Antiviral Research 87 (2010) 230–234

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Use of preconditioned human phagocytes for extracorporeal adsorption of viruses

Felix Oberender[a], Heiderose Oberender[b], Jens Altrichter[a], Emil C. Reisinger[c], Steffen R. Mitzner[a,*]

[a] Division of Nephrology, Department of Medicine, University of Rostock, Rostock, Germany
[b] Division of Virology, Department of Medical Microbiology, Virology and Hygiene, University of Rostock, Rostock, Germany
[c] Division of Tropical Medicine and Infectious Diseases, Department of Medicine, University of Rostock, Rostock, Germany

ARTICLE INFO

Article history:
Received 18 June 2009
Received in revised form 30 March 2010
Accepted 14 May 2010

Keywords:
Extracorporeal
Biohybrid
Bioreactor
Leucocytes
HL-60
Viremia
Enterovirus
HBV

ABSTRACT

Conventional treatment of severe viral disease is limited by the narrow choice as well as the often-significant side effects or lack of clear efficacy of antiviral chemotherapy. At the same time, however, it is known that a reduction in viral load leads to significant clinical improvement in a number of important viral diseases. In this paper we discuss the possibility of using preconditioned human phagocytes in an extracorporeal biohybrid system for adsorption of viral pathogens. We present data from in vitro experiments testing adsorption of an enterovirus and of hepatitis B virus (HBV) by a preconditioned human promyelocytic cell line. While no clearance of HBV could be detected, the results revealed a near elimination of enterovirus with the cell line displaying robust viability. Enterovirus titers of 1000 (reciprocal) were reduced to a mean titer of $10^{0.6}$ $CCID_{50}$ with no virus detectable after adsorption in two out of five samples. Titers of 10 000 (reciprocal) were in turn reduced to a mean of $10^{1.4}$ $CCID_{50}$. The kinetics of the process was remarkable with this near elimination of the pathogen occurring within only 15 min. Extracorporeal viral adsorption by a cellular biohybrid system appears feasible. Pairing target pathogens with suitable cell lines may offer a versatile antiviral technology.

1. Introduction

The exploration of feasibility and potential benefits of treating severe infections by extracorporeal means has become an increasingly important research field in the past two decades. Today, methods ranging from hemofiltration to immunoadsorption to pheresis technologies are being evaluated in this context (Cruz et al., 2007; Haase et al., 2007; Schefold et al., 2007). While the focus of much of the current research lies predominantly on bacterial sepsis as a preeminent clinical challenge, we would like to offer a concept for addressing the equally confronting problem of virus-induced critical illness.

In contrast to bacterial sepsis, conventional treatment options for severe viral disease, i.e. antiviral medication, are extremely limited. Apart from the very small number of pathogens for which effective treatment exists, the risk profile of medications often imposes restrictions on their use against important viruses as seen for example with ganciclovir in cytomegalovirus (CMV) infection or with ribavirin in respiratory syncytial virus (RSV) infection. At times, side effects outweigh benefits to the point that therapies are rendered too hazardous and/or of too doubtful efficacy to be considered as for instance cidofovir for treatment of severe adenovirus infection (McIntosh, 2007; Walls et al., 2003).

On the other hand, the quantitative relationship between pathogen and disease has been described in far greater detail and with much more clarity for viral illness as compared to bacterial sepsis. In case of HIV, the relationship between viral load and disease has long been established with a significant reduction in viral load known to correlate to CD4 count recovery and subsequent improvement or prevention of clinical symptoms (Mocroft and Lundgren, 2004). While certain pathogens, such as hepatitis C virus, appear to defy the correlation between viremia and clinical disease (Zellos et al., 1999), a close relationship between viral load and clinical course has been described for a number of viruses such as hepatitis B virus (HBV), CMV, herpes simplex virus (HSV), adenovirus, Epstein–Barr virus (EBV) and varicella-zoster virus (VZV) (Berger and Preiser, 2002; de Jong et al., 2000; Emery, 1999; Kimura et al., 2002; Merlino et al., 2003; Mommeja-Marin et al., 2003; Schilham et al., 2002).

Reducing viral load consequently offers the genuine possibility to significantly impact the disease process. Founded on the phagocyte-based immune support introduced by Mitzner et al. (2001), we propose the extension of the concept to viral adsorption with the view to reducing or even eliminating viral pathogens from plasma by way of an extracorporeal biohybrid system. In this system, plasma of the patient is perfused through the cell compartment of an extracorporeal bioreactor where

* Corresponding author at: Ernst-Heydemann-Str. 6, 18055 Rostock, Germany. Tel.: +49 381 494 7353; fax: +49 381 494 7354.
E-mail address: steffen.mitzner@med.uni-rostock.de (S.R. Mitzner).

0166-3542/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2010.05.007

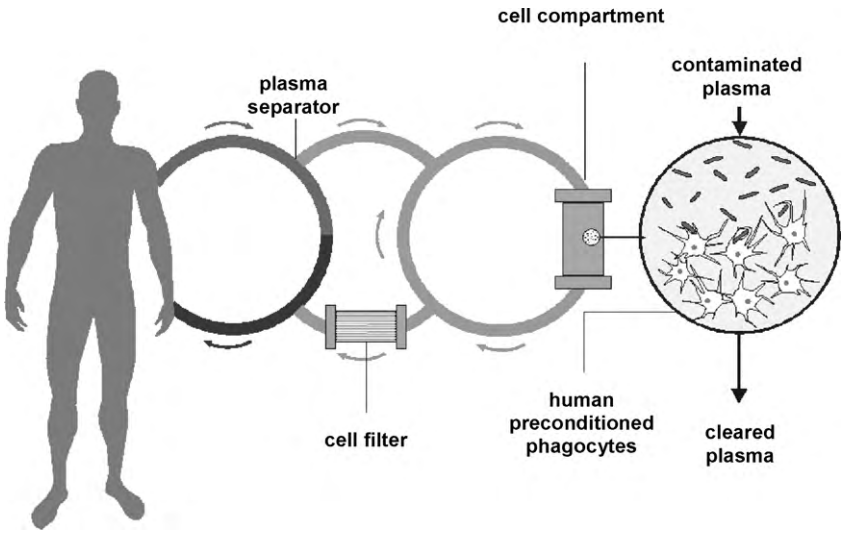

Fig. 1. Extracorporeal immune support system (Mitzner et al., 2001).

reactor-based human phagocytic cells interact with pathogens (Fig. 1).

In this paper, we present data of in vitro experiments to evaluate the potential scope and efficacy of viral adsorption by human preconditioned phagocytes.

2. Materials and methods

2.1. Phagocyte cell cultures and stimulation

The human promyelocytic cell line HL-60 (ATCC CCL-240) was cultivated in RPMI 1640 medium substituted with fetal calf serum (10%) (Invitrogen Life Technologies, Karlsruhe, Germany) under standard conditions (37 °C, 5% CO_2). Granulocytic cell differentiation was induced by all-trans retinoic acid (ATRA; Sigma–Aldrich, Deisenhofen, Germany) at a concentration of 5×10^{-5} mol/l for 5 days. Prior to entering the test series, the cells were centrifuged and resuspended in phosphate-buffered saline.

2.2. Virus source and quantification

Virus adsorption was investigated with two pathogens exemplifying two viruses of substantially different structure: an enterovirus (non-enveloped RNA virus) and HBV (enveloped DNA virus). Both viruses as well as anti-HBV antibody for opsonization were obtained from the collection of the Institute of Medical Microbiology, Virology and Hygiene at Rostock University, Germany.

The enterovirus (ECHO 30 virus) source was an isolate of pediatric cerebrospinal fluid and measured semiquantitatively through logarithmic titration on FL-cells (ATCC CLL-62). Cytopathic effects in the cell culture due to enterovirus were determined by microscopy daily over 5 days.

HBV was obtained through use of sera of patients who had tested positive for both hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg). For opsonization, anti-HBs positive serum of an HBsAg negative patient was used (titer > 1000 mIU/ml). Quantification of HBV was performed using the Digene Hybrid Capture System (Murex Diagnostica GmbH, Burgwedel, Germany). In this test, HBV is hybridized with homologous RNA, immobilized with polyclonal antibody and subsequently detected as well as quantified by chemiluminescence (peroxidase–antibody conjugate and substrate).

2.3. Test series 1: adsorption of enterovirus

0.1 ml of virus (1000 cell culture 50% infective doses – $CCID_{50}$ – determined according to Kaerber, 1931) were added to 0.9 ml of HL-60 cell suspension (10^6 cells/ml) and incubated for 15 min at standard conditions (37 °C, 5% CO_2). After centrifugation, the supernatant was titrated onto FL-cells. Five test sets were carried out whereby every set was titrated onto two rows of wells with FL-cells. Controls consisted of untreated virus as well as of virus subjected to incubating conditions and centrifugation without the presence of HL-60 cells. The test series was repeated using 10 000 $CCID_{50}$.

2.4. Test series 2: adsorption of HBV

In the first part of this test series, adsorption of HBV was examined using 5 HbsAg/HBeAg positive sera. 100 μl of serum were added to 100 μl of HL-60 suspension (10^6 cells/ml) and incubated for 15 min at standard conditions (37 °C, 5% CO_2). Supernatant was obtained after centrifugation and HBV DNA content analyzed.

In the second part of the test series, HBV in five different sera was opsonized by adding 50 μl of anti-HBs positive serum to 50 μl of HBsAg/HBeAg positive serum. The suspension was incubated for 1 h at standard conditions. 100 μl of HL-60 cell suspension was added thereafter and the test conducted in the same fashion as in the first part. Controls consisted of the untreated HBsAg/HBeAg positive sera as well as of sera subjected to all steps of the test without exposure to HL-60 cells.

2.5. Cell viability and activity

Cell viability and activity before and after adsorption were assessed using the Cell Proliferations Kit II (XTT, Roche Applied Science, Germany). The test measures the metabolic activity of viable cells (mitochondrial dehydrogenases) based on the reduction of the tetrazolium salt XTT to formazan salt with a specific UV-absorption spectrum. HL-60 cells (exposed and unexposed) were washed in normal saline. 100 μl of cell suspension were incubated with 50 μl of XTT-reagent (0.3 mg/ml) in a 96-well microtiter plate (every sample tested in quintuplicate) and incubated for 5 h followed by photospectrometric measurement.

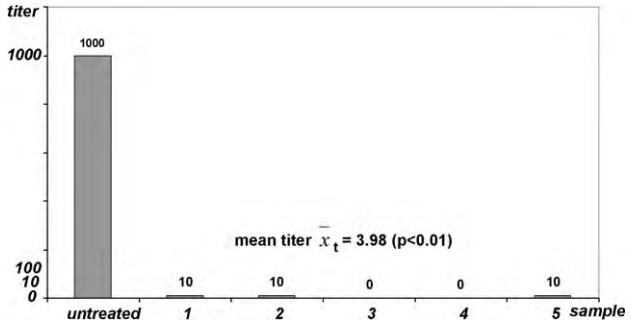

Fig. 2. Titer (reciprocal) before and after deployment of HL-60 cells against 1000 $CCID_{50}$ enterovirus for 15 min.

2.6. Statistics

Corresponding samples were compared using the Mann–Whitney *U*-test while grouped data were analyzed with the Kruskal–Wallis test (results denoted as p_{kw}). Means of titers ($\bar{x}_t$) were calculated as geometric means with $\bar{x}_t = 10^{\text{mean of 1:10 dilution}}$.

3. Results

3.1. Test series 1: adsorption of enterovirus

Deploying HL-60 cells against 1000 $CCID_{50}$ of enterovirus for 15 min (Fig. 2), a reduction in (reciprocal) viral titer from 10^3 to 10^1 was recorded in three samples. No virus was detectable in two samples leading to a geometric mean titer of $10^{0.6}$ $CCID_{50}$ ($p < 0.01$). It should be noted that the test could not identify titers smaller than 10^1. For the purpose of calculating the geometric mean, these results were recorded as 10^0.

Incubating HL-60 cells with 10 000 $CCID_{50}$ for 15 min (Fig. 3), titer reduction from 10^4 to 10^2 was recorded in two samples while three samples registered a reduction to 10^1 with the resulting mean titer after deployment of HL-60 cells being $10^{1.4}$ $CCID_{50}$ ($p < 0.01$).

3.2. Test series 2: adsorption of HBV

In the first part of this test series, five different sera were co-incubated with HL-60 cells for 15 min. All measurements were obtained from a single assay according to a single standard curve in order to exclude inter-assay variance. The untreated sera had HBV DNA concentrations ranging from 418 to 3607 pg/ml, registering with 397–2541 pg/ml in their controls (subjected to all test processes except exposure to HL-60 cells). After deployment of HL-60 cells, HBV DNA concentrations ranged from 424 to 2394 pg/ml (Fig. 4).

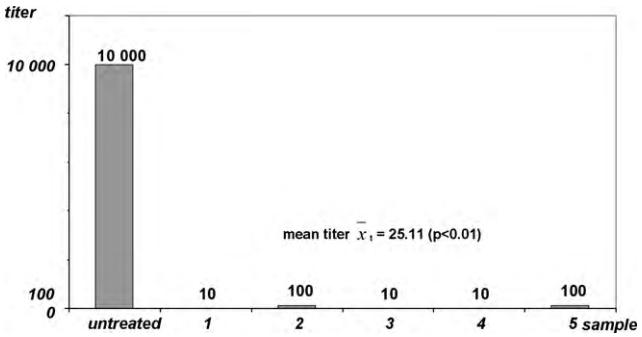

Fig. 3. Titer (reciprocal) before and after deployment of HL-60 cells against 10 000 $CCID_{50}$ enterovirus for 15 min.

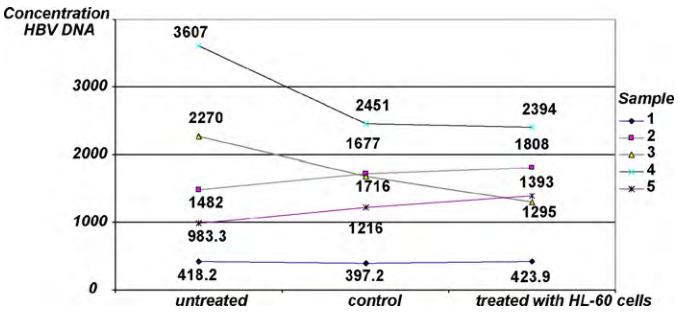

Fig. 4. HBV content in pg/ml before (untreated) and after 15-min exposure to HL-60 cells.

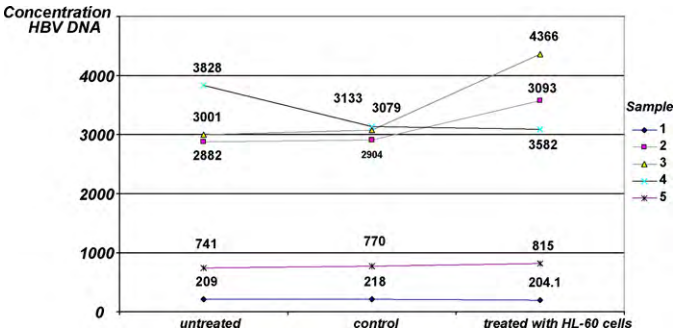

Fig. 5. HBV content in pg/ml before (untreated) and after 15-min exposure to HL-60 cells preceded by opsonization with anti-HBs antibody.

Statistical analysis found no significant difference among untreated, treated and control groups ($p_{kw} = 0.99$). No adsorption of HBV by HL-60 cells could thus be detected.

In the second part of this series, the test was repeated following opsonization of HBV with anti-HBs antibody (Fig. 5). The untreated sera had HBV DNA concentrations ranging from 209 to 3828 pg/ml. The controls had 218–3079 pg/ml while 204–3500 pg/ml were measured after 15 min of exposure to HL-60 cells. Statistical analysis again showed no significant difference between the groups ($p_{kw} = 0.76$). Therefore no adsorption following opsonization of HBV could be confirmed.

3.3. Cell viability and activity

The aim of the XTT-test was to gauge both viability and activity of HL-60 cells as a result of their exposure to the pathogens. As no adsorption of HBV was detected, the XTT was performed only to assess the effect of enterovirus on the cells using 10 000 $CCID_{50}$ (Fig. 6). Unexposed HL-60 cells in the XTT-test had a mean optical density of 0.063 (±0.006). The controls (subjected to all test processes except exposure to enterovirus) registered a mean optical

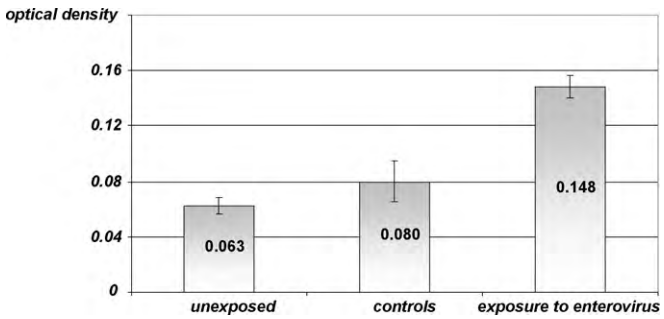

Fig. 6. XTT-test: optical densities as expression of viability and activity of unexposed HL-60 cells, controls and HL-60 cells exposed to 10 000 $CCID_{50}$ of enterovirus.

density of 0.08 (±0.015). Exposure to enterovirus resulted in the cells showing a mean optical density of 0.148 (±0.008). The difference between unexposed cells and the control was not significant ($p = 0.14$), whereas the optical density measured after exposure to enterovirus was significantly higher when compared to both controls and unexposed cells ($p_{kw} < 0.01$).

4. Discussion

Organ support by use of extracorporeal bioreactors represents a relatively young research field. Best evidence derives from clinical investigations of cell-based devices for liver failure (Allen et al., 2001; Demetriou et al., 2004; Strain and Neuberger, 2002) and kidney failure (Saito et al., 2006; Tumlin et al., 2008). The extracorporeal immune support for bacterial sepsis using a granulocyte bioreactor is currently on the verge of clinical studies after animal experiments suggested safe and efficient function (Sauer et al., 2009).

Extracorporeal adsorption of viral antigen by human phagocytes is a novel concept. While lymphocytes and immunoglobulins take the center stage in antiviral immunity, phagocytes interact with viruses at a number of levels. In addition to cytokine production (Yasui et al., 2005) and phagocytosis of infected cells (Fujimoto et al., 2000), phagocytes adsorb or internalize viral antigen either as a function of cellular immunity or by being included in the target spectrum of the virus (Dimitrov, 2004; McCullough et al., 1988). The complexities of viral adsorption were not investigated in this study as it focused first and foremost on feasibility and quantitative potential of the treatment concept.

With regard to the potential capacity of the system, the results are very encouraging. In the test series with enterovirus, excellent adsorption rates were obtained with both 1000 as well as with 10 000 $CCID_{50}$. The kinetics of the process is remarkable with near elimination of the pathogen in only 15 min. Speed and efficacy of viral adsorption are known to vary considerably depending on the specific virus and target cells (Dimitrov, 2004). Kinetic data for the ECHO 30 virus used in this test series had not previously been established. Marjomäki et al. (2002) had found the closely related ECHO 1 virus in SAOS cells (human osteosarcoma cells) after 15 min of incubation, however, their study had been of a qualitative, not quantitative, nature. Adsorption kinetics has been described for several other viruses with time frames varying from seconds to several minutes. Both adeno-associated virus (AAV, non-enveloped) and influenza virus (enveloped) attain access to their respective target cells within seconds (Lakadamyali et al., 2004; Seisenberger et al., 2001) while HIV-1, for example, requires several minutes to more than half an hour for this process (Dimitrov et al., 1992; Raviv et al., 2002). Our results clearly establish the adsorption of enterovirus by HL-60 cells as very rapid. Therapeutic exploitation of the mechanism in an extracorporeal bioreactor therefore seems feasible.

In addition to questions regarding the release of cytokines which future research will need to answer, viral replication in HL-60 may be a principal concern. The design of the test series takes this into account by quantifying enterovirus in the supernatant after exposure to the cells and would therefore also detect newly synthesized particles. Even if replication of virus has taken place, however, the remarkable decline in titer proves the system's excellent net viral clearance. Qualitative analyses published in the literature, moreover, make viral replication in the target cells during incubation unlikely. Vuorinen et al. (1996) demonstrated the ECHO-related coxsackie B virus in HL-60 cells and showed that adsorption was not followed by virus replication. Interestingly, other cells of the granulocytic–monocytic line in their study supported enteroviral replication. This process, however, required several hours and thus substantially more time than would be allowed for viral adsorption with our design.

With regard to the XTT-test, its primary purpose was to examine the viability of HL-60 cells following exposure to enterovirus. This was unequivocally confirmed. The signal rise seen as a result of the interaction with the virus is intriguing and implies that viral adsorption leads to a significant increase in mitochondrial metabolic activity. The non-specific nature of the XTT-test, however, does not allow drawing more detailed conclusions regarding particular aspects or areas of cellular activity. Consequently, the results underline the importance of detailed cytokine analysis as part of the future development of this treatment concept.

Overall the system confirmed the expectation that viral clearance would depend on the host spectrum of the pathogen. Both picorna viruses (ECHO virus) and hepadna viruses (HBV) are internalized following binding to surface receptors of their target cells with the spectrum of suitable receptors defining the tropism of the virus (Dimitrov, 2004). Accordingly, HL-60 cells showed excellent uptake of enterovirus, which displays a broad tropism that includes leukocytes (Pallansch and Roos, 2007). In contrast, elimination of HBV with its narrow target cell spectrum was not detected. It is of interest that opsonization of the enveloped virus did also not lead to measurable phagocytosis or adsorption. A limitation to be noted in this regard, however, is the relatively low sensitivity of the hybridization assay used for quantification of HBV DNA. It can therefore not be ruled out that some degree of adsorption of HBV may have been missed. The employment of real-time PCR with its much higher sensitivity (Servoss and Friedman, 2006) would provide more robust data and allow for a more confident assessment of the interaction between HBV and HL-60 cells. At the same time, investigating the quantitative potential of hepatocytes, the prime target cells of HBV, to adsorb the virus will be one of the next logical steps in this area of research.

Generally, viral adsorption by cellular material is a much more specific process compared to the elimination of larger particles such as bacteria or yeast. Capacity and kinetics will therefore depend on the interaction of a specific virus with a specific cell line. Actual clinical benefit derived from reducing the viral load by extracorporeal means will subsequently need to be critically investigated in clinical trials. With important research questions regarding this model thus emerging, pairing target pathogens with suitable cell lines for extracorporeal adsorption holds the promise of developing this treatment concept into an effective and versatile antiviral technology.

5. Conclusion

In conclusion, our in vitro data demonstrate the feasibility and potentially very high capacity of extracorporeal adsorption of virus by preconditioned human phagocytes. Viral clearance is dependent on the specific interaction between pathogen and target cells. It did not occur between HL-60 cells and Hepatitis B virus to a degree being detectable with our assay but proved to be rapid and near complete in the case of HL-60 cells and enterovirus. HL-60 cells as a reproducible cell line proved robust in terms of viability throughout the test series.

Future development of the concept should focus on cytokine studies during viral adsorption as part of the evaluation of safety and efficacy while determining optimal pathogen–cell pairings offers significant possibilities for this concept.

References

Allen, J.W., Hassanein, T., Bhatia, S.N., 2001. Advances in bioartificial liver devices. Hepatology 34, 447–455.

Berger, A., Preiser, W., 2002. Viral genome quantification as a tool for improving patient management: the example of HIV, HBV, HCV and CMV. J. Antimicrob. Chemother. 49, 713–721.
Cruz, D.N., Perazella, M.A., Bellomo, R., de Cal, M., Polanco, N., Corradi, V., Lentini, P., Nalesso, F., Ueno, T., Ranieri, V.M., Ronco, C., 2007. Effectiveness of polymyxin B-immobilized fiber column in sepsis: a systematic review. Crit. Care 11, R47.
de Jong, M.D., Weel, J.F., Schuurman, T., Wertheim-van Dillen, P.M., Boom, R., 2000. Quantitation of varicella-zoster virus DNA in whole blood, plasma, and serum by PCR and electrochemiluminescence. J. Clin. Microbiol. 38, 2568–2573.
Demetriou, A.A., Brown Jr., R.S., Busuttil, R.W., Fair, J., McGuire, B.M., Rosenthal, P., Am Esch, J.S., Lerut, J., Nyberg, S.L., Salizzoni, M., Fagan, E.A., de Hemptinne, B., Broelsch, C.E., Muraca, M., Salmeron, J.M., Rabkin, J.M., Metselaar, H.J., Pratt, D., De La Mata, M., McChesney, L.P., Everson, G.T., Lavin, P.T., Stevens, A.C., Pitkin, Z., Solomon, B.A., 2004. Prospective, randomized, multicenter, controlled trial of a bioartificial liver in treating acute liver failure. Ann. Surg. 239, 660–667.
Dimitrov, D.S., 2004. Virus entry: molecular mechanisms and biomedical applications. Nat. Rev. Microbiol. 2, 109–122.
Dimitrov, D.S., Willey, R.L., Martin, M.A., Blumenthal, R., 1992. Kinetics of HIV-1 interactions with sCD4 and CD4+ cells: implications for inhibition of virus infection and initial steps of virus entry into cells. Virology 187, 398–406.
Emery, V.C., 1999. Viral dynamics during active cytomegalovirus infection and pathology. Intervirology 42, 405–411.
Fujimoto, I., Pan, J., Takizawa, T., Nakanishi, Y., 2000. Virus clearance through apoptosis-dependent phagocytosis of influenza A virus-infected cells by macrophages. J. Virol. 74, 3399–3403.
Haase, M., Bellomo, R., Morgera, S., Baldwin, I., Boyce, N., 2007. High cut-off point membranes in septic acute renal failure: a systematic review. Int. J. Artif. Organs 30, 1031–1041.
Kaerber, G., 1931. Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Arch. Exp. Pathol. Pharmakol. 162, 480–487.
Kimura, H., Ito, Y., Futamura, M., Ando, Y., Yabuta, Y., Hoshino, Y., Nishiyama, Y., Morishima, T., 2002. Quantitation of viral load in neonatal herpes simplex virus infection and comparison between type 1 and type 2. J. Med. Virol. 67, 349–353.
Lakadamyali, M., Rust, M.J., Zhuang, X., 2004. Endocytosis of influenza viruses. Microbes Infect. 6, 929–936.
Marjomäki, V., Pietiäinen, V., Matilainen, H., Upla, P., Ivaska, J., Nissinen, L., Reunanen, H., Huttunen, P., Hyypiä, T., Heino, J., 2002. Internalization of echovirus 1 in caveolae. J. Virol. 76, 1856–1865.
McCullough, K.C., Parkinson, D., Crowther, J.R., 1988. Opsonization-enhanced phagocytosis of foot-and-mouth disease virus. Immunology 65, 187–191.
McIntosh, K., 2007. Adenoviruses. In: Kliegman, R.M. (Ed.), Nelson Textbook of Pediatrics. Saunders Elsevier, Philadelphia, pp. 1393–1394.
Merlino, C., Cavallo, R., Bergallo, M., Giacchino, F., Bollero, C., Negro Panzi, A., Cavallo, G., 2003. Epstein–Barr viral load monitoring by quantitative PCR in renal transplant patients. New Microbiol. 26, 141–149.
Mitzner, S.R., Freytag, J., Sauer, M., Kleinfeldt, T., Altrichter, J., Klöhr, S., Koball, S., Stange, J., Ringel, B., Nebe, B., Schmidt, H., Podbielski, A., Noeldge-Schomburg, G., Schmidt, R., 2001. Use of human preconditioned phagocytes for extracorporeal immune support: introduction of a concept. Ther. Apher. 5, 423–432.
Mocroft, A., Lundgren, J.D., 2004. Starting highly active antiretroviral therapy: why, when and response to HAART. J. Antimicrob. Chemother. 54, 10–13.
Mommeja-Marin, H., Mondou, E., Blum, M.R., Rousseau, F., 2003. Serum HBV DNA as a marker of efficacy during therapy for chronic HBV infection: analysis and review of the literature. Hepatology 37, 1309–1319.
Pallansch, M.A., Roos, R.P., 2007. Enteroviruses: polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses. In: Knipe, D.M., Howley, P.M. (Eds.), Fields Virology. Lippincott Williams & Wilkins, Philadelphia, pp. 849–850.
Raviv, Y., Viard, M., Bess Jr., J., Blumenthal, R., 2002. Quantitative measurement of fusion of HIV-1 and SIV with cultured cells using photosensitized labeling. Virology 293, 243–251.
Saito, A., Aung, T., Sekiguchi, K., Sato, Y., Vu, D.M., Inagaki, M., Kanai, G., Tanaka, R., Suzuki, H., Kakuta, T., 2006. Present status and perspectives of bioartificial kidneys. J. Artif. Organs 9, 130–135.
Sauer, M., Altrichter, J., Kreutzer, H.J., Lögters, T., Scholz, M., Nöldge-Schomburg, G., Schmidt, R., Mitzner, S.R., 2009. Extracorporeal cell therapy with granulocytes in a pig-model of Gram-positive sepsis. Crit. Care Med. 37, 606–613.
Schefold, J.C., von Haehling, S., Corsepius, M., Pohle, C., Kruschke, P., Zuckermann, H., Volk, H.D., Reinke, P., 2007. A novel selective extracorporeal intervention in sepsis: immunoadsorption of endotoxin, interleukin 6, and complement-activating product 5a. Shock 28, 418–425.
Schilham, M.W., Claas, E.C., van Zaane, W., Heemskerk, B., Vossen, J.M., Lankester, A.C., Toes, R.E., Echavarria, M., Kroes, A.C., van Tol, M.J., 2002. High levels of adenovirus DNA in serum correlate with fatal outcome of adenovirus infection in children after allogeneic stem-cell transplantation. Clin. Infect. Dis. 35, 526–532.
Seisenberger, G., Ried, M.U., Endress, T., Büning, H., Hallek, M., Bräuchle, C., 2001. Real-time single-molecule imaging of the infection pathway of an adeno-associated virus. Science 294, 1929–1932.
Servoss, J.C., Friedman, L.S., 2006. Serologic and molecular diagnosis of hepatitis B virus. Infect. Dis. Clin. North Am. 20, 47–61.
Strain, A.J., Neuberger, J.M., 2002. A bioartificial liver – state of the art. Science 295, 1005–1009.
Tumlin, J., Wali, R., Williams, W., Murray, P., Tolwani, A.J., Vinnikova, A.K., Szerlip, H.M., Ye, J., Paganini, E.P., Dworkin, L., Finkel, K.W., Kraus, M.A., Hume's, H.D., 2008. Efficacy and safety of renal tubule cell therapy for acute renal failure. J. Am. Soc. Nephrol. 19, 1034–1040.
Vuorinen, T., Vainionpää, R., Vanharanta, R., Hyypiä, T., 1996. Susceptibility of human bone marrow cells and hematopoietic cell lines to coxsackievirus B3 infection. J. Virol. 70, 9018–9023.
Walls, T., Shankar, A.G., Shingadia, D., 2003. Adenovirus: an increasingly important pathogen in paediatric bone marrow transplant patients. Lancet Infect. Dis. 3, 79–86.
Yasui, K., Baba, A., Iwasaki, Y., Kubo, T., Aoyama, K., Mori, T., Yamazaki, T., Kobayashi, N., Ishiguro, A., 2005. Neutrophil-mediated inflammation in respiratory syncytial viral bronchiolitis. Pediatr. Int. 47, 190–195.
Zellos, A., Thomas, D.L., Mocilnikar, C., Perlman, E.J., Boitnott, J.K., Casella, J.F., Schwarz, K.B., 1999. High viral load and mild liver injury in children with hemophilia compared with other children with chronic hepatitis C virus infection. J. Pediatr. Gastroenterol. Nutr. 29, 418–423.