, and new medium was added. Cultures were shifted to 37°C, and supernatant fluids were removed at 0, 1, or 2 h. The acid-soluble radioactivity of each sample was determined and used as an indication of viral uncoating. Both neutralizing serum and F(ab')$_2$ fragments decreased the uncoating of labeled virus by fibroblasts. (B) Uncoating of ^{35}S-labeled strain 1514 virus in macrophages. Neutralizing serum increased the uncoating of virus 104% above the control value. Uncoating with F(ab')$_2$ fragments was similar to uncoating with the control. The data represent the means ± the standard errors of four replicates for fibroblasts and eight replicates for macrophages. NEUT., Neutralizing.

indicated that the binding between the virus and the antibodies was more easily dissociated in macrophages than in fibroblasts. It was of interest that the complexes between virus and immunoglobulin or virus and F(ab')$_2$ could remain quiescent in the macrophages for several days before breakthrough into full virus replication. The mechanisms of antibody-mediated sequestration of virus in macrophages is not understood. This study emphasized that antibody-virus interactions in host cells have different biological outcomes when one of the cell types under investigation is the macrophage. It was evident that the potency of antibodies in protection may be overexaggerated when non-macrophage cell types are used. This may contribute in part to the anomalous finding that the agent persists in vivo in the presence of high titers of neutralizing antibodies, whose titers are measured in vitro by protection of non-macrophage cells such as fibroblasts or even lymphocytes. Whereas such antibodies may indeed protect lymphocytes, they may enhance the early stages of infection in macrophages but somehow retard the production of infectious particles. The data clearly suggest that in lentiviral infections neutralizing antibodies may have only a nominal efficacy in protecting macrophages from infection. It may be that more antibody molecules may be required to protect a macrophage than a fibroblast. Therefore, in the development of effective vaccines against lentiviral agents consideration must be given not only to the virus and antibody molecules but also to the cells used to measure the efficacy of such antibodies.

TABLE 1. Neutralizing titers of immune serum and F(ab')$_2$ fragments in fibroblast and macrophage cultures

Treatment	Neutralizing titer in:			
	Fibroblasts on day:		Macrophages on day:	
	5	12	5	12
Immune serum	1:5,000	1:5,000	1:5,000	1:320
F(ab')$_2$ fragments	1:800	1:800	1:400	<1:100

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