Research Article

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Naïve CD8 T cell activation by liver bone marrow-derived cells leads to a "neglected" IL-2^{low} Bim^{high} phenotype, poor CTL function and cell death

Lauren E. Holz[1,†], Volker Benseler[1,‡], Michelle Vo[1], Claire McGuffog[1], Nico Van Rooijen[2], Geoffrey W. McCaughan[1], David G. Bowen[1,*,§], Patrick Bertolino[1,*,§]

[1]*AW Morrow Gastroenterology and Liver Centre, Centenary Institute, Royal Prince Alfred Hospital and University of Sydney, Camperdown, NSW 2050, Australia;* [2]*Department of Molecular Cell Biology, Vrije Universiteit, VUMC, Amsterdam 1081BT, The Netherlands*

See Editorial, pages 718–719

Background & Aims: The occurrence of primary CD8 T cell activation within the liver, unique among the non-lymphoid organs, is now well accepted. However, the outcome of intrahepatic T cell activation remains controversial. We have previously reported that activation initiated by hepatocytes results in a tolerogenic phenotype characterized by low expression of CD25 and IL-2, poor cytotoxic T lymphocyte (CTL) function, and excessive expression of the pro-apoptotic protein Bim.

Methods: To investigate whether this phenotype was due to activation in the absence of co-stimulation, we generated bone marrow (bm) radiation chimeras in which adoptively transferred naïve transgenic CD8 T cells were activated in the presence of co-stimulation by liver bm-derived cells.

Results: Despite expressing pro-inflammatory cytokines, high levels of CD25 and CD54, donor T cells activated by liver bm-derived cells did not produce detectable IL-2 and displayed poor CTL function, suggesting incomplete acquisition of effector function. Simultaneously, these cells expressed high levels of Bim and died by neglect. Transfer of Bim-deficient T cells resulted in increased T cell numbers.

Conclusions: These results imply that expression of CD25 and CD54 is co-stimulation dependent and distinguishes T cell activated by hepatocytes and liver bm-derived cells. In contrast, low expression of IL-2, poor CTL function and excess Bim production represent a more universal phenotype defining T cells undergoing primary activation by both types of hepatic antigen presenting cells (APC). These results have important implications for transplantation, in which all liver antigen presenting cells contribute to activation of T cells specific for the allograft.

Introduction

The liver possesses a remarkable capacity to retain and activate naïve CD8 T cells recognizing their cognate antigen in this organ [1,2]. Hepatocytes, Kupffer cells (KC), liver sinusoidal endothelial cells (LSEC), dendritic cells (DC), and stellate cells have all been demonstrated to act as potential antigen presenting cells for naïve CD8 T cells [3,4]. This unique ability of the liver to activate naïve T cells has been implicated in oral tolerance, the propensity for liver allografts to be accepted, and the failure of adaptive immune responses to clear HBV and HCV in many cases [3,5,6].

The phenotype and fate of naïve T cells activated in the liver remain controversial. We have shown that most naïve T cells activated in the liver were deleted [1,2,7,8], while other reports have indicated that they acquired full effector function [9,10]. These discrepancies might be due to the different experimental models used and/or the variety of liver cells endowed with antigen-presenting ability. Using T cell receptor (TCR) transgenic (Tg) T cell models, we have recently shown that regardless of the type of liver APC expressing the antigen, most naïve CD8 T cells activated intrahepatically were rapidly cleared by a non-apoptotic process. Within the first few hours after transfer, recipient-reactive T cells entered hepatocytes and were rapidly degraded in lysosomes, a process that we termed "suicidal emperipolesis (SE)" [11]. Some T cells survived suicidal emperipolesis and

Keywords: Kupffer cells; Hepatocytes; Apoptosis; Suicidal emperipolesis; Death by neglect.

Received 28 February 2012; received in revised form 3 May 2012; accepted 17 May 2012; available online 1 June 2012

☆ DOI of original article: http://dx.doi.org/10.1016/j.jhep.2012.07.010.

* Corresponding authors. Tel.: +61 2 9565 6186; fax: +61 2 9565 6101.

E-mail addresses: d.bowen@centenary.org.au (D.G. Bowen), p.bertolino@centenary.org.au (P. Bertolino).

† Current address: Immunology Section, Liver Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Department of Health and Human Services, Bethesda, MD, USA.

‡ Current address: Department of Surgery, University of Regensburg, Bavaria 93053, Germany.

§ Equal last author contributors.

Abbreviations: IL-2R, interleukin-2 receptor; bm, bone marrow; IL-2, interleukin-2; KC, Kupffer cells; LSEC, liver sinusoidal endothelial cells; DC, dendritic cells; APC, antigen presenting cells; ICAM-1, intercellular adhesion molecule-1; LN, lymph node; TCR, T cell receptor; CTL, cytotoxic T lymphocyte; ALT, alanine aminotransferase; MHC, major histocompatibility complex; IFN, interferon; TNF, tumor necrosis factor.

proliferated within the liver, but did not recirculate during the first 48 h post-activation [11].

When cognate antigen expression was restricted to hepatocytes, T cells surviving SE acquired a unique cell surface phenotype characterized by low expression of the interleukin 2 receptor (IL-2R) alpha chain (CD25) and intercellular adhesion molecule-1 (ICAM-1) [12]. In addition, these cells were poor CTLs, unable to produce IL-2, IFNγ, and TNF-α, and subsequently underwent Bim-dependent death by neglect [12].

As hepatocytes do not express co-stimulatory molecules, it remains unclear whether the "neglected" phenotype was due to activation in the absence of co-stimulation. To address this question, we used a previously described model in which naïve Tg CD8 T cells were rapidly retained in the liver and activated *in situ* by bone marrow (bm) derived-cells [2]. We show here that in contrast to hepatocyte-activated CD8 T cells, T cells activated by liver bm-derived APCs expressed high levels of CD25 and ICAM-1 at 48 h, consistent with activation in the presence of co-stimulation. However, despite the high expression level of CD25, these cells were poor CTLs, did not produce detectable levels of IL-2, and expressed high levels of Bim. These results suggest that the low CD25/ICAM-1 phenotype is only acquired following activation by hepatocytes while the poor CTL function/IL-2^{low} Bim^{high} "neglected" phenotype is a universal phenotype acquired as a result of primary T cell activation within the liver environment.

Materials and methods

Mice

Des, Des-RAG, BimDes, Met-K^b, and 178.3 Tg mice have been described elsewhere [1,2,12] and were maintained at the Centenary Institute animal facility under specific pathogen free conditions. B10.BR and C57BL/6 mice were purchased from the Animal Resource Centre (Perth, WA, Australia). All experiments were approved by the University of Sydney Animal Ethics Committee.

Generation of chimeric mice

B10.BR mice were irradiated with 9.5 Gy and reconstituted with 5×10^6–10^7 bm cells from either B10.BR or syngeneic 178.3 mice. Irradiated recipients were injected with Des-RAG, Des or BimDes T cells, 6 weeks after hematopoietic reconstitution, as previously described [1,12].

Adoptive transfer experiments

Single cell suspensions of pooled lymph node (LN) cells were labeled with 5-carboxyfluorescein di-acetate succinimidyl ester (CFSE, Molecular Probes, Eugene, OR, USA) or ^{51}Cr as previously described [8,13]. 2×10^6 labeled Des-RAG lymphocytes or 5–15 $\times 10^6$ Des or BimDes labeled lymphocytes were injected into the tail veins of recipient mice. In some experiments, liver phagocytic cells were depleted from recipient mice 48 h prior to adoptive transfer of T cells by i.p. injection of 200 µl of clodronate liposomes. Clodronate liposomes (Roche Diagnostics GmbH, Mannheim, Germany) and control PBS liposomes were prepared, as previously reported [14].

Radioactivity analysis

Four hours following the transfer of radiolabeled T cells, blood was extracted by cardiac puncture. To remove residual blood, livers were perfused with PBS via the inferior vena cava. Liver, lungs, intestine, pooled LNs, spleen, and the remaining carcass were harvested and retained radioactivity quantified using a gamma counter (PerkinElmer Wallac, Turku, Finland).

Functional assays and flow cytometric analysis

Leukocytes were prepared from blood, liver, LNs, and spleen as previously described [7,8,12]. Monoclonal antibodies specific to leukocyte surface markers were purchased from Becton Dickinson (Franklin Lakes, NJ, USA) or Cell Signaling Technology (Danvers, MA, USA). PE-conjugated antibody recognizing the cleaved form of caspase 3 (clone C92–605) was also purchased from Becton Dickinson. Antibodies specific for the clonotypic Tg TCR Desiré were prepared in-house. MAb 3C5, which recognizes all isoforms of Bim [15], was purchased from Alexis (Lausen, Switzerland). Surface, cytokine and intracellular staining were carried out as previously described [1,12]. *In vivo* CTL assays were conducted at day 2 and 5 post-transfer as previously described [12]. Flow cytometric analysis was performed using a FACSCanto or LSRII flow cytometer (Becton Dickinson). Analysis was performed using FlowJo software (TreeStar Inc, San Carlos, CA, USA) on a Macintosh computer (Apple Computers, Cupertino, CA, USA).

Serum ALT measurement

Serum was collected and analyzed for the concentration of serum alanine aminotransferase (ALT) using a Hitachi 917 automated analyzer (Roche Diagnostics, Sydney, Australia).

Immunohistochemistry

For paraffin sections, tissues were immediately fixed in 10% formalin (Sigma, St. Louis, MO, USA). Following fixation in formalin, tissues were embedded in paraffin and 5-µm sections cut and stained with hematoxylin and eosin (H&E).

Statistics

Prism 5 (GraphPad software Inc., La Jolla, CA) was used to perform (i) 2 way ANOVA followed by Bonferroni's post-test for analysis of 3 or more groups and (ii) Mann–Whitney test for analysis of two groups. p values of less than 0.05 were considered significant.

Results

Unlike liver bone marrow-derived cells (identified as MHC II+F4/80+ cells), hepatocytes do not express co-stimulatory molecules (Supplementary Fig. 1). To investigate whether the Bim^{high} IL-2^{low} phenotype acquired by hepatocyte-activated T cells resulted from lack of co-stimulation during primary activation, we used a previously published model in which CD8 T cells are activated by liver resident bm-derived cells [2]. B10.BR animals were irradiated and reconstituted with bm from 178.3 mice (B10.BR mice expressing Tg H-$2K^b$ ubiquitously). Following hematopoietic reconstitution, we have previously demonstrated by immunohistochemistry and flow cytometry that expression of H-$2K^b$ was restricted to bm-derived cells in 178.3bm → B10.BR chimeric mice (178.3 bm chimeras) [2]. B10.BRbm → B10.BR chimeric mice (B10.BR bm chimeras) served as controls.

Antigen expression on phagocytic cells promotes retention of naïve CD8 T cells in the liver

Consistent with previous data [2,13], adoptive transfer of radiolabeled naïve CD8 T cells specific for H-$2K^b$ (Des-RAG T cells) into 178.3 bm chimeric mice resulted in the majority of radioactivity (and hence donor T cells) being detected in the liver within 4 h, despite the expression of cognate antigen being dispersed on bm-derived cells in all tissues (Fig. 1A). As a result of this efficient intrahepatic T cell retention, little radioactivity was retained in spleen or LNs of 178.3 bm chimeras (Fig. 1A). As expected, the majority of radioactivity was retained within lymphoid tissues