Clinical Immunology (2012) 144, 159–171

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

T-lymphocyte perturbation following large-scale apheresis and hematopoietic stem cell transplantation in HIV-infected individuals

Borislav Savkovic [a], Janet L. Macpherson [b], John Zaunders [c], Anthony D. Kelleher [c,d], Alison E. Knop [e,f], Susan Pond [g], Louise Evans [h], Geoff Symonds [c], John M. Murray [a,d,*]

[a] School of Mathematics and Statistics, University of New South Wales, Sydney 2052, Australia
[b] Department of Cell & Molecular Therapies, Royal Prince Alfred Hospital, Missenden Rd., Camperdown, New South Wales, Sydney 2050, Australia
[c] St. Vincent's Centre for Applied Medical Research, Darlinghurst, New South Wales, Sydney 2010, Australia
[d] Kirby Institute, University of New South Wales, Sydney, Australia
[e] Calimmune Australia, 405 Liverpool St., Darlinghurst, NSW, 2010, Australia
[f] George Institute for Global Health, Australia
[g] University of Sydney, New South Wales 2006, Sydney, Australia
[h] Department of Immunology, Liverpool Hospital, Elizabeth Street, Liverpool, New South Wales 2170, Australia

Received 30 January 2012; accepted with revision 7 June 2012
Available online 21 June 2012

KEYWORDS
Lymphocyte distribution and trafficking;
HIV;
Compartment model;
Apheresis;
Granulocyte-colony stimulating factor;
Stem cell transplantation

Abstract Analysis and mathematical modeling of T-lymphocyte perturbation following administration of granulocyte colony stimulating factor (G-CSF) and two large-scale aphereses are reported. 74 HIV-1 positive antiretroviral-treated individuals were infused with gene- or sham-transduced CD34+ hematopoietic stem cells (HSC) in a Phase II clinical trial. T cell numbers were examined in four phases: 1) during steady state; 2) increases in peripheral blood (PB) following G-CSF administration; 3) depletion post-aphereses and 4) reconstitution post HSC infusion. The present analysis provides the first direct estimate of CD4+ T cell distribution and trafficking in HIV-infected individuals on stable HAART, indicating that CD4+ T lymphocytes in PB represent 5.5% of the pool of CD4+ T lymphocytes that traffic to PB.

Abbreviations: PB, Peripheral blood; LT, Lymph tissue; G-CSF, Granulocyte colony stimulating factor; HSC, Hematopoietic stem cell; SSE, Sum-of-squares error; OZ1, tat–vpr specific anti-HIV ribozyme.

* Corresponding author at: School of Mathematics and Statistics, University of New South Wales, Sydney 2052, NSW, Australia. Fax: +61 2 9385 7123.
E-mail address: J.Murray@unsw.edu.au (J.M. Murray).

1521-6616/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.clim.2012.06.004

1. Introduction

Distribution and trafficking patterns of T lymphocytes between blood and tissue in humans remain poorly understood, with current estimates of T lymphocyte distribution in healthy individuals based on extrapolations from animal models [1,2]. Estimates from rats report that 2% of the total T lymphocytes in the body reside in peripheral blood (PB) [1,2], whereas estimates employing noninvasive in-vivo imaging in nonhuman primates with SIV have recently reported a figure of 0.4% [3]. The primary limitation of these studies relates to the employment of animal models, and as such may have limited relevance to T lymphocyte distribution in humans.

In the present study we are concerned with T lymphocyte distribution and trafficking in HIV-infected individuals. Knowledge of T lymphocyte distribution in HIV-infected individuals is of considerable interest, since PB CD4+ T lymphocyte counts have limited prognostic value with regard to the clinical outcome of HIV infection [4]. More precise knowledge of the total CD4+ T lymphocyte distribution during HIV infection may lead to an enhanced understanding of HIV pathology, as well as improved therapies against HIV.

Here, employing mathematical modeling, we provide the first direct estimate of T lymphocyte distribution and trafficking in HIV-infected individuals based on human in-vivo data from a recent Phase II clinical trial [5] undertaken by members of our group. In the trial an anti-HIV ribozyme was delivered to CD34+ hematopoietic stem cells (HSC) in HIV infected individuals in whom viral load was well controlled by HAART [5]. Previous studies have used perturbation of HIV viral load in response to antiretroviral therapy or apheresis to determine the dynamical aspects of the disease, such as the half-life of a HIV infected cell [6–8]. Here we use the perturbations in this study (granulocyte colony stimulating factor (G-CSF) mobilization, high volume apheresis and HSC infusion) to better understand the dynamics of PB T cell numbers, particularly their trafficking and homeostatic mechanisms in HIV-infected individuals. We analyze the dynamic changes in CD4+ and CD8+ T cell numbers in PB in response to G-CSF administration and aphereses for 74 HIV-infected individuals on stable HAART with undetectable viral load [5]. Four aspects of T cell modulation were investigated:

1. The extent of mobilization of T cells from lymph tissue (LT) to PB due to G-CSF,
2. Trafficking of T cells between PB and LT at steady-state and as a consequence of G-CSF mobilization, aphereses and infusion,
3. Relative proportions of T cells in PB, as a percentage of T cells in the body capable of trafficking to PB either normally or under the influence of G-CSF,
4. Causes of T cell depletion in PB following apheresis and the dynamics of the subsequent relatively protracted recovery in T cell numbers.

2. Materials and methods

2.1. Subjects and clinical trial

As previously reported [5], a total of 74 HIV-1 infected individuals receiving HAART (mean duration of 4.3 years), were enrolled in a randomized, placebo-controlled double-blind study of a retroviral *tat–vpr* specific anti-HIV ribozyme (OZ1) delivered using autologous CD34+ HSC. Of the total, 38 individuals received OZ1, the remaining 36 received sham transduced CD34+ HSC. A substudy examining T cell subsets was conducted at one clinic in 9 sequentially recruited and consented study participants (2 patients receiving OZ1 and 7 patients receiving placebo), and were compared to 5 healthy HIV negative individuals.

The timeline of events and data collection points relevant to this analysis are shown in Fig. 1A, with time denoted relative to infusion of CD34+ HSC on day 0. From day −7 to day −3, G-CSF (Filgrastim, subcutaneous injection) was administered at a daily dosage of 30 μg/kg/day (Fig. 1B). Two aphereses were performed on days −4 and −3 respectively for the purpose of harvesting autologous HSC. The duration of each apheresis was approximately 5 h (i.e. approximately 0.2 days) per patient. The first apheresis was performed from day −4 to day −3.8 and the second apheresis from day −3 to day −2.8. Subsequent to the second apheresis, harvested CD34+ cells were transduced with OZ1 or sham-transduced, and reinfused on day 0. Baseline (BL) is taken as day −7, which is the first day of G-CSF administration. While the study continued until week 100 post-infusion, the present analysis covers the period from day −37 until day 141 post-infusion (i.e. up to week 20), the period during which all subjects remained on HAART. Substudy data were collected on days −7, 0, 8, 28 and 84.

While the total number of lymphocytes in the apheresis product was evaluated, CD4+ and CD8+ T cell percentages (of total lymphocytes in the apheresis product) were not determined. Previous studies (in healthy individuals undergoing mobilization with G-CSF, 10 μg/kg/day dosage) reported no significant difference in CD4+ percentages between PB at steady-state and the apheresis product [9], while a significant (albeit small) difference in the CD8+ percentage was reported (30% of lymphocytes in PB were CD8+ T cells and 35% of lymphocytes in apheresis product were CD8+ T cells). Accordingly we assumed that CD4+ and CD8+ T cell percentages (of total lymphocytes in the apheresis product) were equal to the baseline CD4+ and CD8+ T cell percentages of the total lymphocytes in PB. However in order to explicitly account for uncertainty in the number of CD4+ and CD8+ T cells in the apheresis product, a sensitivity analysis of our results was performed under the assumption of ±30% uncertainty in the numbers of CD4+ and CD8+ T cells in the apheresis product of each patient (see Results and Discussion).

2.2. Substudy T cell immunophenotyping

T lymphocytes in samples of anti-coagulated fresh peripheral blood were stained for cell surface and intracellular markers and subsets were analyzed by 4-color flow cytometry on an EPICS XL flow cytometer (Beckman-Coulter, Hialeah, FL) as previously described [10]. The monoclonal antibodies used were CD3-PerCP, CD8-PE, CD38-PE, HLA-DR-FITC, Ki-67-FITC, Bcl-2-PE, CD62L-FITC and CD45RA-PE (BD Biosciences, San Jose, CA); and CD4-ECD (Beckman Coulter, Hialeah, FL, USA). Intracellular staining was performed using FACSlyse and FACSPermeabilizing Reagents (BD Biosciences) according to the manufacturer's directions, and analyzed as previously described [11].