Filtracon Sizes of Human Immunodeficiency Virus Type 1 and Surrogate Viruses Used To Test Barrier Materials

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Viruses are being used to evaluate barrier materials (1–4, 12–17, 20, 21, 24, 27, 29, 30). For safety and cost considerations, bacteriophages are being used as surrogate viruses for human pathogenic viruses, e.g., human immunodeficiency virus (HIV), hepatitis B virus, or herpes simplex virus (HSV) (1,20,21). The property of virus particles which is expected to most influence penetration through barrier materials is size. It is generally accepted (11) that a virus particle may pass through a hole whose diameter is greater than the diameter of that particle measured from electron micrographs. The purpose of the present study was to verify this statement by using filters with well-defined holes to determine the effective (hydraulic) diameters in buffer of HIV type 1 (HIV-1) and four bacteriophages (φX174, T7, PRD1, and φ6), which may serve as surrogate viruses for testing barrier materials. Bacteriophages φ6 and PRD1 most closely model human immunodeficiency virus type 1 in filtration size.

Filters with well-defined holes were used to determine the effective diameters in buffer of human immunodeficiency virus type 1, herpes simplex virus type 1, and four bacteriophages (φX174, T7, PRD1, and φ6), which may serve as surrogate viruses for testing barrier materials. Bacteriophages φ6 and PRD1 most closely model human immunodeficiency virus type 1 in filtration size.

The viruses, their host cells, and their compositions are listed in Table 1. The enveloped bacteriophages φ6 and PRD1 were chosen as possible surrogates for HIV-1. Standard procedures were used for virus growth and for quantitative assay of virus titers, with plaque assays used for all viruses (18,19) except HIV-1, for which both reverse transcriptase and antigen capture assays were used (6,23,25,26,28). The test virus suspensions were used at concentrations of 0.4 × 10⁴ to 1.5 × 10⁴ PFU/ml for the bacteriophages, 6 × 10⁴ tissue culture infective doses per ml for HIV-1, and 1.4 × 10⁴ PFU/ml for HSV-1.

We used the filtration end point method of Elford and coworkers (5,6) as modified by Hsiung (11) and subsequently by He et al. (10). The size of the virus is bracketed by filter hole sizes which just pass or just block that virus. In brief, the viruses in 3 ml of Dulbecco’s phosphate-buffered saline were filtered through 25-mm Nuclepore polycarbonate membrane filters (which are coated during manufacture to have a nonionic surface that is low binding for proteins) with quoted pore diameters of 15, 30, 50, 80, 100, 200, 400, 600, 800, and 1,000 nm. The pore diameters quoted by the manufacturer define the maximum pore diameter, with the median diameter being approximately 10% less than the quoted value. The filtration rate was controlled by attaching a hypodermic needle to the downstream side of the filter and pushing the needle into a Vacutainer except (for safety reasons) with HIV-1, for which the filtration was manually controlled and the filtrate passed through a cannula into a tube. The virus titers were determined before and after filtration, and the fractions which passed through the filters were calculated.

We evaluated factors which might confound interpretation of the results, such as binding of the viruses to the filters or effects of cellular debris in virus stocks. Previously, we found that, in Dulbecco’s phosphate-buffered saline, these bacteriophages would not be transmitted through protein-binding filters unless sufficient extraneous protein (2% serum) was present (21). No evidence of virus binding or other inhibitory action by the Nuclepore filters was discovered in preliminary studies (not shown) in which manually controlled, very slow filtration through larger pore sizes did not decrease the titers of φX174, T7, PRD1, φ6, or HSV-1. The virus stocks (cleared by low-speed centrifugation) did contain small cellular debris which was substantially diluted with the viruses for the experiments. Since the filters were low binding for proteins and viruses, the filtration of the viruses should not have been affected by this extraneous protein. Furthermore, since the buffer containing virus and cellular debris passed through the filters at appropriate rates, there was no evidence of blocking of filter holes by the extraneous protein.

The results are shown in Fig. 1 and summarized in Table 2. In most cases, the size of the virus in electron micrographs was reflected in the filtration results. He et al. (10) showed that, when the electron microscopic diameter of the virus is approximately the same as the quoted filter pore diameter, the virus fraction transmitted through the filter is about 1%. Our results confirm that finding in the cases of φ6 (at 80 nm) and HIV-1 (at 100 nm), and the data for φX174 and T7 are consistent with that statement. The low transmission values probably result from a combination of there being only a few pores as large as the quoted diameter and the virus particles being a hydration shell (boundary layer) associated with surface proteins.

Filtration data with the 80-nm filter for bacteriophage PRD1 were almost identical to data for φ6, indicating that PRD1 behaved as though it were effectively 80 nm in diameter rather than the 65 nm seen in electron micrographs. The presence of spikes could explain this anomaly, although no evidence of spikes on PRD1 has been reported (23a).

Both enveloped human viruses, HIV-1 and HSV-1, had

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lower than expected transmission through the larger pores (200 to 600 nm). The filtration data with HSV-1 were not consistent with the size determined by electron microscopy (120 to 150 nm), but were consistent with other, reported filtration data which place the effective diameter of HSV at 200 to 250 nm (6, 11). Only a few percent of HIV-1 passed through the 200-nm pores, and less than 20% passed through 400- and 600-nm pores, in general agreement with the results of He et al. (10) for a different retrovirus, Friend murine leukemia virus. It is not known which factors contributed to poor passage of these viruses through the larger pores.

In summary, as expected, the filtration sizes of the bacteriophages we tested were similar to those seen in the electron microscope. All of these bacteriophages may serve as surrogates for HIV-1 or HSV-1 since they represent a conservative test of barrier materials since they all pass through pores at higher rates than HIV-1 and HSV-1. Finally, for filtration size, bacteriophages φ6 and PRD1 most closely model HIV-1.

REFERENCES


