Affinity Hemodialysis for Antiviral Therapy with Specific Application to HIV

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We propose an artificial lymph node to improve immune function in fighting viral diseases. The device is based on hemodialysis using a cartridge containing a solid phase affinity resin. Virus capture is mediated by a collection of broad specificity antibodies covalently coupled to agarose. Viral proteins, which can directly damage uninfected cells, are also efficiently removed. Immobilized antisense DNA provides a mechanism to remove infectious viral nucleic acids. Theoretical calculations suggest that the device could effectively remove virus, toxic viral proteins and infectious viral nucleic acids from the blood thereby limiting disease by preventing reinfection of new cells. In the absence of newly infected cells, previously infected cells are cleared by the immune system. For a typical immobilized antibody, calculations predict a pseudo-first order rate of capture \( t_{1/2} \approx 10 \text{ min} \) with viral load reduction \( \approx 660\)-fold at equilibrium. Theoretical calculations of a diffusion limited process predict \( t_{1/2} \approx 2.8 \text{ h} \). Measured transport rates for latex particles in a prototype device are significantly faster than the theoretical diffusion limit suggesting that transport is primarily convective and sufficient to allow rapid virus clearance. Since the device is highly selective it can be used in conjunction with drug therapy and other treatments.

Keywords: Virus load; Replication rate; Clearance rate; Affinity hemodialysis; Virus diffusion; Mathematical models

INTRODUCTION

Antiviral treatments have long been sought to help eradicate important viral diseases. While the body nearly always generates a defensive response, for many pathogenic viruses that response is inadequate to prevent debilitating disease or death. The first effective treatments were vaccines based on weakened or killed viruses. Vaccines such as those developed by Jenner for smallpox, Pasteur for rabies and Salk and Sabin for polio have been highly successful in eradicating viral diseases. More recently, effective vaccines for measles, mumps, rubella and Hepatitis B virus have been developed. However, the search for new vaccines is almost always difficult. Moreover, vaccines for chronic diseases are rare (e.g. HBV vaccine) and to date no successful vaccines exist for chronic diseases caused by highly mutable viruses. For HIV, which introduces up to three mutations in each newly synthesized virus and multiple quasi-species, the search for an effective vaccine is now in its second decade (Gallo and Schupbach, 2000; Menzo et al., 2000; Cohen, 2001).

Although a vaccine may be the best long-term hope for curing chronic viral infections, results to date have been disappointing. The search for antiviral chemotherapeutics has had less success in spite of intensive research. Conventional therapies using small molecule drugs have grown steadily more effective, particularly those directed against HIV infection and AIDS (Baum et al., 1999; Bower et al., 1999; Clifford et al., 1999; Mocroft et al., 1999). In spite of advances in the treatment of HIV using highly active antiretroviral therapy (HAART), recent studies have shown that live viruses persist in the system even after years of treatment (Chun et al., 1999; Zhang et al., 1999; Ramratnam et al., 2000). Currently 28.5% of patients are drug resistant, a population that is expected to increase to over 40% by 2005 (Blower et al., 2001). In addition, latently infected cells and macrophages provide a treatment resistant reservoir that is long lived (Aquaro et al., 1998; Chun and Fauci, 1999). To make things more complicated, host factors also play a role in both disease progression and drug resistance (Cohen et al., 1997).
As a result, although patient condition is improved, life is prolonged an average of only 1.8 years (Bozette, 2001) and the treatment ultimately fails due to the development of drug resistance. Recognition of these difficulties has recently led to substantial changes in the Federal Treatment Guidelines on HIV infections (Fauci et al., 2001).

Taken together, these observations indicate that alternative antiviral therapeutic modalities need to be explored. We propose an alternative treatment based on a device that augments the function of the immune system through the physical removal of viruses, toxic viral proteins and potentially infectious viral nucleic acids from the blood of infected patients. Physical removal of pathogens is precisely what biology accomplishes through humoral and cellular immune responses. Antibodies generated to viral antigens bind to the virus and mediate physical clearance from the body, which in turn reduces the re-infection rate. At the same time, previously infected cells are cleared by the immune system, die or become quiescent. When sufficient amounts of the pathogen are cleared from the blood, the viral infection recedes.

Recent drug studies provide evidence that preventing the re-infection of new host cells by circulating infectious virus is an effective means of reducing viral load and inhibiting disease progression. Entry inhibitors, the latest class of new antiviral drugs, are designed to prevent mature, infectious virus from entering new cells. For HIV, the most clinically advanced of the entry inhibitors is T-20, a peptide that binds to gp41 in the viral envelope and prevents fusion of HIV envelope with the host cell membrane (Kilby et al., 1998). A number of other entry inhibitors are currently in or near clinical trials.

The proposed device is a modified hemodialysis cartridge containing immobilized antibodies and antisense DNA. The device functions as an artificial lymph node, increasing the rate of virus clearance and reducing the bystander and superantigen effects of intrinsic viral proteins. The technique is based on hemodialysis, a widely used and highly successful therapy most commonly used to treat kidney failure. The basic cartridge has been in clinical use for over 30 years. The use of multiple antibodies directed against conserved epitopes in the viral coat proteins makes the development of resistant mutations very difficult. Since the affinity matrix elements are covalently immobilized, side effects should be minimal. And since the technique is highly specific, the treatment could be used in conjunction with drug therapy and most other treatments, in contrast to expensive, cumbersome and less effective blood replacement techniques.

In this paper, we describe the principles of the device and discuss the theoretical basis for its operation using HIV as a model infection. While HIV is the focus of this article, we recognize that the technique may be as important in treating fatal, acute viral infections.

**DESCRIPTION OF THE AFFINITY HEMODIALYSIS DEVICE**

Our device is based on hemodialysis and a novel hollow-fiber affinity dialysis cartridge designed to boost immune system function by efficiently removing viruses and toxic viral proteins from the blood of infected patients. Effective removal of infectious virus from circulation prevents the reinfection of new host cells, analogous to entry inhibitor drugs. As newly infected cells are reduced, previously infected cells die or are cleared by the immune system.

At the same time, viral proteins are reduced, thereby preventing immune cell loss due to the “bystander effect” and superantigenicity. Although there are several theories for the mechanism by which HIV causes AIDS (cf. Cloyd et al., 2000), both the bystander effect and superantigenicity are well documented. HIV envelope proteins have been shown to cause CD4+ T cells to undergo apoptosis (Koga, 1996; Medina et al., 1999). Binding of gp120 to CD4+ cells in the presence of anti-envelope antibodies and complement opsoninizes the cells, targeting them for clearance (cf. Chaplin, 1999). The combined effect is the destruction of uninfected immune cells known as the “bystander effect” (Ameisen et al., 1995). In addition, HIV envelope proteins function as superantigens, which first overstimulate then deplete B cells (Schnittman et al., 1986; Nair et al., 1988; Muller and Kohler, 1997). Perhaps equally germane, viral surface proteins can directly damage neuronal cells (Krucker et al., 1998; Lipton, 1998; Gray et al., 2001).

Thus the device would function as an artificial lymph node, increasing the rate of virus clearance and reducing the toxic effects of the viral proteins. Moreover, the device is expected to be highly selective for the virus and hence compatible with conventional drug therapy and most other treatments.

The basic platform for the device is hemodialysis. Hemodialysis and plasmapheresis are widely available techniques. While hemodialysis is generally associated with treatment for kidney failure, it has also been used for direct removal of toxins and heavy metals (Ambrus and Horvath, 1987; 1988; Ambrus et al., 1987; Anthone et al., 1995). Circulating immune complexes are also routinely removed by plasmapheresis (Furst et al., 2000). Recent studies have suggested that extracorporeal treatments may have value in treating HIV and AIDS. Simple hemodialysis can produce a small but measurable reduction in viral load (Ahuja et al., 1999). Beretta et al. (2000) report immunologic improvement in partial HAART responders after ex-vivo removal of circulating monocytes. Blick et al. (1998) suggest that passive immunotherapy and frequent plasma donation may delay disease progression in asymptomatic HIV-infected individuals.

For all these reasons, hemodialysis using adsorptive media capable of physically reducing viral load may represent an attractive adjunct to current therapy. While clearly this type of treatment is intended only for patients who are likely to become extremely ill or die as the result
of infection, it is also clear that these are the diseases whose solution is most highly sought after. Perhaps equally important is the fact that a significant population of HIV+ patients already receive dialysis treatments as a result of HIV-associated nephropathy (Winston and Klotman, 1998; Saha and Agarwal, 2001).

The selectivity of the device is provided by an affinity matrix comprised of a collection of covalently immobilized antibodies directed against conserved epitopes in the viral coat proteins. Immunoaffinity chromatography has been widely employed to purify biological macromolecules as described by Cuatrecasas et al., 1968 (cf. Areson et al., 1980). Large particulate antigens, in particular viruses have been purified in this fashion (Lawrence, 1993; Hauswirth et al., 2000). Studies using affinity methods to remove viruses from blood have typically employed variants of direct binding to ion-exchange resins or immunochromatography in which the blood or plasma is passed directly over the resin. (Terman et al., 1980; Wang and Tsao, 1988; Takashima et al., 1991; Lopukhin Iu et al., 1991; 1992; Tepic and Laissue, 1996; Motomura et al., 1997). While successful in some cases, these methods have not achieved the desired results with virus removal from blood. One plausible explanation for this observation is that many viruses (e.g. HIV) are relatively fragile, shedding viral surface proteins with minimal provocation. As a result, the virus can be stripped from its binding sites by shear forces resulting from fluid flow when blood or plasma is treated directly. In addition, serious side effects such as hemolysis and complement activation may occur when blood cells directly contact the affinity sorbent.

To overcome these difficulties, we use a hollow-fiber dialysis cartridge that contains an affinity resin on the dialysate side of the membrane (Scamurra and Ambrus, 1999). Figure 1 shows how the device is designed to operate.

In the device, the affinity matrix containing immobilized antibodies is located in the free space between the hollow-fibers and set apart from the bulk fluid flow. As blood flows through the hollow-fibers, the virus is transported by a combination of diffusion and convection through pores in the hollow-fibers to reach the affinity matrix. In this situation, antibody-bound viral particles exist outside the blood flow, allowing even relatively weak interactions to capture them. The covalent coupling of antibodies to the affinity matrix prevents them from entering the general circulation, potentially allowing the use of non-human antibodies.

**HIV AS A MODEL VIRUS**

HIV is a good model virus for chronic viral disease since a great deal is known about the biology of the virus (cf. Fauci et al., 1996; Gallo and Schupbach, 2000; Braunwald et al., 2001). While it is not the purpose of this article to review these findings, there are a few facts

**FIGURE 1** Operation of a Hypothetical Artificial Lymph Node Based on an Affinity Hemodialysis Cartridge. The device is a disposable, hollow-fiber hemodialysis cartridge designed to fit on existing hemodialysis machines. The affinity matrix consisting of antibodies covalently-coupled to agarose or glass beads is located in the free space between the hollow-fibers and the dialysate compartment is sealed. Virus and free viral proteins in the blood are transported via convection and diffusion through 200 nm pores in the hollow fibers to the matrix where they are captured. Formed elements of the blood including red and white cells are excluded.
worthy noting. First, while HIV preferentially infects CD4+ T cells and macrophages, other cell types can also be infected. These include bone marrow derived stem cells, microglia, fibroblasts, Langerhan’s cells, testicular cells and epithelial cells (cf. Pearce-Pratt and Phillips, 1993; Kulkosky et al., 2000). Thus viral reservoirs other than memory T cells and macrophages may be important. Second, HIV is generally considered non-infectious until the virion has budded off the surface of the infected cell and matured. Maturation may be prevented by protease inhibitors that inhibit viral polyprotein cleavage (Wiegers et al., 1998; Jardine et al., 2000). This may be an important factor in determining the spatial distribution of infectious virus. Third, in blood less than 1% of CD4+ T cells are HIV+ and a large proportion of the circulating viral particles appear to be non-infectious (Schnittman et al., 1989; Bourinbaiar, 1994). In cell culture, as little as 0.001% of the virions may be capable of infecting new cells (Layne et al., 1992). These defective particles can also have deleterious effects not associated with virus growth (Kameoka et al., 1997a,b).

The replication rate of HIV in humans is estimated at $10^{10}$ virions per day with a doubling time of 10 h and a half life in blood of $t_{1/2} \sim 6$ h (Ho, 1995; Ho et al., 1995; Wei et al., 1995; Ogg et al., 1998; Little et al., 1999). Infected T cells have an estimated lifetime of 1.88 days (Lafeuillade et al., 1996a,b). In patients undergoing HAART treatment, the doubling time is estimated at 2–3 days (Neumann et al., 1999). Studies using therapeutic plasma exchange have also been used to estimate the maximal viral doubling times and clearance half times at 3.5–4 h. In plasma exchange studies, treatment times range from 1 to 2 h. Due to the short duration of treatment, it is likely that the measured rates have a substantial contribution from virus entry into the blood from extravascular reservoirs in addition to new viral replication.

The levels of HIV-1 viral particles in blood strongly correlate with the clinical status of infected patients (Ferre et al., 1995; O’Brien et al., 1996). Typical measured plasma HIV concentrations are on the order of $10^4$ to $10^6$ viral copies per ml (cpm). During HAART therapy, the circulating virus population can drop to undetectable amounts (<100 cpm). Even when undetectable by most clinical tests, infectious virus is still present in circulation and can reactivate on removal of the drugs. The decay of latent HIV is inversely correlated with the extent of residual viral replication (Ramratnam et al., 2000) and viral infection is maintained by re-infection of new host cells (Furtado et al., 1999).

Previous studies have suggested that a physical device capable of removing virus would have a beneficial effect. Kapadvanjwala and Sofer (1989) developed a detailed model of the natural history of HIV and the effect of a hypothetical machine that removes the virus from circulation. The model predicts that small increases in the rate of virus removal cause a significant decrease in virus levels. A virus removal device would be even more effective when used in conjunction with drug therapy and other treatments.

More recent models describe the initial infection and establishment of the virus in a steady state (cf. Bonhoeffer et al., 1997; Tuckwell and Le Corfec, 1998; Wodarz et al., 1999). Stafford et al. (2000) provide an explicit model for the early stages of HIV infection and the establishment of the set point or asymptomatic phase in which viral particle production is in steady state with viral particle clearance. The expressions that describe the virus concentration as a function of time are given by a series of non-linear differential equations

\[
\frac{dT(t)}{dt} = \lambda - dT - kTV \]

\[
\frac{dT^*(t)}{dt} = kTV - 8T^* \]

\[
\frac{dV(t)}{dt} = \pi T^* - cV
\]

where $T$ is the number of CD4+ T cells (cells/ul), $T^*$ concentration of activated CD4+ T cells (cells/ul), $V$ is the viral load (copies/ul), $\lambda$ is the rate of CD4 T cell production, $k$ is the rate of infection of T cells, $d$ is the death rate of normal T cells, $\delta$ is the death rate of productively infected cells, $\pi$ is the rate of virus production (virions/cell/day) and $c$ is the clearance rate constant for the virus.

Using these expressions we calculated the effect of increasing clearance rates on the viral load using a numerical approach. The results, presented graphically in Figure 2, have several interesting features. As Stafford et al. (2000) have already shown, the viral load shows a damped oscillation with time corresponding to changes in viral load shown in patient studies. The results also show that as little as a three-fold increase in clearance rate causes major changes in both the time of appearance of the initial viremic spike and the steady state level of virus. In this model, an increase in viral clearance corresponds to continuous dialysis in a hospital setting ($c = 9$). In practice treatment patterns will be with either continuous (for brief periods of time) or intermittent (a few hours every few days) as is currently the case for kidney dialysis. Continuous dialysis for a period of time early in infection could serve to prevent the establishment of a large viral reservoir, thereby reducing the viral set point and delaying the onset of frank disease.

**CONSIDERATIONS IN THE DESIGN OF AN AFFINITY HEMODIALYSIS Device**

The design of an affinity hemodialysis device requires consideration of several key factors. First, the rate and extent of virus binding to the cartridge must be sufficient to remove a major fraction of the viral load in a single
treatment. Second, the rate of diffusion and convective transport of the virus across the membrane must be fast enough to allow effective binding to the affinity matrix. And third, the device must effectively reduce viral load without damage to the blood.

RATE AND EXTENT OF VIRUS BINDING TO IMMOBILIZED ANTIBODIES

The first criterion requires that the binding capacity of the cartridge substantially exceed whole body viral load. To accomplish this, the binding affinity of the immobilized antibodies must be high enough to assure efficient capture of the virus and the kinetics of binding of the virus must be fast enough so that the procedure can be performed in a few hours on the entire blood volume.

The minimum virus-binding capacity of the cartridge is determined by the steady state levels of the virus, which in turn depends on the rates of viral reproduction and clearance. Based on the daily production of $10^{10}$ virions, one would minimally require $1.6 \times 10^{-14}$ moles of specific antibody to bind the virus. The cartridges we envision will contain 10 mg of MAb immobilized on 10 ml of solid support with a maximum capacity of $4 \times 10^{15}$ virions. This capacity is 400,000 times higher than the capacity needed to remove the total daily production of the virus. Given that good monoclonals have binding affinities $K_a \sim 10^{10}$ high capture efficiencies can be expected. Given a reasonable concentration of MAb within the cartridge of $7 \times 10^{-8}$ M, the equilibrium concentration of the free virus would be $1.3 \times 10^{-18}$ M vs. an initial concentration of $8 \times 10^{-16}$ M. At equilibrium, this would result in 660 fold reduction in both virus concentration and free viral surface proteins (e.g. gp120).

For efficient capture, the rate of the reaction is also important. The kinetics of virus binding must be fast enough so that the procedure can be performed quickly. The influence of immobilization on antibody reaction kinetics has been studied in detail (Nygren et al., 1987). Typical monoclonal antibodies have on-rate constants averaging $k_{on} \sim 10^5$ M$^{-1}$ s$^{-1}$. Since the antiviral antibodies are in vast excess over circulating virus, the reaction becomes pseudo-first order. Using the conditions given above, we can calculate a $t_{1/2} \sim 100$ s for the reaction. Under these conditions, binding should be effectively complete in 17 min (10 half-lives).

This analysis has an important corollary in that since the reaction is pseudo-first order, the half-reaction time depends only on the initial concentration of the immobilized antibody and is independent of the concentration of the virus. Thus if the rate limiting step is virus binding to the affinity matrix, even at the lowest virus concentrations, 50% will be removed in ~2 min. This should be true even in cases where the concentration of the virus is below the limit of detection of current testing.

Another consequence of viral infection is the presence of infectious viral nucleic acids derived from cell lysis. Many viral nucleic acids are known to be directly infectious. Since nucleases are widespread in nature and occur in measurable concentrations in blood, it would seem unlikely that the concentrations of viral nucleic acids in blood would be significant. However, evidence
is accumulating that circulating free DNA and RNA are present in significant amounts in plasma and serum. In fact, the initial observations of cell free circulating RNA and DNA were made by Mandel and Metais over 50 years ago. Current estimates place normal circulating DNA levels at 13 ng/ml with values climbing to over 180 ng/ml in some disease states (Anker, 2000). RNAs of several types including viral messenger RNAs have also been found (Su et al., 2001).

While current evidence suggests that HIV requires a complete, mature virion for infectivity, it would not be surprising to learn that HIV RNA has some level of direct infectivity. In order to prevent any potentially infectious viral nucleic acid from contributing to the spread of the disease, we have incorporated into the design of the device an antisense DNA affinity matrix to capture viral RNA and RNA fragments.

The rate of capture of free viral RNA or DNA can be predicted (cf. Meinkoth and Wahl, 1984). The rate expression for a pseudo-first order hybridization reaction (with the capture strand in sequence excess) is given by

\[
C_0t_{1/2} = \frac{1.98 \times 10^{-6} N}{L^{0.5}}
\]

where \( C_0 \) is the concentration of the oligonucleotide (in moles nucleotide/l), \( N \) is the complexity of the DNA and \( L \) is the length of the DNA in bases. Using this formula, one can estimate the half-time for a hybridization capture under essentially physiological conditions. For a 20 base capture probe at 50 ug/ml, the calculated \( t_{1/2} \) is \( \sim 0.056 \) s. Thus, on the basis of direct reaction rates it is reasonable to expect that an affinity hemodialysis cartridge could efficiently remove circulating viral nucleic acids in times on the order of minutes.

**HEMODIALYSIS AND VIRAL DIFFUSION**

The second design consideration is that the transport of the virus through the pores in the membrane be fast enough to allow efficient removal. Diffusion is typically a slow process and is often rate limiting in chemical and enzymatic reactions (Cussler, 1998). Diffusion theory predicts an inverse proportionality to the square root of the molecular weight. On this basis alone, viruses will diffuse 5–10 times more slowly than most proteins. Thus transport of the virus may become rate limiting. Virus transport through membranes has been the subject of several mathematical studies related to the permeability of gloves and condoms (Davis, 1998).

In hemodialysis, blood flows through 200 μ capillaries at a linear flow rate \( \sim 1 \) cm/min. Under these conditions, viral particles in the blood are likely to be entrained in fluid flow through the lumen of the fibers with only a small portion retained near the walls in a relatively stagnant boundary layer. On the other hand, red blood cells and lymphocytes tend to push the smaller viral particles to the periphery due to volume exclusion effects. Within the lumen, viral particles are transported through 200 nm pores in the membrane by a combination of thermal diffusion and convection driven by pressure differentials. When the virus enters the pores, diffusion effects may become most important. Since the pores are on average 40 μ long and only 200 nm wide, virus particles in the pores can also diffuse to the walls and interact with the membrane.

In order to determine the effects of diffusion on the process, we first calculated the rate of approach to equilibrium based on the diffusion of a 100 nm particle across a porous membrane into an infinitely large dialysis reservoir (\( V_1 \gg V_2 \)) using the formula \( \Delta C_t = \Delta C_0 e^{-D \beta t} \), where \( \Delta C_t \) is the concentration difference across the membrane at time \( t \). \( D_0 \) is the diffusion coefficient at infinite dilution and \( \beta = A/\lambda^* \left( 1/V_{in} + 1/V_{out} \right) \), where \( A \) is the surface area of the membrane in contact with the fluid, \( \lambda \) is the length of the diffusion path (i.e. the width of the membrane) and \( V \) is the volume of the solution on either side of the membrane (Cussler, 1998). We model the effect of irreversible capture of the virus by setting volume inside the cartridge to be very much larger than the volume of the solution to be dialyzed (\( V_{in} \gg V_{out} \)) which reduces \( \beta \) to \( A/\lambda^* \left( 1/V_{out} \right) \).

For purposes of calculation we have used the properties of a typical hemodialysis cartridge. \( D_0 \) for HIV was estimated at \( 2.9 \times 10^{-8} \) cm²/s based on a spherical virus with a diameter of 110 nm. The results presented graphically in Figure 3 gave an estimated \( t_{1/2} \) of 2.5 or 11 h to 95% equilibration. Thus in a diffusion limited

**FIGURE 3** Transport of 100 nm Fluorescent Latex beads in a Hollow-fiber Device vs Calculated Diffusion. The cartridge was 0.5 cm × 10 cm containing polyethersulfone hollow fibers (500 μ ID; 40 μ wall thickness; nominal pore size 500 nm; estimated membrane surface area of 5 cm²; intraluminal volume 80 μl; extraluminal volume 450 μl) circulating volume was 20 ml containing 0.02% red and 0.04% HIV. Measured -- ---; calculated □ — □.
device with essentially irreversible virus binding, viral load would be reduced 20 fold in 11 h.

In a hollow fiber dialysis device, diffusion may be less important than convection. In order to test the actual performance of the device we performed a model experiment to measure the rate of transport of 100 nm carboxylate-modified fluorescent latex beads across a 500 nm pore polyethersulfone hollow-fiber membrane. Carboxylate modified beads were used to mimic a virus particle with a negative charge at physiological pH. In this experiment, 1000 nm red beads and 100 nm green beads were mixed in a 1:1 ratio in PBS and the diffusion rate observed in a 0.5 ml volume hollow-fiber dialysis device. Hemodiafiltration showed that the membranes in the device allowed 0.1 u green particles to pass freely while completely excluding the 1 u red particles, indicating that the membranes were intact and operating properly.

With the dialysate compartment sealed, the red and green bead mixture was circulated through the device at a flow rate of 10–20 ml/min. At intervals, the pump was stopped and the cartridge isolated from the external circulation by means of the shutoff valves. The external compartment, containing any beads that had entered, was then flushed with PBS and the concentration of the green and red beads noted. The valves were then restored to the original configuration condition and flow resumed. The results, also shown in Figure 3, showed a rapid accumulation of 100 nm green beads in the dialysis compartment and depletion from the external circulation. Accumulation appeared to be virtually complete in 90 min with a _t_1/2 ~ 30 min. Since this is substantially faster than the estimated rate of accumulation due to diffusion alone, particle transport in this system must involve a large convection component driven by trans-membrane pressure differentials.

To further explore the properties of the affinity hemodialysis device, we measured the capture of replication defective HIV from cell culture media. First we constructed an antibody capture matrix in which a mixture of anti-gp-120 antibodies were bound to protein G agarose and subsequently immobilized with disuccinimidyl suberimidate. A hemopurifier cartridge was then constructed using the Microkros cartridge described in the section above and loaded in the extraluminal space with the immobilized antibody matrix. As shown in Figure 4, passaging the virus isolated from the HL 2/3 cell culture fluid from through the cartridge resulted in a rapid decrease in virus as judged by p24 ELISA. After 1 h approximately 82% of the virus was removed vs. 12% for the control. Similar results were obtained with gp120 from the same cell culture system (data not shown). A more detailed analysis including in depth experimental details and results have been published elsewhere.

Thus, measured transport rates for fluorescent latex particles and viral capture from cell culture fluids support the contention that hemodialysis can be an effective means of capturing circulating virus from model fluids. Further experiments will be required to confirm the efficiency of the device against natural virus populations in blood.

**POTENTIAL PROBLEMS**

There are several potential problems which confront the design of artificial organs in general, and artificial lymph nodes in particular. Humoral antibodies specific to the virus could bind to viral surface proteins and block viral capture. Viral mutations might also allow the virus to evade capture. In addition to the biology of the host virus interactions, the device itself could generate undesirable host responses.

In almost every instance of viral infection, the body generates antibodies against viral proteins many of which target the virus coat. For HIV, these antibodies are generally of non-neutralizing and inadequate in concentration to control the infection. In order to overcome this challenge, it is necessary to select antibodies that have higher affinity for the virus than those generated by the patient. It is also possible to genetically engineer antibodies with very high affinity constants as well as significantly enhanced on rates.

Similarly, viral mutations can alter targeted epitopes allowing the virus to evade capture. In order to prevent the development of resistant mutations, we propose incorporating a collection of broad-spectrum antibodies, which react with all known viral clades. Since HIV diagnostics face the same challenge, many broad-spectrum antibodies are already known which would be directly useful.

![Figure 4](image-url)  
**FIGURE 4** Capture and removal of HIV from HL2/3 Cell Culture Supernatants Using an Affinity Hemodialysis Device. The cartridge was as described in Figure 3 modified to contain ~ 0.5 ml a mixture of anti-gp120 monoclonal antibodies covalently crosslinked to protein G agarose using DSS (disuccinimidyl suberimidate). HL 2/3 cell culture supernatant was manually passed over the cartridge for 1h. p24 gag was then measured by quantitative ELISA (NEN-DuPont). Control = albumin Sepharose. Spnt = HL 2/3 cell culture supernatant before treatment.
Clearly, virus removal will provide selective pressure toward resistant mutants just as it does with drugs and natural antibodies. However, it is a simple matter to replace antibodies in the cartridge as opposed to the development and testing of a new drug, primarily because the antibodies in the cartridge are covalently immobilized and thus prevented from entering the general circulation. This flexibility makes it possible to consider actually tailoring the cartridge to the individual patient.

Another potential problem is protein leakage from the device. Antibodies injected directly into the blood stream are already used for therapeutic purposes. We have selected a covalent coupling methodology that is highly resistant to leakage. Thus, the relatively small amounts of protein or peptide entering the blood stream from the device should not present an insurmountable problem.

Cartridge mediated damage to formed blood cells and the generation of cellular activation factors including complement is a continuing concern in hemodialysis. Early on it was observed that blood contact with synthetic membranes resulted in hemolysis, leucopenia and complement activation resulting in severe complications and death for some patients. More modern membrane formulations have substantially decreased these side effects. While some issues such as β2-microglobulin build-up remain a problem, these cartridges have been FDA approved and generally considered safe and effective in over 30 years of use.

Complement activation is perhaps the most likely problem for a cartridge of the type we have proposed. Complement activation is stimulated by the presence of immobilized antibodies and antibody complexes. The process is initiated by complement binding to the Fc region of antibodies. However, devices that trap antibody complexes are already in general use. One such device is the Prosorba column developed by Cypress Biosciences and currently marketed as an FDA approved device for the removal of antibody complexes from the blood of patients with severe, non-responsive forms of rheumatoid arthritis (Furst et al., 2000). The device consists of Protein A covalently linked to diatomaceous earth (primarily silica) enclosed in a plastic cartridge. The device removes large amount of immunoglobulins and antibody complexes without harmful levels of complement activation. Thus it is likely that the proposed device, which is very similar in structure, would not be any less safe.

CONCLUSIONS

Chronic viruses present a continuing challenge to current drugs and vaccines. We propose a device based on hemodialysis and immunoaffinity capture designed to augment the function of the immune system to treat viral infections. The device is highly selective and can be used in conjunction with other treatments. Based on the available data, it is likely that an effective affinity hemodialysis device can be developed which effectively and safely removes pathogenic viruses and viral proteins directly from the blood.

References


