Antiviral Research 81 (2009) 93–102

Contents lists available at ScienceDirect

Antiviral Research

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

Effect of macrophage depletion on viral DNA rebound following antiretroviral therapy in a murine model of AIDS (MAIDS)

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ARTICLE INFO

Article history:
Received 9 May 2008
Received in revised form 25 July 2008
Accepted 29 September 2008

Keywords:
Drug delivery
MAIDS
HIV reservoirs
Loaded erythrocytes
Macrophages

ABSTRACT

In the attempt to eradicate HIV-1 infection, a strategy to eliminate macrophages, one of the most important cellular reservoirs in sustaining virus replication during HAART, could be of great benefit in the suppression of viral rebound. Aware of the ability of clodronate to cause macrophage depletion, the effect of the administration of clodronate encapsulated in erythrocytes on disease progression and on viral rebound was evaluated in a murine model of AIDS (MAIDS). One group of LP-BM5 retroviral complex-infected C57BL/6 mice received oral administrations of azidothymidine and dideoxyinosine daily for 12 weeks; two other groups received in addition, either clodronate-loaded erythrocytes or free clodronate at 7–10 day intervals. At the end of the treatment, the three groups maintained parameters characterizing disease progression similar to those of uninfected mice and showed a significantly lower level of BM5d DNA than infected mice in all organs and cells tested. To assess the viral rebound, some animals were left for an additional 4 month period without any treatment. After this time, the BM5d DNA content in blood leukocytes increased in all groups, but the group having received clodronate-loaded erythrocytes, in addition to transcriptase inhibitors, showed a significant delay in viral rebound.

1. Introduction

Human immunodeficiency virus type 1 (HIV-1) eradication is one of the most important aims of novel antiretroviral therapies. In fact, highly active antiretroviral therapy (HAART) is able to suppress plasma viremia to undetectable levels, but it cannot eradicate HIV-1. This is mainly due to the existence of cellular reservoirs. The best characterized cellular reservoirs of HIV-1 are the latently infected resting memory CD4+ T cells, which, containing an integrated copy of HIV-1 genome, become an extremely stable HIV-1 reservoir that can survive in the patient for many years (Chun et al., 1997).

Resting memory CD4+ T cells may archive the wild type and drug-resistant HIV-1 strains, which appear throughout the years of HAART and can produce HIV-1 upon the subsequent activation by the antigen. Apart from latent HIV-1 infection of the resting memory CD4+ T cells providing long-term viral persistence, residual viral replication can also occur in these cells. Such residual replication contributes to viral rebound following cessation of HAART. Unfortunately, the latent reservoir decays slowly, with a half-life of up to 44 months, making it the principal, known obstacle in the eradication of HIV-1 infection (Sedaghat et al., 2008).

Another important cellular reservoir of HIV-1 is represented by the cells of macrophage lineage which were shown to be the first to be infected. Both blood monocytes and tissue macrophages can harbour HIV-1, although monocytes are significantly less susceptible than macrophages. Following infection, these cells are resistant to the cytopathic effects of HIV-1 and hence can persist in the tissues for a long period of time (even in the presence of HAART), support viral replication and contribute to the pathogenesis of disease. Whilst the kinetics of virus release from cells of macrophage lineage are slower in comparison to CD4+ T cells, the lack of HIV-induced cytopathicity enables macrophages to continue to secrete HIV-1 for a longer period of time (Chun and Fauci, 1999; Crowe and Sonza, 2000). Hence, tissue macrophages can act as long-term stable reservoirs for HIV-1 capable of disseminating the virus in other tissues, thus contributing to viral reservoir pools that ultimately lead to disease progression (Shehu-Xhilaga et al., 2005).

Viral compartmentalization has been reported not only within circulating immune cells, but also in various biological fluids and organs such as the brain, the cerebrospinal fluid (CSF), seminal plasma, and lymph nodes, where differences in the concentration of

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doi:10.1016/j.antiviral.2008.09.006

HIV inhibitors have been demonstrated (Pomerantz, 2003; Shehu-Xhilaga et al., 2005).

Recent findings have proposed the regulatory Nef protein, expressed by HIV-1 in the early stages of the infection cycle, to counteract the HIV-induced apoptosis in macrophages, thus having a role in the in vivo establishment of the HIV macrophage sanctuary (Olivetta and Federico, 2006). Some researchers have proposed STAT-1 and its activation upon HIV infection in macrophages as a possible survival factor contributing to the role of macrophages as persistent viral reservoirs (Magnani et al., 2003). Other authors have shown that the nerve growth factor (NGF), a neurokine involved in the survival, development and functions of peripheral and central neurons (Levi-Montalcini, 1987), is produced and released by HIV-1-infected macrophages during the first hours/days of infection (Garaci et al., 1999) and is implicated in inhibiting the cytopathic effects normally induced by this virus in other cells.

Besides this well established role in harboring the virus, acting as reservoirs of virions (Gartner et al., 1986), macrophages play an important role in the regulation of T cell apoptosis following HIV-1-infection. In fact, infected activated macrophages are able to trigger the apoptosis of uninfected T cells (Badley et al., 1997; Herbein et al., 1998), and to protect HIV-infected T cells from apoptosis favoring their recruitment and productive infection (Mahlknecht et al., 2000). All these findings clearly demonstrate that new therapeutic approaches targeting macrophages may be relevant in the therapy of HIV infection and, in particular, the transient and organ-specific suppression of their functions may be beneficial, eliminating the so-called cellular sanctuary that plays a role in HIV-1 persistence.

Macrophage depletion by means of liposome encapsulation of the bisphosphonate clodronate is a well documented technique to obtain the transient suppression of macrophage functions (van Rooijen and van Kesteren-Hendrikx, 2002). Clodronate (dichloromethylenebisphosphonate, Clod) is a non-nitrogen containing bisphosphonate widely used in the treatment of metabolic bone diseases such as hypercalcemia in malignant forms, and osteolytic diseases resulting from bone metastases (Fleisch, 1991), Paget's disease (Khan et al., 1996) and osteoporosis (Meunier et al., 1999). Depletion of macrophages through liposome-encapsulated clodronate is shown to prevent corneal graft rejection (Slegers et al., 2003), to increase graft survival after cardiac xenotransplantation in rats, preventing anti-graft antibody production (Koyamada et al., 2005), and to reduce neointimal hyperplasia and restenosis after mechanical arterial injury (Danenberg et al., 2002). Furthermore, it has been studied in several preclinical models of rheumatoid arthritis (van Lent et al., 1996) and neurological disorders (Tran et al., 1998). Moreover, applications of liposomes as drug delivery systems for the treatment of AIDS have already been reported (Lanao et al., 2007).

Despite the wide spectrum of applications and the encouraging evidence, the only application of liposome-encapsulated clodronate that has reached clinical use is in the depletion of synovial macrophages (Barrera et al., 2000).

As an alternative to liposomes, we propose erythrocyte-mediated drug delivery to selectively target clodronate to the macrophage compartment, exploiting the phagocytic capacity of macrophages.

As to erythrocytes (RBC) as drug carriers, these are readily available in large quantities, biocompatible (when autologous RBC are used), and completely biodegradable. They have a large capacity, so that a significant amount of drug can be encapsulated. Furthermore, it is possible to achieve a selective targeting of drugs to macrophages without affecting the non-phagocytic cells. To specifically target the drug-containing RBC to the phagocytic cells, in particular to the monocyte-derived macrophages, it is possible to artificially induce senescent signals on the RBC membrane, thus promoting the macrophages to exploit their physiologic role (Magnani et al., 1992).

In our previous experiences, we demonstrated that the administration of Clod-loaded RBC both in vitro and in vivo was able to eliminate macrophage cells (Rossi et al., 2005). Prompted by these considerations, we propose to take advantage of this strategy to obtain the elimination of viral macrophage reservoirs in a murine model of immunodeficiency. Murine AIDS (MAIDS) is a severe immunodeficiency syndrome induced by a complex of retroviruses, called LP-BM5 murine leukemia virus (MuLV), in susceptible strains of mice (Morse et al., 1992). LP-BM5 retroviral isolates consist of pathogenic defective murine retrovirus (BM5def) that requires replication-competent ecotropic helper viruses (e.g. BM5eco) for its entry into cells and proliferation in vivo. The LP-MB5 infection of C57BL/6 mice is considered an efficient mouse model for human AIDS due to the similarity of symptoms (Morse et al., 1992). Some of these similarities include development of profound immunodeficiency characterized by deficits in B- and T-lymphocyte function as well as deficiencies in macrophage functions. The early stage of the disease is characterized by early-onset hypergammaglobulinemia and polyclonal activation of lymphocytes and proliferation associated with progressive lymphoadenopathy and splenomegaly (Chattopadhyay et al., 1991). In advanced stages of the disease, infected mice become increasingly immunodeficient resulting in increased susceptibility to opportunistic infections (Doherty et al., 1995) and development of secondary neoplasm, especially B-cell lymphomas (Buller et al., 1987); in fact, both CD4 T cells and B cells are required for disease induction and progression. It has been reported that the main targets of initial LP-BM5 retrovirus infection are B cells, and to some extent, macrophages and T cells.

In this study, we have evaluated the effects of macrophage depletion induced by the periodical administration of Clod-loaded RBC in immunodeficient LP-BM5-infected C57BL/6 mice treated with two antiretroviral drugs (azidothymidine, AZT and dideoxyinosine, DDI) to suppress viral replication taking place in activating/proliferating CD4+ T cells. To this end, infected mice were treated with AZT plus DDI in drinking water and received weekly administrations of Clod by means of RBC or given free. Comparative studies were performed with mice treated with the two antiretrovirals alone or with only Clod. After 12 weeks of treatment, drug administrations were interrupted and the mice, left without drugs for a further four months, were used to assess some signs of the disease and the viral load. In fact, the primary aim of this study was to evaluate if the depletion of the macrophage population could have some effect on disease progression and, in particular, on viral rebound in mice treated with the combination of AZT + DDI.

2. Materials and methods

2.1. Virus and animals

The LP-BM5 viral mixture was kindly provided by Robert Yetter (Veterans Administration Hospital, Baltimore, MD, USA) and was maintained in a persistently infected SC-1 cell line as previously described (Mosier et al., 1987). Five-week-old female C57BL/6 mice (Harlan, Italy) were inoculated with 0.250 ml of the virus stock containing 0.33 Units (IU) of reverse transcriptase by means of two consecutive intraperitoneal injections at 24-h interval. Mice were housed in specified conditions at $22 \pm 1\,^{\circ}C$ with a 12-h light/dark cycle, $60 \pm 5\%$ humidity, and 12-h air changes/hour. The use and care of the animals used in this study were approved by the Ethical Committee of the University of Urbino "Carlo Bo", Italy.