Different expression of Blimp-1 during HIV infection may be used to monitor disease progression and provide a clue to reduce immune activation and purge viral reservoirs

Francesco NICOLI, Fabio SFORZA and Riccardo GAVIOLI

Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy

Correspondence to Prof. Riccardo Gavioli, PhD, r.gavioli@unife.it

Blimp-1 is an important factor involved in the generation of terminally differentiated B and T lymphocytes [1]. Recent studies have demonstrated that this molecule is differently expressed during the course of viral infections, including HIV, and proper levels of Blimp-1 are necessary for viral control and to avoid T cell exhaustion [2-4]. This suggests that Blimp-1 expression may be modulated and/or used to monitor disease progression as well as response to therapy.

In particular, in this issue of AIDS, Beisel at al. report an inverse correlation between Blimp-1 expression in activated memory B cells and viral load in HIV-infected individuals suggesting that low levels of Blimp-1 in this particular B cell subset are associated with disease progression. Interestingly, in a previous issue, de Masson et al. showed lower Blimp-1 expression in central memory $CD4^+$ T cells of subjects with high cellular HIV DNA levels suggesting that induction of Blimp-1 may reduce the size of HIV reservoirs [5]. Noteworthy, other studies have shown that Blimp-1 contrasts the development of central memory and follicular memory $CD4^+$ T cells [1], two sanctuary of HIV latency. Thus, high levels of Blimp-1 seem to be associated, in both $CD4^+$ and B lymphocytes, with slower or no progression to AIDS. In contrast to these findings, a previous study performed by Seddiki et al. on the whole $CD4^+$ T cell subset reported higher

Blimp-1 levels in progressors compared to long-term non-progressors or healthy controls [6]. These conflicting results may be due both to the cellular subset analyzed and cohort of analysis. Indeed, de Masson et al. compared two groups of long-term non-progressors while Seddiki et al. compared viremic progressors with long-term non-progressors. Moreover, Seddiki et al. analyzed Blimp-1 expression in total $CD4^+$ T cells, which include, especially during progression to AIDS, a large proportion of activated $CD4^+$ T cells. This suggests that Blimp-1 is differently expressed along the course of HIV infection according to the activation status of the cells and the progression stage of HIV-infected individuals. At present, it is not possible to exclude that this different expression may result in a dual role of Blimp-1 in HIV disease progression. A dual role of Blimp-1 has also been observed in $CD8^+$ T cells: in chronic infections high Blimp-1 expression promotes the up-regulation of PD-1 resulting in exhausted $CD8^+$ T cells [2], in contrast to acute infections where Blimp-1 represses the expression of PD-1 [4].

The molecular mechanisms involved in the modulation of Blimp-1 during HIV infection remain to be elucidated and may depend on the cell type as well as the activation status of cells. HIV infection may affect Blimp-1 levels by modulating those factors involved in the physiological control of Blimp-1 expression such as miR-9, IL2 or IL21 [1, 6, 7], which are dysregulated in HIV-infected individuals. Alternatively, HIV-derived products may directly act on the signaling pathways involved in Blimp-1 expression. To this regard, it has been demonstrated that Blimp-1 is induced in $CD4^+$ T cells activated by HIV-exposed dendritic cells [2]. Furthermore, we have recently demonstrated that the HIV-1 Tat protein induces an over-expression of Blimp-1 in activated, but not in resting, $CD8^+$ T cells [8]. Moreover, Tat also affects Blimp-1 expression in $CD4^+$ T cells (Gavioli et al., unpublished results). Of note, we and others have demonstrated that Tat up-regulates, in both activated $CD4^+$ and $CD8^+$ T cells, the release of IL2 [8], which is an

inducer of Blimp-1 [1]. In addition, Tat is known to up-regulate proteasomal activity [9] as well as the expression of CD80 [10], a ligand of CTLA-4 which, through the Hippo pathway, favors the proteasomal degradation of Yap, a suppressor of Blimp-1 expression [3]. This latter mechanism requires $CD8^+$ T cells in an activation status. Of note, Tat favors, *in vitro* and *in vivo*, the activation of $CD8^+$ T cells and the development of effector memory $CD8^+$ T lymphocytes [8, 11-13], which are two hallmarks of immune activation and both require Blimp-1 expression [1]. Consistently, induction of anti-Tat immune responses by a Tat-based vaccine reduces immune activation and restores the levels of central memory T cells in HIV-infected individuals [14]. It would be interesting to assess whether vaccination with Tat affects Blimp-1 levels in B and T lymphocytes.

Thus, taking into consideration the pattern of expression of Blimp-1 in T cells during the different phases of HIV infection, we hypothesize that, during the acute phase and active progression, high levels of Blimp-1 favor immune activation, exhaustion of T cells and activation of $CD4^+$ T lymphocytes thus promoting viral spread. Molecular mechanisms behind these phenomena include Blimp-1 induction by Tat, miR-9, IL2, IL21 and cell-to-cell contact among activated T cells. Conversely, during latent chronic infection, we can speculate that high Blimp-1 levels may promote the reduction of $CD4^+$ T cells harboring the integrated provirus by both contrasting the development of $CD4^+$ T cell reservoirs and favoring the re-activation and, thus, elimination of HIV from latently infected cells.

Blimp-1 is crucial for plasma cell differentiation, memory B cell development and antibody production. However, as highlighted by Beisel and colleagues, studies assessing the role of Blimp-1 in B cell dysregulation, hypergammaglobulinemia and HIV-specific humoral responses are largely missing, although important to our understanding of B cell dysfunctions during HIV infection. Tat has been proposed to be implicated in B cell alterations and activation induced by

CD40 [11, 15], a molecule involved in Blimp-1 up-regulation. However, the role of Tat on B cells dysfunctions and Blimp-1 expression needs further elucidations.

In conclusion, Blimp-1 expression may be a marker to monitor the course of HIV infection as well as response to therapy. It is tempting to speculate that Blimp-1 expression may be modulated by immunotherapeutic strategies, although this approach needs strong caution due to its dual effect according to the diseases phase. Indeed, as already proposed [3], Blimp-1 expression may be reduced during active progression to decrease immune activation, viral spread and reservoir establishment. On the contrary, Blimp-1 induction in chronically HIV-infected HAART-treated individuals may favor reservoir purging.

[1] Crotty S, Johnston RJ, Schoenberger SP (2010) Effectors and memories: Bcl-6 and Blimp-1 in T and B lymphocyte differentiation. *Nat Immunol* **11:** 114-120

[2] Larsson M, Shankar EM, Che KF, Saeidi A, Ellegard R, Barathan M, Velu V, Kamarulzaman A (2013) Molecular signatures of T-cell inhibition in HIV-1 infection. *Retrovirology* **10:** 31

[3] Thaventhiran JE, Fearon DT (2013) Control of HIV infection: escape from the shadow of Blimp-1. *Eur J Immunol* **43:** 323-326

[4] Lu P, Youngblood BA, Austin JW, Mohammed AU, Butler R, Ahmed R, Boss JM (2014) Blimp-1 represses CD8 T cell expression of PD-1 using a feed-forward transcriptional circuit during acute viral infection. *J Exp Med* **211:** 515-527

[5] de Masson A, Kirilovsky A, Zoorob R, Avettand-Fenoel V, Morin V, Oudin A, Descours B, Rouzioux C, Autran B (2014) Blimp-1 overexpression is associated with low HIV-1 reservoir and transcription levels in central memory CD4+ T cells from elite controllers. *AIDS*

[6] Seddiki N, Phetsouphanh C, Swaminathan S, Xu Y, Rao S, Li J, Sutcliffe EL, Denyer G, Finlayson R, Gelgor L, Cooper DA, Zaunders J, Kelleher AD (2013) The microRNA-9/B-lymphocyte-induced maturation protein-1/IL-2 axis is differentially regulated in progressive HIV infection. *Eur J Immunol* **43:** 510-520

[7] Ding BB, Bi E, Chen H, Yu JJ, Ye BH (2013) IL-21 and CD40L synergistically promote plasma cell differentiation through upregulation of Blimp-1 in human B cells. *J Immunol* **190:** 1827-1836

[8] Sforza F, Nicoli F, Gallerani E, Finessi V, Reali E, Cafaro A, Caputo A, Ensoli B, Gavioli R (2014) HIV-1 Tat affects the programming and functionality of human CD8+ T cells by modulating the expression of T-box transcription factors. *AIDS*

[9] Gavioli R, Gallerani E, Fortini C, Fabris M, Bottoni A, Canella A, Bonaccorsi A, Marastoni M, Micheletti F, Cafaro A, Rimessi P, Caputo A, Ensoli B (2004) HIV-1 Tat protein modulates the

generation of cytotoxic T cell epitopes by modifying proteasome composition and enzymatic activity. *J Immunol* **173:** 3838-3843

[10] Fanales-Belasio E, Moretti S, Fiorelli V, Tripiciano A, Pavone Cossut MR, Scoglio A, Collacchi B, Nappi F, Macchia I, Bellino S, Francavilla V, Caputo A, Barillari G, Magnani M, Laguardia ME, Cafaro A, Titti F, Monini P, Ensoli F, Ensoli B (2009) HIV-1 Tat addresses dendritic cells to induce a predominant Th1-type adaptive immune response that appears prevalent in the asymptomatic stage of infection. *J Immunol* **182:** 2888-2897

[11] Nicoli F, Finessi V, Sicurella M, Rizzotto L, Gallerani E, Destro F, Cafaro A, Marconi P, Caputo A, Ensoli B, Gavioli R (2013) The HIV-1 Tat protein induces the activation of CD8(+) T cells and affects in vivo the magnitude and kinetics of antiviral responses. *PLoS One* **8:** e77746

[12] Gavioli R, Cellini S, Castaldello A, Voltan R, Gallerani E, Gagliardoni F, Fortini C, Cofano EB, Triulzi C, Cafaro A, Srivastava I, Barnett S, Caputo A, Ensoli B (2008) The Tat protein broadens T cell responses directed to the HIV-1 antigens Gag and Env: implications for the design of new vaccination strategies against AIDS. *Vaccine* **26:** 727-737

[13] Sicurella M, Nicoli F, Gallerani E, Volpi I, Berto E, Finessi V, Destro F, Manservigi R, Cafaro A, Ensoli B, Caputo A, Gavioli R, Marconi PC (2014) An Attenuated Herpes Simplex Virus Type 1 (HSV1) Encoding the HIV-1 Tat Protein Protects Mice from a Deadly Mucosal HSV1 Challenge. *PLoS One* **9:** e100844

[14] Ensoli B, Bellino S, Tripiciano A, Longo O, Francavilla V, Marcotullio S, Cafaro A, Picconi O, Paniccia G, Scoglio A, Arancio A, Ariola C, Ruiz Alvarez MJ, Campagna M, Scaramuzzi D, Iori C, Esposito R, Mussini C, Ghinelli F, Sighinolfi L, Palamara G, Latini A, Angarano G, Ladisa N, Soscia F, Mercurio VS, Lazzarin A, Tambussi G, Visintini R, Mazzotta F, Di Pietro M, Galli M, Rusconi S, Carosi G, Torti C, Di Perri G, Bonora S, Ensoli F, Garaci E (2010) Therapeutic immunization with HIV-1 Tat reduces immune activation and loss of regulatory T-cells and improves immune function in subjects on HAART. *PLoS One* **5:** e13540

[15] Lefevre EA, Krzysiek R, Loret EP, Galanaud P, Richard Y (1999) Cutting edge: HIV-1 Tat protein differentially modulates the B cell response of naive, memory, and germinal center B cells. *J Immunol* **163:** 1119-1122