Virology 377 (2008) 239–247

Contents lists available at ScienceDirect

Virology

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

Persistence of restricted CD4 T cell expansions in SIV-infected macaques resistant to SHIV89.6P superinfection

M.-D. Salha [a,b], R. Cheynier [b,c], R. Halwani [b,c], H. McGrath [b,c], T.Y. Langaee [b,c], B. Yassine Diab [b,c], J. Fournier [d], M. Parenteau [d], J. Edgar [d], D. Ko [e], A. Sherring [e], D. Bogdanovic [e], R.-P. Sekaly [a,b,c,*,1], E.W. Rud [d,e,f,g,1]

[a] Department of Microbiology and Immunology, McGill University, Montreal, Canada PQ H3A 2B4
[b] Centre de Recherche du Centre Hospitalier de l'Université de Montreal (CHUM), Montreal, Canada PQ H2W 1T8
[c] Département de Microbiologie et d'Immunologie, Université de Montreal, Montreal, Canada PQ H3C 3J7
[d] Animal Resource Division, Health Canada, Ottawa, Ontario Canada K1A 0L2
[e] National Laboratory for HIV Pathogenesis, Health Canada, Ottawa, Ontario Canada K1A 0L2
[f] Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ontario Canada K1H 8M5
[g] McGill AIDS Center, Lady Davis Institute, Jewish General Hospital, Montreal, Canada PQ H3T 1E2

ARTICLE INFO

Article history:
Received 4 December 2007
Returned to author for revision
29 February 2008
Accepted 24 April 2008

Keywords:
TRBV repertoire
CD4
Primary infection
SHIV89.6P
Viral challenge
Protection

ABSTRACT

Attempts to evaluate the protective effect of live attenuated SIV vaccine strains have yielded variable results depending on the route of immunization, the level of attenuation, the level of divergence between the vaccine candidate and the challenge. The protective mechanisms induced by these vaccines are still not well understood. In an effort to address whether the diversity of the CD4+ T cell repertoire in cynomolgus macaques plays a role in the immunological protection following SIVmacC8 infection, we have performed a longitudinal follow-up of the CD4 repertoire by heteroduplex tracking assay in macaques mock-infected or infected with either the attenuated SIVmacC8 or its homologous SIVmacJ5 and challenged with simian-human immunodeficiency virus (SHIV89.6P). Viral load and CD4 absolute counts were determined in these animals and the presence of SHIV89.6P virus in challenged animals was evaluated by PCR and serology. In all macaques that were protected against the challenging virus, we demonstrated a reduced diversity in the CD4+ TRBV repertoire and a few dominant CD4+ T cell clones during early primary infection. In contrast, CD4 TRBV repertoire in unprotected macaques remained highly diverse. Moreover, some of the CD4 T cell clones that were expanded during primary SIV infection re-emerged after challenge suggesting their role in protection against the challenging virus. These results underline the importance of maintaining the CD4 T cell repertoire developed during acute infection and point to the restriction of the CD4 response to the vaccine as a correlate of protection.

Introduction

Immunological responses generated against persistent viral infections are characterized by the activation and expansion of CD4 and CD8 antigen-specific T lymphocytes. Although cytotoxic T cell responses are responsible for clearance of most viral infections (Shin and Wherry, 2007; Purtha et al., 2007; Kuroda et al., 1999), CD4 T cells play a major role in supporting antibody production, initiating and maintaining CTL activity as well as performing direct effector activity through the production of specific cytokines (Ribeiro, 2007; Davenport et al., 2007). Following viral suppression through immunological control, these T cells decline in activity and number mostly through apoptosis (McHeyzer-Williams and Davis, 1995; Gupta and Gollapudi, 2007; Badovinac et al., 2002), leaving the host with a sufficient number of pathogen-specific memory T cells. The major role of memory T cells is to ensure protection upon re-exposure to pathogens through rapid clonal proliferation and functional activation. This has been demonstrated in both CD8 (Walker et al., 1996; Levitsky et al., 1998; Maryanski et al., 1996; Sourdive et al., 1998; Blattman et al., 2000; Roberts and Woodland, 2004; Geginat et al., 2003) and CD4 positive T cells (Bitmansour et al., 2001; seder and Ahmed, 2003; Sallusto et al., 2004; Zaph et al., 2004). Several factors including the stimuli, the host or the environment can all contribute to create variations between antigen-selected repertoires. These factors can also influence whether a potent antigen-specific repertoire is selected, whether it is persistent and protective.

Attempts to generate a vaccine against human immunodeficiency virus (HIV) have led to the evaluation of attenuated strains as candidates. Macaques challenged with pathogenic simian immunodeficiency virus (SIV) several months after being infected with live attenuated SIV show better protection than monkeys immunized by

* Corresponding author. Centre de Recherche du CHUM, Hôpital Saint-Luc, 264, René-Lévesque est, Bureau 1317, Montréal (Québec), Canada H2X 1P1. Fax: +1 514 412 7415.
E-mail address: rafick-pierre.sekaly@umontreal.ca (R.-P. Sekaly).
[1] The two authors have equally contributed to the manuscript.

0042-6822/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2008.04.031

any other vaccine strategy (Tenner-Racz et al., 2004; Johnson et al., 1999; Mori et al., 2001; Koff et al., 2006). The use of attenuated SIV viruses as vaccines has provided complete or near complete protection from challenge with wild type SIV (Koff et al., 2006). Better protection was obtained with homologous challenge than with heterologous challenge (Johnson and Desrosiers, 1998). The most common strategy of attenuating SIV was achieved by the complete or partial deletion of nef (SIVΔnef or SIVmacC8, respectively) or the deletion of both nef and vpr (SIVΔ3). The use of these viruses has provided strong protection against pathogenic SIV challenge (Johnson and Desrosiers, 1998). Another way of attenuating SIV was achieved through the deletion of V1–V2 region of the envelope protein (Env; SIVΔV1–V2). This virus has also conferred potent protection from intravenous challenge by wild type SIVmac239 (Cole et al., 2004).

The correlates and mechanisms of protection induced by attenuated viruses, however, are poorly understood. Stebbings et al. demonstrated using CD8 T cells depletion techniques, that CD8 T cell responses alone are not central to the protection against acute superinfection conferred 20 days after vaccination with attenuated SIVmacC8 (Stebbings et al., 2005). This conclusion was consistent with the findings of other investigators who were unable to identify a correlation between SIV-specific CD8 CTL responses elicited by inoculation with live attenuated SIV and protection against superinfection (Sharpe et al., 2004; Dittmer et al., 1999).

The persistence of the CD4 helper function was shown in several model systems to be essential for the maintenance of memory CD8 responses during chronic infection as well as the generation of neutralizing antibodies to viral escape mutants and for the control of viremia (Suvas et al., 2003; Sun et al., 2004; Sun and Bevan, 2003; Matloubian et al., 1994; von Herrath et al., 1996; Zajac et al., 1998; Wodarz and Jansen, 2001; Ahmed et al., 1988; Kalams et al., 1999). While the rapid turnover of CD4 T cells characterize HIV/SIV infection, it is not clear to what extent HIV/SIV infections can drive an expansion rather than a depletion of antigen-specific CD4 T cells. Assessing T cell receptor (TCR) repertoire during HIV/SIV infection will add to our understanding of the way T cells respond to this infection and contribute in mediating protection. Chen et al. have shown using CDR3 profile and sequence analysis of CD4 T cell receptor repertoires that infection of macaques with SIV can result in prolonged periods of clonal dominance of CDR3-restricted CD4 T cell clones despite the decline of CD4 T cell count (Chen et al., 2000).

In this study we evaluated the impact of the breadth and persistence of CD4 T cell repertoire stimulated during primary infection with attenuated SIVmacC8 and pathogenic SIVmacJ5 strains on the resistance to subsequent challenge with the highly pathogenic simian-human immunodeficiency virus (SHIV89.6P) strain.

Results

Virological and immunological evolution following SIVmacJ5 or SIVmacC8 infection

In order to analyze the relationship between the breadth of the CD4 repertoire induced during primary infection (PI) by attenuated SIV strains and the susceptibility to subsequent superinfection, cynomolgus macaques were infected with either the pathogenic SIVmac strain SIVmacJ5 or the attenuated strain SIVmacC8 (Cranage et al., 1992). Twenty weeks post-primary infection, animals were challenged with the pathogenic SHIV89.6P strain.

In all SIVmacJ5-infected macaques the CD4 count transiently dropped 2 weeks post-infection and returned to normal values at following time points (Fig. 1A). In these animals viral load peaked simultaneously to the CD4 decline (~10^8 copies per ml) and settled at week 6 below the limit of detection, with some occasional viral blips.

In the SIVmacC8-infected group, macaques C93054F and C94042F had both lower CD4 counts at study entry compared to other macaques in that group and did not show any significant perturbations in their CD4 counts during acute infection (Fig. 1B). Except for a

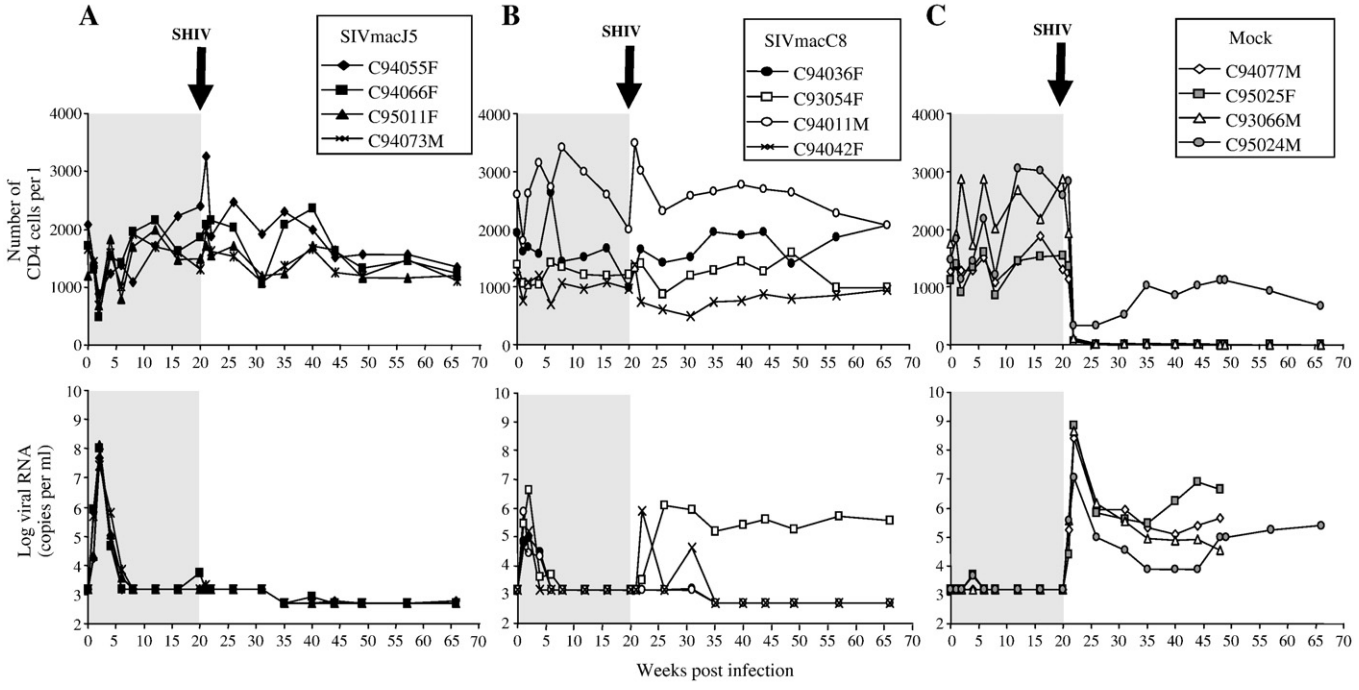

Fig. 1. Disease status in SIV-infected animals. CD4 absolute count (right panels) and plasma SIV RNA detected by branched DNA (left panels) from groups A—SIVmacJ5, B—SIVmacC8, and C—mock-infected macaques. Animals infected with SIVmacJ5 or SIVmacC8 show slow progressor patterns. Following challenge at week 20 with SHIV89.6P, two SIVmacC8-infected macaques show a rebound in viral load and a progressive drop in CD4 counts. SIV viral load target probes, designed to hybridize with the *pol* region of the SIVmac groups of strains were used to quantify SIV viral load. Results were quantified by comparison with purified and quantified *in vitro*-transcribed SIV *pol* RNA and were plotted on a log(10) scale. The detection limit of this assay was 1500 copies of SIVmac RNA per ml until week 35 and 500 thereafter. White blood cell counts were obtained from a hematology workstation and were used to calculate the absolute CD4 counts.