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NOTES

Antibodies to Varicella-Zoster Virus Modulate Antigen Distribution but Fail To Induce Viral Persistence In Vitro

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Varicella-zoster virus (VZV) persists in human sensory ganglia. One of the hypotheses to explain the induction or the maintenance of VZV latency is that it could be promoted by the immune response itself. It is known that in the case of viruses which bud off the infected cell membrane, virus-specific antibodies can induce antigenic modulation, i.e., spatial redistribution of viral antigens and modulation of their synthesis. To determine whether antigenic modulation occurs during VZV infection in vitro and could possibly be involved in viral persistence, we have grown infected cells in the presence of anti-VZV antibodies either transiently or permanently. The distribution of immune complexes and viral proteins was then analyzed. In transient immunomodulation experiments, the distribution of one or more viral antigens was modified not only in the cytoplasmic membranes but also in the cytoplasm and nucleoplasm of infected cells. When infected cells were kept permanently in the presence of antibodies, the same pattern of redistribution of immune complexes was observed and the localization of internal viral glycoproteins was significantly modified. However, antibodies did not prevent the lytic effect of infection; they altered neither the infectious virus yield nor the Western immunoblot pattern of viral proteins, suggesting that immunomodulation is not the primary effector of viral persistence.

Varicella-zoster virus (VZV) is a human herpesvirus which causes a primary infection in childhood, becomes latent in dorsal root ganglia, and can be reactivated many years later to produce shingles in adults (10). Investigation of mechanisms by which viruses become persistent and remain latent in infected cells progresses along two paths: (i) searches for a viral gene that is directly or indirectly responsible for latency and (ii) identification of host factors that induce viral latency. In the latter case, the main factor of interest is the host immune system. It has been shown that both in vitro and in vivo, persistence of a virus which buds at the plasma membrane of infected cells can be induced by the presence of antiviral antibodies at the time of virus maturation (20–23). Antibody treatment can induce a redistribution of membrane antigens which are assembled in patches and then in caps, as has been well documented for enveloped viruses budding at the plasma membrane, such as measles virus (8, 9, 13, 18), a retrovirus (19), or influenza virus (24). Studies of measles virus have demonstrated that the effect of antibodies on the viral replication cycle is not restricted to a mechanical hindrance of the budding process. In fact, antibodies modulate through an unknown mechanism the synthesis of the viral glycoproteins expressed on the cell surface before budding (5–7, 14). Regulation of gene expression has also been described for a parvovirus (1) and a coronavirus (3). The phenomenon of specific regulation of gene expression by antibodies was first described for cellular structural proteins and called immunomodulation by Stackpole et al. (25). Such an immunomodulating effect on viral gene transcription could lead to persistence (17) and could account, on a molecular basis, for the data obtained for the induction of persistence by antibody pressure.

At first sight, such a phenomenon may seem rather unlikely for viruses of the family *Herpesviridae*, since they are known to mature at the inner nuclear membrane and in cytoplasmic vesicles. However, viral glycoproteins are expressed on the plasma membrane during infection. These proteins do not participate in the assembly of the viral particle, but they are fully accessible and can thus be targets for antiviral antibodies. During latency, high anti-VZV antibody levels can be detected (2), but their role in the induction or maintenance of a quiescent state is unknown. Indeed, in the case of herpes simplex virus infection, it is not clear whether the binding of immunoglobulins to virus-induced Fc receptors plays (4) or does not play (11) a role in the inhibition of virus growth. Even though our laboratory studies can only mimic the in vivo situation, it is worth controlling the direct effect of antibodies on the course of viral infection. We have thus tested the immunomodulation hypothesis in order to confirm it or to rule it out, at least under in vitro conditions.

MRC-5 cells (human embryonic lung fibroblasts; Biomérieux, Marcy l'Étoile, France) were maintained in Eagle's basal medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% fetal calf serum, 100 U of

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TABLE 1. Molecular weights and identities of polypeptides recognized by monoclonal antibodies

Monoclonal antibody	Mol wt (10^3)	Identity
Biosoft 215-84/101	49 and 39	gpII
VL1	49 and 39	gpII
VL8	70, 63, and 57	gpI

penicillin per ml, 100 mg of streptomycin per ml, and 0.15% $NaHCO_3$.

The original strain of VZV used in this study was isolated by sterile aspiration of vesicular lesions of a patient with chicken pox. Restriction analysis of the purified DNA showed the typical pattern of VZV Ellen strain. Infection was propagated by cocultivation of MRC-5 cells with VZV-infected MRC-5 cells (ratio of 1 infected cell to 3 uninfected cells).

Human anti-VZV antibodies were prepared by ammonium sulfate precipitation of a non-convalescent-phase human plasma with high anti-VZV antibody activity (≥12,800 in enzyme immunoassay) and dialysis at 4°C in phosphate-buffered saline (PBS). Anti-VZV rabbit hyperimmune serum was obtained by immunization with VZV purified from infected MRC-5 cells followed by three steps of absorption on uninfected cells to decrease reactivity against nonviral antigens. Monoclonal antibodies were either obtained from Biosoft (Paris, France) (215-84/101) or produced in our laboratory (VL1 and VL8). The molecular weights of the viral polypeptides recognized by these monoclonal antibodies are listed in Table 1. The specificity of each antiserum or monoclonal antibody was verified by immunohistology or Western blotting (immunoblotting) analysis on infected and uninfected cells.

MRC-5 cells infected by cocultivation with VZV-infected or mock-infected cells were treated with human anti-VZV antibodies (1/20 in cell culture medium) either transiently (2 h) or permanently (for 72 h) (Fig. 1).

Viral antigens were detected by surface immunolabeling on cells fixed with 4% (wt/vol) paraformaldehyde in PBS (pH 7.6) (20 min at 20°C). For intracellular labeling, cells were fixed in acetone for 5 min (−20°C), dried, and stored at −20°C until use or fixed in paraformaldehyde, permeated with 95% ethanol (15 min at −20°C), and stored at 4°C. Just before use, cells were rehydrated in PBS at 20°C for 5 min. After saturation with skimmed milk (15 g/liter in PBS) for 30 min at 20°C, viral antigens were labeled by incubation either with rabbit anti-VZV antibody (1:50 in PBS, 60 min at 37°C) or with a monoclonal antibody or a human plasma with high anti-VZV antibody activity (1:40 in PBS, 60 min at 37°C). After four washes with PBS, endogenous peroxidase was inactivated by a treatment with 0.03% (vol/vol) hydrogen peroxide in PBS for 15 min. Then goat anti-rabbit immunoglobulin (Ig), rabbit anti-mouse IgG, or rabbit anti-human IgG antibody coupled to peroxidase (Dako, Glostrup, Denmark) (1:40 in PBS, 60 min at 37°C) was added. The presence of peroxidase was revealed with diaminobenzidine tetrachloride (0.5 mg/ml in PBS) and 0.01% (vol/vol) hydrogen peroxide.

Viral antigens were analyzed by Western blotting (26). They were produced from infected cells by washing the cells several times with serum-free culture medium, mechanically scraping the cells off the plastic substrate in PBS, and centrifugating the cells for 10 min at 400 × *g*. The pellets corresponding to 75 cm^2 of cells were then resuspended either in 0.5 ml of RIPA mixture (12) or in the same volume of PBS prior to ultrasonication for 1 min. Finally, the material was centrifuged at 200 × *g* for 15 min. Antigens were treated 1:1 (vol/vol) in a denaturing mix (0.125 M Tris-HCl [pH 6.8], 4% [wt/vol] sodium dodecyl sulfate [SDS], 20% [vol/vol] glycerol, 10% [vol/vol] β-mercaptoethanol, 5% [vol/vol] bromophenol blue) and then heated at 100°C for 90 s. Samples (3.75 μg of proteins [16] in each well)

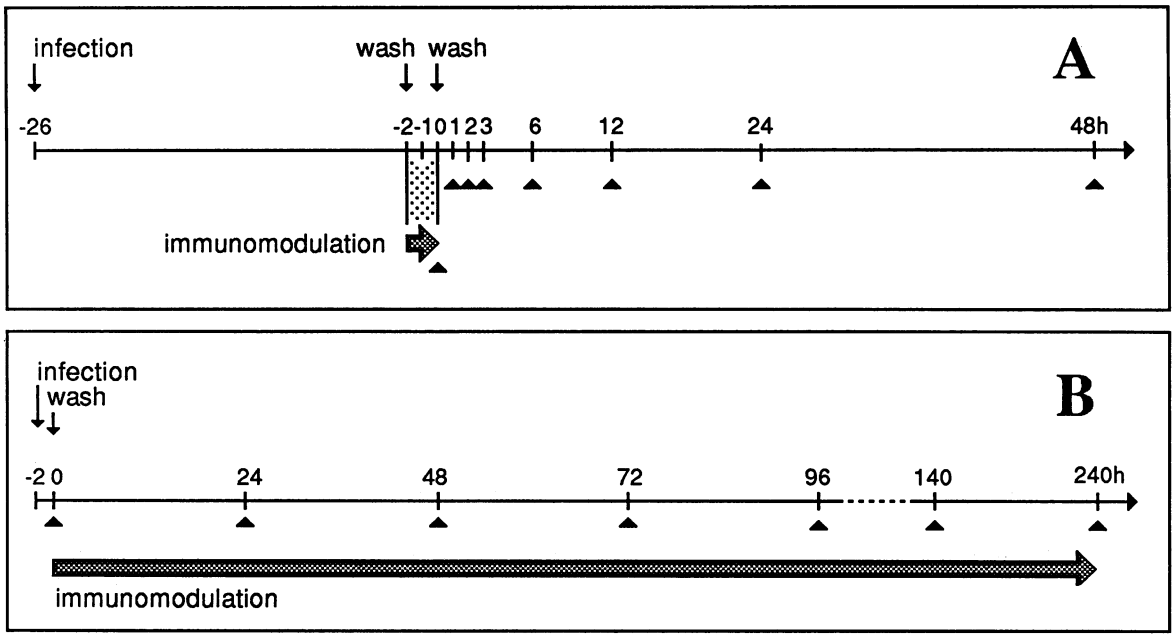

FIG. 1. Schematic representation of transient (A) and permanent (B) immunomodulation treatment protocols. The thick arrow corresponds to duration of antibody treatment. Arrowheads indicate times of sample fixation.

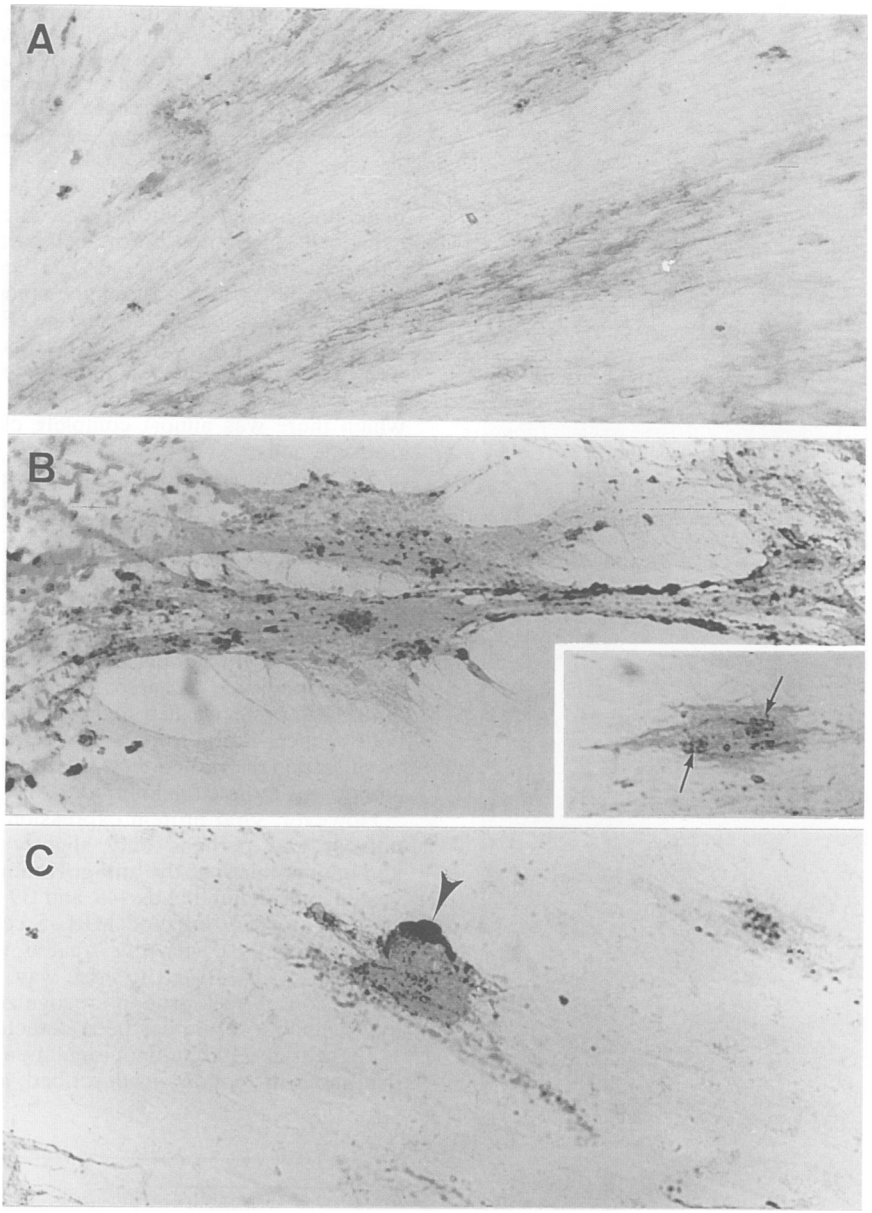

FIG. 2. Detection of antigen-antibody complexes on the surface of VZV-infected cells cultured with anti-VZV human antibodies for 2 h (transient immunomodulation). The complexes were detected by immunoperoxidase with a rabbit peroxidase-labeled anti-human Ig (Dako) on cells fixed 0 (A), 1 (B), or 3 (C) h after immunomodulation treatment. Patches (arrows) and a cap (arrowhead) are indicated. Magnification, ×240.

were applied on polyacrylamide gels (4% [wt/vol] in 0.5 M Tris-HCl [pH 6.8] for the stacking gels; 8% [wt/vol] in 1.5 M Tris-HCl [pH 8.8] for the separating gels). Electrophoresis was run at 150 V in 0.025 M Tris (pH 8.3)–0.2 M glycine–0.1% (wt/vol) SDS. Proteins were transferred onto an Immobilon membrane (Millipore) in a Biolyon dry electroblotter according the manufacturer's instructions (1 h at 24 V).

Transient immunomodulation experiments were performed to verify whether antibodies could bind to viral antigens on living cells and could thus induce modifications of viral expression. Human anti-VZV antibodies were added to the culture medium 24 h after infection, maintained for 2 h, and then removed (Fig. 1A). Mock-infected cells were cultured and treated in the same way. The fate of antigen-antibody complexes was monitored. When cultured infected cells were exposed to antibodies specific of their membrane constituents, the immune complexes formed either were internalized (15) or remained on the cell membrane (20). When cells were fixed at various times after immunomodulation and then incubated with rabbit anti-human IgG antibodies coupled to peroxidase (Dako) (1/40 in PBS, 60 min at 37°C), immune complexes were never detected on the surface of uninfected cells, and a typical patching and capping effect was observed on infected cells. The presence of membranous antigen-antibody complexes formed during the immunomodulation treatment was observed by using a la-

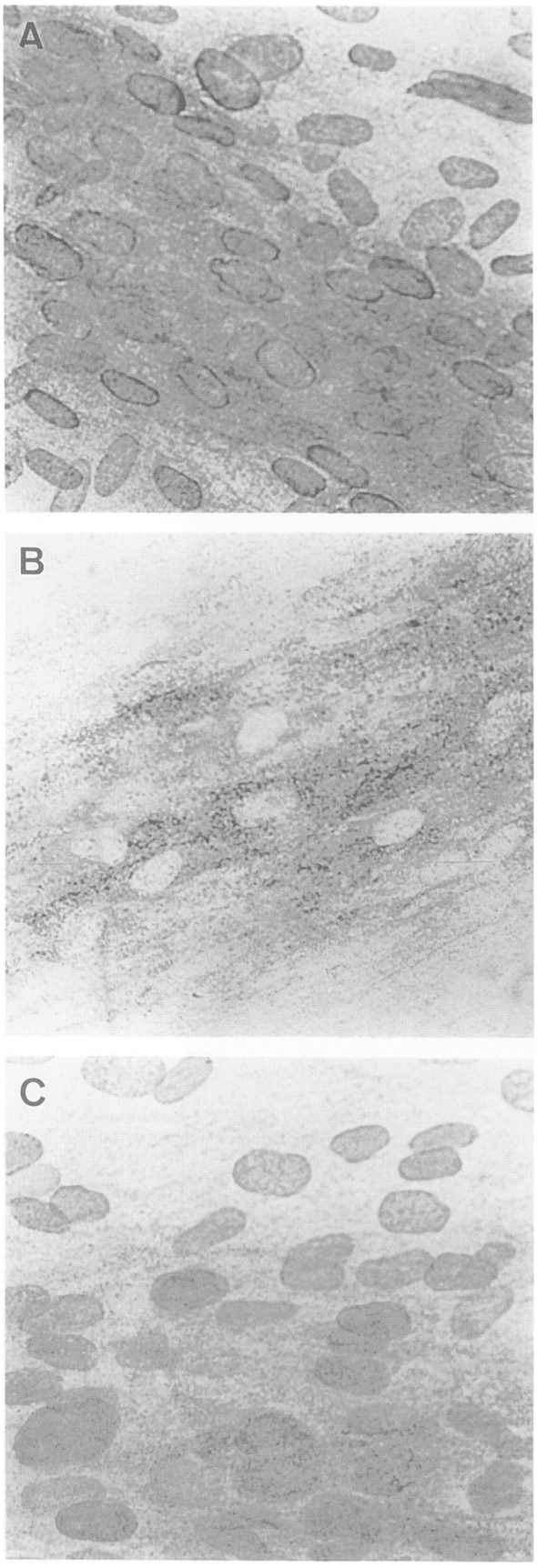

FIG. 3. Immunoperoxidase labeling of viral antigen with a monoclonal antibody (Biosoft 215-84/101) on VZV-infected cells. Cells were cultured without (A) or with (B and C) anti-VZV human antibodies for 2 h, rinsed, and then fixed with acetone immediately (B) or after 1 h (C). Magnification, ×440.

beled anti-human Ig antibody. Such complexes were homogeneously distributed on the cell surface immediately after antibody treatment (Fig. 2A). If cells were allowed to recover after treatment, immune complexes formed patches randomly scattered on the surface (Fig. 2B) and then gathered in a cap (Fig. 2C). Endocytosis followed; no labeling was detected after paraformaldehyde fixation, whereas cytoplasmic labeling was observed after acetone fixation, after which there was almost complete clearance of complexes from the cells.

The effect of antibody treatment was significant when free antigenic sites were observed by immunoperoxidase labeling. The most striking observation was the profound modification of the localization of viral epitopes recognized by an anti-gpII monoclonal antibody after the transient treatment. In untreated cells, an epitope with a nuclear and cytoplasmic distribution was observed (Fig. 3A). This distribution became exclusively cytoplasmic when cells were cultured for 2 h in the presence of anti-VZV antibodies (Fig. 3B). When antibodies were washed away, it took only 60 min for the epitope localization to become nuclear again (Fig. 3C). This modification of viral epitope localization was confirmed with an anti-gpI monoclonal antibody.

However, viral protein expression was unchanged qualitatively and perhaps only slightly delayed by transient immunomodulation; the anti-gpII monoclonal antibodies revealed two major bands (49 and 39 kDa) in Western blot analysis of VZV-infected MRC-5 cells, even in untreated cells and cells transiently treated with human anti-VZV antibodies (Fig. 4). In the same way, no modification of the expression of viral proteins recognized by rabbit anti-VZV hyperimmune serum has been detected.

These transient immunomodulation experiments indicated that under the conditions described, antibodies reacted with

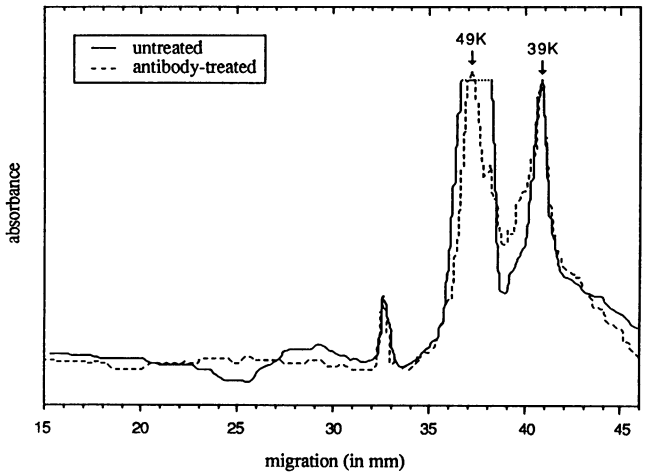

FIG. 4. Electrophoretic profile of viral antigens revealed after Western blotting. Cells were cultured without or with anti-VZV human antibodies during 2 h and recovered 48 h after infection. Viral polypeptides were detected with an anti-gpII monoclonal antibody.

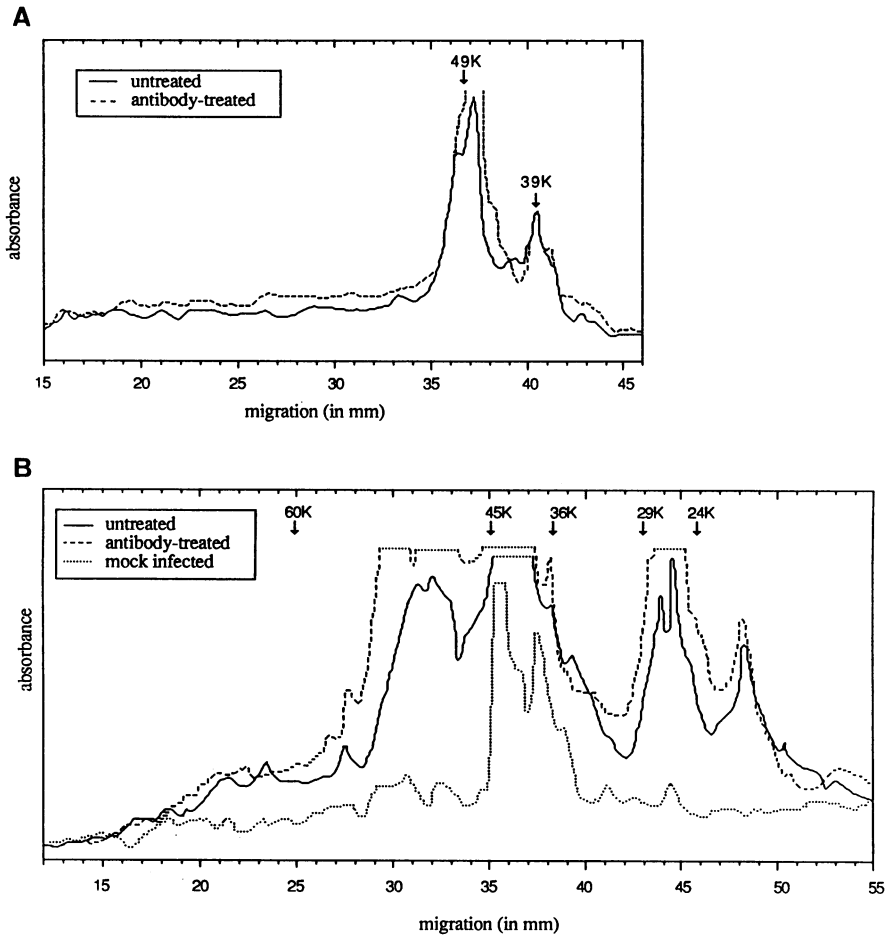

FIG. 5. Electrophoretic profile of viral antigens revealed after Western blotting. Cells were cultured without or with anti-VZV human antibodies during 48 h and then recovered. Viral polypeptides were detected with an anti-gpII monoclonal antibody (A) or a rabbit anti-VZV hyperimmune serum (B).

viral antigens on infected cells in such a manner that they could induce significant modifications in these cells. Permanent immunomodulation experiments could then be undertaken. Infected and mock-infected cells were cultured in the presence of human anti-VZV antibodies for various times and then fixed (Fig. 1B). The effects of immunomodulation were studied by searching for immune complexes on the cell surface, modifications of localization and expression of viral polypeptides, and production of infectious virus. The same pattern of redistribution of immune complexes on the cell surface was observed, and modifications of the localization of internal viral glycoproteins occurred as in transient treatment. Expression of viral polypeptides after 48 h of culture in the presence of anti-VZV antibodies was qualitatively unmodified, as indicated by Western blotting analysis with an anti-gpII monoclonal antibody (Fig. 5A) or with rabbit anti-VZV antibodies (Fig. 5B). Any quantitative differences that appeared seemed to reflect variations in the evolution of infection in cell cultures.

TABLE 2. Infectivity after permanent immunomodulation

Antibody treatment	Duration (h) of immunomodulation	Infectivity (PFU/10^6 cells)
−		1,670
+	48	1,500
−		6,300
+	72	8,000

Treated and untreated cells were scraped, pelleted, and ultrasonicated. Supernatants were inoculated on uninfected MRC-5 cells, and foci were counted after 4 days. Infectious titers remained unchanged and evolved similarly whether cells had been treated or not (Table 2).

Our data indicate that antibody treatment of infected MRC-5 cells, although playing a dramatic role in the intracellular localization of viral glycoproteins, has, surprisingly, no apparent effect on viral maturation and assembly. Since polypeptides present at the cell surface and capable of interacting with antibodies during immunomodulation treatment are glycoproteins, we used antiglycoprotein antibodies for characterization of the immunomodulation effects. However, it remains possible that a change in expression or localization of nonglycosylated polypeptides occurs in response to antibody pressure, but in this case, it does not seem to alter production of infectious particules. Such an immunomodulation treatment is thus obviously unable to induce persistent VZV infection in vitro. The correlation between the permanent presence of anti-VZV antibodies in body fluids during the latent phase of infection and the

induction of a quiescent state is thus weakened by our observations. However, our findings do not exclude a role for humoral immunity in maintaining VZV latency once it has been induced.

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