Available online at www.sciencedirect.com
ScienceDirect
Immunobiology 213 (2008) 39–50
Immunobiology
www.elsevier.de/imbio

Developmental shift in TcR-mediated rescue of thymocytes from glucocorticoid-induced apoptosis

László Pálinkás, Gergely Talabér, Ferenc Boldizsár*, Domokos Bartis, Péter Németh, Timea Berki

Department of Immunology and Biotechnology, University of Pécs, Szigeti út 12, H-7643 Pécs, Hungary

Received 22 September 2006; received in revised form 24 May 2007; accepted 22 June 2007

Abstract

Glucocorticoid hormone (GC) production by thymic epithelial cells influences TcR signalling in DP thymocytes and modifies their survival. In the present work, we focused on exploring details of GC effects on DP thymocyte apoptosis with or without parallel TcR activation in AND transgenic mice, carrying TcR specific for pigeon cytochrome C, *in vivo*. Here we show that the glucocorticoid receptor (GR) protein level was the lowest in DP thymocytes, and it was slightly down-regulated by GC analogue, anti-CD3, PCC and combined treatments as well. Exogenous GC analogue treatment or TcR stimulation alone lead to marked DP cell depletion, coupled with a significant increase of early apoptotic cell ratio (AnnexinV staining), marked abrogation of the mitochondrial function in DP cells (CMXRos staining), and significant decrease in the Bcl-2^{high} DP thymocyte numbers, respectively. On the other hand, the simultaneous exposure to these two proapototic signals effectively reversed all the above-described changes. The parallel analysis of CD4 SP cell numbers, AnnexinV, CMXRos, Bcl-2 and GR stainings revealed, that the GR and TcR signals were not antagonistic on the mature thymocytes. These data provide experimental evidence in TcR transgenic mice, *in vivo*, that when TcR activation and GR signals are present simultaneously, they rescue double positive thymocytes from programmed cell death. The two separate signalling pathways merge in DP thymocytes at such important apoptosis regulating points as the Bcl-2 and GR, showing that their balanced interplay is essential in DP cell survival.

Keywords: Glucocorticoid hormone (GC); Glucocorticoid receptor (GR); Double positive (DP) thymocytes; Apoptosis; Mutual antagonism; Bcl-2

Introduction

The production of immunocompetent (self MHC restricted, tolerant) T lymphocytes is a key element for the balanced function of the immune system (Klein and Hořejší, 1997). The special microenvironment of the thymus has a central role in this process (Ladi et al., 2006). The complex meshwork of thymic stromal cells is composed of thymic epithelial cells, macrophages, dendritic cells and fibroblasts, providing a vast surface for direct cell–cell interactions and producing soluble factors regulating thymocyte development, intrathymic migration and selection processes (Anderson and Jenkinson, 2001). Key molecular players in cell–cell interactions are adhesion molecules, chemokine recep-

*Corresponding author. Tel.: +36 72 536 288; fax: +36 72 536 289.
E-mail address: fboldizsar@hotmail.com (F. Boldizsár).

0171-2985/$ - see front matter © 2007 Elsevier GmbH. All rights reserved.
doi:10.1016/j.imbio.2007.06.004

tors (Savino et al., 2004), Wnt (Pongracz et al., 2003) and MHC (Klein and Kyewski, 2000) molecules.

Thymocyte maturation proceeds through well-defined stages in the thymus, characterised by the sequential expression of T-cell specific surface molecules, including T-cell receptor (TcR), CD3, CD4 and CD8 (Res and Spits, 1999). Bone marrow progenitors are committed to the T-cell direction as a result of Notch-1 receptor signalling (Radtke et al., 2004), and become double negative (DN) thymocytes with no cell surface TcR, CD3, CD4 and CD8, respectively. After successful rearrangement in the α and β TcR gene loci, the developing cells reach the double positive (DP): CD4+, CD8+, TCR+, CD3+ stage, and are selected on the basis of the signal transducing capability and antigen specificity of their TcR (Sebzda et al., 1999). DP thymocytes expressing a TcR with subthreshold avidity die by neglect (Starr et al., 2003). Thymocytes that express a TcR with high avidity for self peptides presented on self MHC molecules are eliminated by activation induced apoptosis (negative selection) (Starr et al., 2003). Cells recognising self MHC bound peptides with medium to low avidity survive (positive selection) (Starr et al., 2003). As a result of these selection processes, a self MHC-restricted and self-tolerant naive T cell repertoire is formed, to be distributed in the peripheral lymphoid organ compartments (Starr et al., 2003).

Several soluble factors are produced locally by the thymic stromal cells, including glucocorticoid hormone (GC) (Lechner et al., 2000). Lymphoid cells, especially the DP thymocytes, are highly sensitive to GC induced apoptosis (Cohen, 1992; Blomgren and Andersson, 1970), but the molecular mechanism(s) in the background are still to be elucidated. Paradoxically, low-to-moderate concentrations of GCs antagonise TcR-mediated apoptosis via the glucocorticoid receptor (GR) (“mutual antagonism theory”) (Vacchio et al., 1999). On the other hand, the role of the GR in thymic diffentiation processes was questioned by works on GR-knockout and GR dimerisation mutant mice where there was no abnormality in T cell development and selection (Godfrey et al., 2000).

Apoptosis is a central element in eliminating the non-functional or potentially self-reactive T-cell clones during thymic maturation. Programmed cell death is a universal chain of events, leading to the cleavage of genomic DNA and the fragmentation of the cells (Hengartner, 2000). Early steps of apoptosis include the loss of membrane phosphatidylserine asymmetry (Vermes et al., 1995) and the activation of upstream caspases (caspase-8 and -10) (Ho and Hawkins, 2005). The central or mitochondrial phase integrates various apoptotic stimuli, and initiates the effector caspases (caspase-9, -3, -6, and -7), mainly through the release of cytochrome C to the cytoplasm (Hengartner, 2000).

Although GC induced apoptosis in thymocytes has been known for a long time, we still do not have an integrated picture of the underlying molecular events. However, several details have been elucidated in the last decade (Frankfurt and Rosen, 2004). Most of the works agree that for apoptosis induction the liganded GR has to translocate to the nucleus, where it acts as a transcription factor, binding to GRE sequences in GC responsive genes. However, recent work by Sionov et al. (2006) showed that mitochondrial translocation, rather than nuclear translocation, of the GR plays a key role in determining GC sensitivity of cells. GC exposure leads to Ca^{2+}-mobilisation, Src and Cdk2 activation, together with phosphatidylinositol-specific phospholipase C phosphorylation, and activation of acidic sphingomyelinase (Smase) with subsequent ceramide generation (Marchetti et al., 2003; Granes et al., 2004). The mitochondrial pro-apoptotic members of the Bcl-2 protein family Bax, tBid, Bim, and Bak are involved in the downstream effector mechanisms (Almawi et al., 2004). The mitochondrial membrane potential decreases, and cytochrome C and Smac/Diablo are released to the cytoplasm leading to the activation of caspase-9 and -3 and endonucleases (Zhang et al., 2000).

The Bcl-2 protein, a member of the anti-apoptotic BH1-4 subfamily, was demonstrated to play a critical role in thymocyte survival, especially at the stage of death by neglect (Zhang et al., 2000). Bcl-2 over-expressing T-cell hybridoma is resistant to GC induced apoptosis (Memon et al., 1995).

Our previous *in vivo* observations have provided *in vivo* evidence in both normal, Balb/c and AND TcR transgenic mice that the four major thymocyte subpopulations (DN, DP, CD4 SP and CD8 SP cells) have different GC sensitivities (Berki et al., 2002; Boldizsar et al., 2003). We demonstrated that the DP cells are the most sensitive to GCs, although they have the lowest GR expression, suggesting the possible participation of non-genomic GC actions in thymocyte development (Berki et al., 2002). We also found that TcR stimulation-induced apoptosis could successfully be inhibited with synthethic GC analogue treatments, in both Balb/c (Berki et al., 2002) and AND TcR transgenic mice (Boldizsar et al., 2003), resulting in a higher ratio of positively selected CD4+ SP cells, providing further *in vivo* evidence for the role of GCs in the positive selection processes (Boldizsar et al., 2003).

In our present work, we further investigate the role of GCs in the apoptosis of thymocytes during the selection processes in the transgenic PCC specific TcR bearing AND mouse model. We characterised the apoptotic processes of thymocytes upon simultaneous or separate activation through TcR and GR by analysing phosphatidylserine translocation to the outer cell membrane layer and mitochondrial membrane potential changes as two early parameters of apoptosis and correlated these