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Selective elimination of cytomegalovirus infected cells by immunotoxins targeted with various antibodies linked to ricin A chain or gelonin. BB Barnett, DF Smee and RW Sidwell. Institute for Antiviral Research, Utah State University, Logan, UT 84322-5500, USA

Cytomegalovirus (CMV) infections in the immunodeficient patient may be suppressed with currently approved drugs. However, in the absence of an effective cytotoxic immune response, infected cells persist and the CMV disease recrudesces upon termination of drug therapy. An additional component that might be beneficial in CMV therapy in immunodeficient patients is the use of selective cytotoxic agents to eliminate CMV-infected cells. Immunotoxins are targeted cytotoxic agents with the potential to accomplish this purpose. Clinical trials of immunotoxins in cancer patients have shown antitumor effects and reasonable patient tolerance. The development of anti-CMV immunotoxins will facilitate testing of combination therapies consisting of specific virostatic drugs and immunotoxins targeted to viral markers displayed on the surface of infected cells. This report describes the selective cytotoxic activity of anti-CMV immunotoxins constructed by linking anti-HCMV and anti-MCMV (murine CMV) antibodies to gelonin or ricin A chain. Activity was measured by [35S]methionine incorporation. Responses of CMV-infected and uninfected cells to immunotoxins were dose and time dependent. The specific cytotoxicity (EC50) of a gelonin-polyclonal immunotoxin to MCMV infected cells was 36 μg/ml; there was insignificant toxicity towards uninfected cells at 200 μg/ml for a selective index (SI) of >6. Immunotoxins consisting of polyclonal or monoclonal antibodies linked to deglycosylated ricin A chain were determined to have EC50 values ranging from 0.4 to 13 μg/ml and SI values ranging from 12 to 56. By increasing the duration of treatment from 24 h to 48 h the EC50 was lowered 6-fold and the selective index increased 3.5-fold. Control immunotoxins towards irrelevant proteins had EC50 values ranging from 60 to >200 μg/ml and SI values between 1 and 2. Immunotoxin binding and internalization proceeded via CMV markers expressed even in the presence of therapeutic levels of ganciclovir. Ganciclovir at 32 μg/ml, which was strongly inhibitory to CMV infection, had no effect on immunotoxin activity. These data suggest immunotoxins have potential for killing CMV-infected cells in the immunodeficient patient during otherwise traditional antiviral therapy.

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Activities of Anti-Murine Cytomegalovirus (MCMV) Immunotoxins Used Alone or in Combination with Antiviral Agents Against MCMV Infections. D.F. Smee, K.M. Okleberry, B.B. Barnett, and R.W. Sidwell. Institute for Antiviral Research, Utah State University, Logan, Utah USA.

Immunotoxins are targeted cytotoxic agents which have the potential to exhibit antiviral activity by selectively killing virus-infected cells. To explore this hypothesis, immunotoxins comprised of deglycosylated ricin A chain (dgA) coupled to monoclonal antibodies toward MCMV have been constructed and evaluated for anti-MCMV activity in cell culture. One antibody, D5.F10.B8, is MCMV-neutralizing, whereas a second antibody, C34.18.F6, is non-neutralizing. Both antibodies are of the IgG_{2a} subtype. When coupled to dgA the resulting immunotoxins, D5-dgA and C34-dgA, inhibited MCMV replication in virus yield assays at ≥1.25 µg/ml in a dose-responsive manner when added to cell cultures starting 24 hours after virus inoculation. Non-specific cell killing of uninfected C127I cells was also dose-responsive and occurred starting at ≥5 µg/ml in parallel assays. The activities of the immunotoxins increased when combined with antiviral agents such as ganciclovir and HPMPC. Immunotoxin concentrations of 0.63 to 2.5 µg/ml were synergistically active with ganciclovir concentrations of 1.25, 2.5, and 5 µM, or with HPMPC concentrations of 0.03, 0.06, and 0.12 µM. These results suggest that immunotoxins may have potential for the treatment of cytomegalovirus infections, particularly when combined with antiviral drugs.

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