Bcl-3 suppresses Tax-induced NF-κB activation through p65 nuclear translocation blockage in HTLV-1-infected cells

JINHENG WANG[1], JUNYING LI[2], YANMEI HUANG[1], XIANGFENG SONG[1], ZHIGUO NIU[1], ZHITAO GAO[1] and HUI WANG[1]

[1]Research Center for Immunology, Xinxiang Medical University, Xinxiang, Henan 453003; [2]Henan Province Red Cross Blood Center, Zhengzhou, Henan 450000, P.R. China

Received August 24, 2012; Accepted October 16, 2012

DOI: 10.3892/ijo.2012.1685

Abstract. Human T cell leukemia virus type 1 (HTLV-1) Tax-induced persistent activation of the NF-κB pathway is perceived as the primary cause of adult T cell leukemia (ATL), an aggressive leukemia caused by HTLV-1. Although elevated oncoprotein Bcl-3 levels are found in many HTLV-1-infected T cell lines and ATL cells, the role of Bcl-3 in the malignant progression caused by HTLV-1 retrovirus remains poorly understood. We confirmed, in the present study, that the Tax-induced NF-κB activation involves the regulation of Bcl-3. Both knockdown and overexpression of Bcl-3 inhibit the Tax-induced NF-κB activation. Similarly, excessive Bcl-3 inhibits the NF-κB/DNA binding activity and significantly decreases Tax-induced p65 nuclear translocation. The present results demonstrate the pleiotropic roles of Bcl-3 in Tax-induced NF-κB activation and indicate that a balance in the aberrant Bcl-3 expression may be established to play an important role in the maintenance of proliferation and inhibition of apoptosis in HTLV-1-infected and ATL cells.

Introduction

The human T cell leukemia virus type 1 (HTLV-1) is a complex human retrovirus that causes an aggressive leukemia known as adult T cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (1-3). The HTLV-1-encoded oncoprotein Tax alters the expression of numerous genes associated with cell proliferation, immune and inflammatory response, apoptosis and other biological processes through the activation of NF-κB pathway (4-6). The pathophysiology of ATL and other neurological disorders is largely attributed to the persistent activity of the NF-κB factors. Tax promotes the nuclear translocation of the NF-κB factors through dissociation from the IκB proteins and then this oncoprotein links p65 to the co-activators to enhance the transcriptional activity (7). The target genes include several IκB family members such as *p100, IκBζ* and *bcl-3* (8). p100 and p52 are strongly expressed in numbers of Tax-positive HTLV-transformed cells, with Tax is regarded as the inducer. The aberrant expressions of p100 and p52 were also observed in the Tax-negative ATL cells (9), indicating that the noncanonical NF-κB pathway is also involved in the development of ATL since p52 is a primary member in that pathway.

Bcl-3 (B-cell chronic leukemia protein 3), an oncoprotein, is also a member of the IκB family of NF-κB inhibitors characterized by a conserved central ankyrin repeat domain (10). Aberrant Bcl-3 leads to increased cell proliferation, cell survival and malignant potential (11-13). Bcl-3 is a nuclear protein that is specifically associated with the homodimers of p50 and p52 (14) and functions as a co-activator by binding to p50 and p52 to regulate the transcription of target genes because of its two cooperative transactivation domains (15-17). This protein was also reported to inhibit DNA binding of p50 and p52, thereby negatively regulating gene transcription (16,18-20). Early (21) and recent reports (22) have also shown that Bcl-3 increases the p50 homodimer binding to NF-κB sites without causing co-activation. Bcl-3 increases the p50 homodimer NF-κB site occupancy, thereby indirectly repressing the NF-κB target gene transcription. Interestingly, Bcl-3 can repress its own transcription by forming a complex with p50 at the NF-κB sites within the Bcl-3 promoter and intronic enhancer HS3 (23). Thus, Bcl-3 is a multifaceted modulator of the NF-κB activity and plays multiple functions.

Previous studies have shown that Tax transcriptionally upregulates the Bcl-3 expression mainly through NF-κB pathway (24) and Bcl-3 is constitutively expressed in many HTLV-1-infected T cell lines (25). Given the importance of NF-κB activation and the strong correlation between Bcl-3 and HTLV-1, we investigated the function of Bcl-3 in Tax-induced NF-κB activation. Both knockdown and overexpression of Bcl-3 inhibit NF-κB activation in Tax-positive cells. Ectopic expression of Bcl-3 dramatically suppresses Tax-induced NF-κB activation through a mechanism associated with reversing Tax-mediated p65 nuclear translocation. Bcl-3 overexpression reduces the NF-κB/DNA binding activity. These data reveal pleiotropic functions of Bcl-3 in Tax-induced NF-κB activation.

Correspondence to: Dr Hui Wang, Research Center for Immunology, Xinxiang Medical University, Xinxiang, Henan Province 453003, P.R. China
E-mail: wanghui@xxmu.edu.cn

Key words: human T cell leukemia virus type 1, Tax, Bay 11-7082, Bcl-3, NF-κB activation, p65 nuclear translocation

Materials and methods

Plasmid construction. Flag-tagged human Bcl-3-expressing plasmid (pCMV-entry-Bcl-3) was obtained from Origene (China). To construct a plasmid expressing full-length Bcl-3 without tag, the CDs of Bcl-3 was subcloned from pCMV-entry-Bcl-3 into the *Eco*RI and *Xho*I sites of the pcDNA3.0 vector (Invitrogen, USA). Bcl-3 shRNA and p65 shRNA plasmids were purchased from Santa Cruz Biotechnology (USA). pNF-κB-luc, pCMV-Bam-Tax and pCMV-Bam plasmids were kindly provided by Haojiang Luan and Shoji Yamaoka, respectively.

Western blot analysis. Whole cell lysates were extracted from cells suspended in radio immune precipitation buffer supplemented with 1 mM PMSF (Beyotime, China). The cytoplasmic and nuclear extracts were obtained using the Cytoplasmic and Nuclear Extract kit (Beyotime) according to the manufacturer's instructions. The lysates were resolved by electrophoresis on polyacrylamide gels containing 0.1% SDS (SDS-PAGE) and then transferred to the nitrocellulose membranes. The blots were incubated with the appropriate primary antibody diluted by TBST and then exposed to the appropriate second antibody conjugated with horseradish peroxidase after being washed with TBST. The bands on the membrane were visualized and captured using the ECL reagent (Beyotime) and X-ray films (Kodak, USA). The pixel densities of proteins were quantitated using ImageJ 1.44 software (National Institute of Health). Graphs represent the pixel density for different proteins normalized to β-actin.

Luciferase reporter assay. The cells were transfected using pNF-κB-luc, pSV-β-Gal plasmids and the appropriate plasmid. The enzymatic activities were assayed using the Luciferase Assay System (Promega, USA) and 20/20^n Luminometer (Turner BioSystems, USA) according to the manufacturer's instructions. The luciferase activity was expressed as the fold of the relevant control of each experiment. The β-galactosidase Enzyme Assay System (Promega) was used to detect β-galactosidase activity, which normalized the transfection efficiency. All reporter assays were performed in triplicate and repeated in 3 independent experiments.

Electrophoretic mobility shift assay (EMSA). The biotin-labeled NF-κB consensus double stranded oligonucleotides (5'-AGT TGAGGGGACTTTCCCAGGC-3') were purchased from Beyotime. The DNA-protein complexes were resolved on a 4% non-denaturing polyacrylamide gel. The gels were subsequently transferred to nylon membranes and blocked with 5% bovine serum albumin (BSA) in TBST. The membranes were incubated with the appropriate streptavidin conjugated with horseradish peroxidase. The bands were visualized and captured using the ECL reagent (Beyotime) and X-ray film (Kodak).

Immunofluorescence staining. The cells were fixed with chilled 95% ethanol. The fixed cells were washed with TBS and transferred to a slide. The cells were locked with TBS containing 5% BSA and washed twice with TBS containing 0.1% Triton X-100. The cells were incubated overnight with TBS containing anti-p65 rabbit pAb at 4˚C. The cells were incubated with TBS containing FITC-conjugated goat anti-rabbit IgG. The nuclei were stained with 10 μg/ml DAPI. Finally, the cells were washed twice with PBS and observed using the Olympus FluoView™ FV1000 microscope. The data were analyzed using the FV10-ASW1.6 Olympus software.

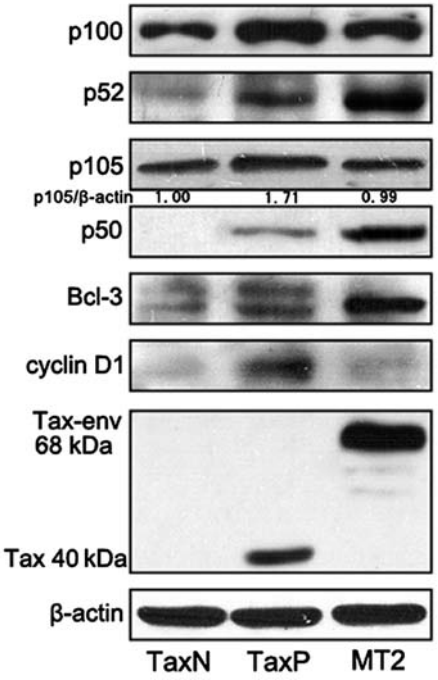

Figure 1. The expression of various proteins induced by Tax. Whole cell lysates were extracted from TaxN, TaxP and MT2 cells. Western blot analysis was performed with antibodies against β-actin, Tax, Bcl-3, cyclin D1, p105/p50 and p100/p52. Analysis of β-actin protein was included as a loading control.

Data analysis. Statistical significance for the luciferase reporter assays was determined using the Student's t-test or one-way ANOVA and a $p<0.05$ was regarded as statistically significant. The standard errors were demonstrated by the bar in the figures.

Results

Tax induces the expression of p105, p100, Bcl-3 and cyclin D1. A Tax-positive subline of Jurkat (TaxP) was established (26), which stably expresses Tax and a Tax-negative subline of Jurkat (TaxN) was established as the control to investigate the role of Tax in carcinogenesis. We analyzed the expression of Bcl-3, p100/p52, p105/50 and cell cycle-associated protein cyclin D1 in TaxN, TaxP and a HTLV-1-infected cell line MT2 (Fig. 1) to understand the transcriptional targets of Tax. Tax could be detected in the cell line TaxP and a known fusion between the envelope and the Tax-coding sequence (Tax-env) (27,28) was observed in MT2 cells. Interestingly, the Bcl-3 protein was abundantly expressed in MT2 cells and only slightly increased Bcl-3 was detected in TaxP cells compared with that in TaxN cells. As a transcriptional target of Bcl-3 and p52, cyclin D1 was expressed in lower levels in MT2 cells, which expressed high levels of Bcl-3 and p52 proteins. TaxP cells, which expressed a low level of Bcl-3, expressed a higher level of cyclin D1. Similarly, the p100 and p105 protein levels expressed in TaxP cells are higher than those in the other two cell lines, suggesting

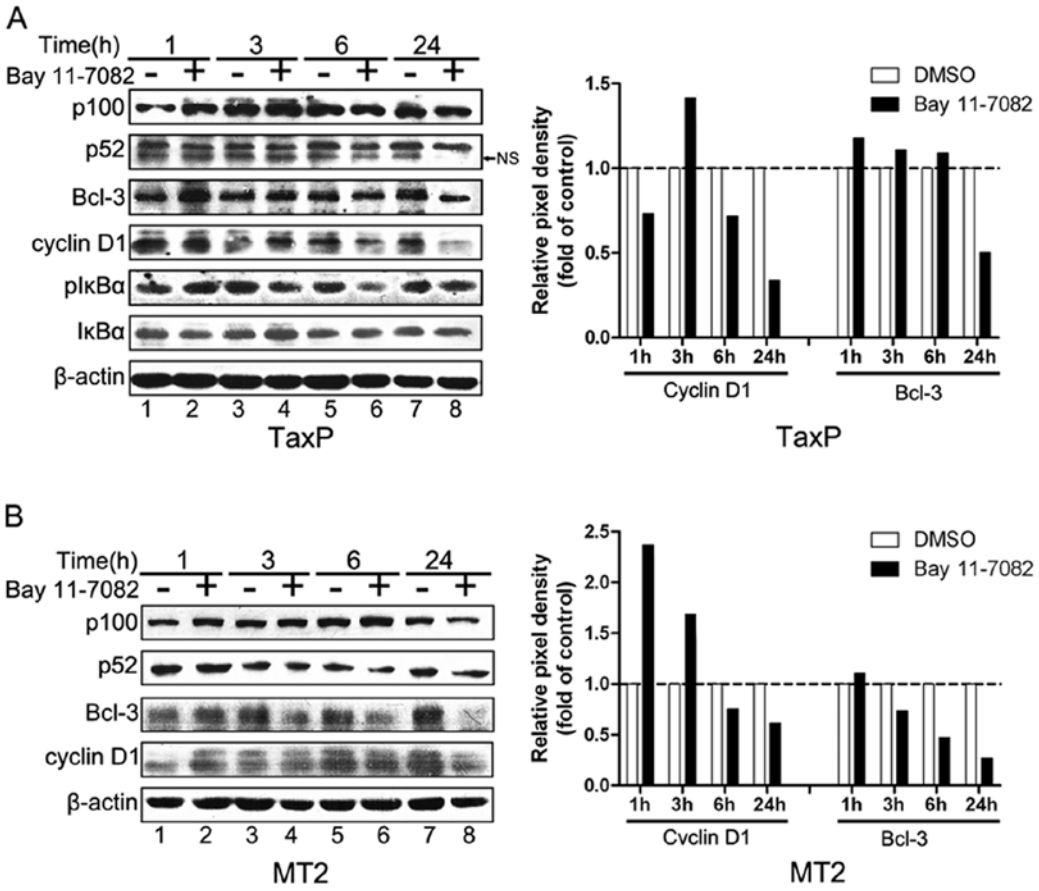

Figure 2. Effect of Bay 11-7082 on the expression of various gene products in Tax-expressing cells. (A) Time course of Bcl-3, cyclin D1, IκBα and p100/p52 protein expression in TaxP cells treated with 5 μM Bay 11-7082. Total lysates were extracted from TaxP cells following treatment with or without Bay 11-7082 for the indicated periods. The phosphospecific antibodies to P-Ser32 were used in the detection of phosphorylated residues of IκBα. (B) Total lysates were extracted from MT2 cells following treatment with or without 5 μM Bay 11-7082 for the indicated points. The expression levels of Bcl-3, cyclin D1, p100/p52 protein were examined by western blot analysis. The analysis of β-actin protein was included as a loading control. NS, non-specific band.

that p100 and p105 are also induced by Tax alone. Western blot analysis also showed the elevated p52 and p50 protein in TaxP and MT2 cells.

Bay 11-7082 downregulates the expression of cyclin D1 and Bcl-3. An NF-κB inhibitor Bay 11-7082 was introduced to determine the roles of NF-κB pathway in Tax-induced Bcl-3 expression. Bay 11-7082, an IκBα phosphorylation inhibitor, blocks the nuclear translocation of NF-κB factors and suppresses the activation of the genes containing NF-κB binding site. The effect of NF-κB inhibition by Bay 11-7082 (5 μM) was assessed using western blot analysis to detect the protein expression of Bcl-3, p100/p52, p105/p50 and cyclin D1 after treatment in TaxP (Fig. 2A) and MT2 cells (Fig. 2B). Increased Bcl-3 expression was observed after 1 h of Bay 11-7082 treatment (Fig. 2, compare lanes 1 and 2), followed by a gradual decrease in the Bcl-3 expression after 24-h treatment in both TaxP and MT2 cells. Similarly, increased levels of phosphorylated IκBα (Ser32) were also found after 1-h treatment for in TaxP cells (Fig. 2A, compare lanes 1 and 2). The phosphorylation of IκBα was significantly inhibited by Bay 11-7082 after 3, 6 and 24 h of treatment (Fig. 2A, lanes 4, 6 and 8). The expression levels of p100, another NF-κB target gene and p52 protein were not remarkably changed in the presence of 5 μM Bay 11-7082. By contrast, cyclin D1 was significantly downregulated by Bay 11-7082 in the both Tax-positive cell lines. The p105 and p50 protein levels were not significantly changed after treatment in MT2 cells. These results suggest that the NF-κB pathway is involved in the Tax-induced Bcl-3 expression in HTLV-1-infected cells. The Bay 11-7082-induced downregulation of cyclin D1 was accompanied by the decrease of Bcl-3.

Knockdown of Bcl-3 suppresses the Tax-induced NF-κB activation. Unlike other IκB family members, Bcl-3 can either promote or inhibit NF-κB activation (12). Bcl-3 expression was remarkably reduced in TaxP and MT2 cells after transfection of Bcl-3 shRNA plasmid (Fig. 3A). The activity of pNF-κB-luc reporter was lower in the Bcl-3-knocked down cells compared with the control shRNA-transfected cells regardless of the presence of Tax (Fig. 3B). Bcl-3-knockdown induced the inhibition of Tax-induced NF-κB activation in a dose-dependent manner. A total of 200 ng Bcl-3 shRNA inhibited the NF-κB activation in the three cell lines (Fig. 3C), confirming that Bcl-3 is involved in the Tax-induced NF-κB activation and plays a positive role although Bcl-3 shRNA failed to inhibit the Tax-induced NF-κB activation specifically. Bcl-3 promotes NF-κB activity mainly through the non-canonical pathway and Tax induces NF-κB activation through both non-canonical

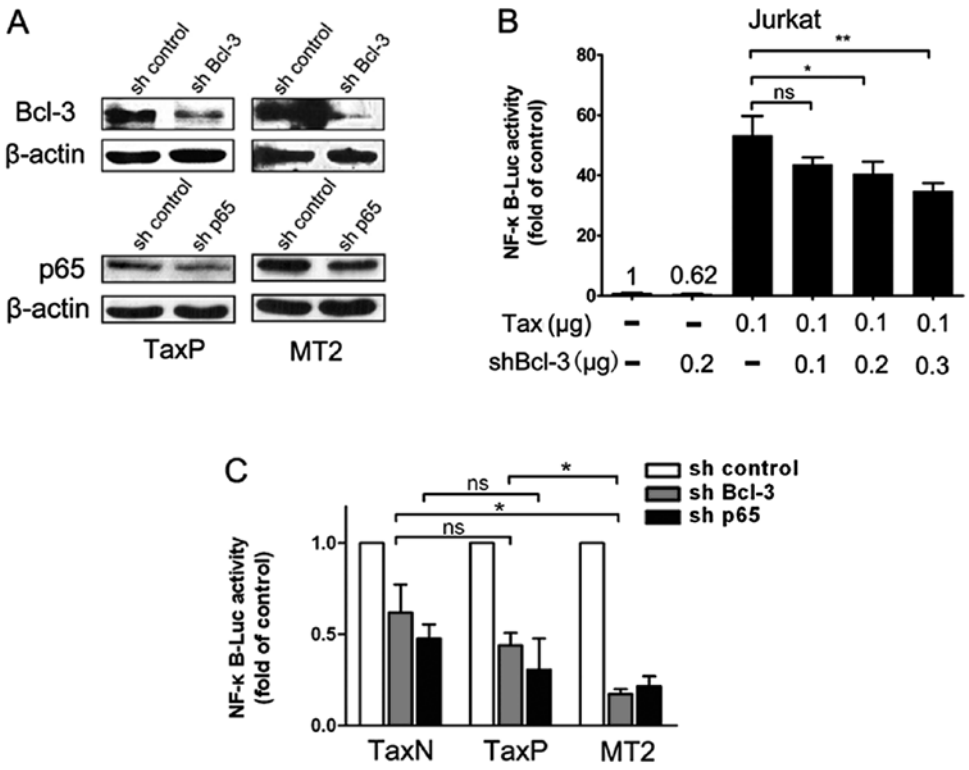

Figure 3. Knockdown of cell endogenous Bcl-3 inhibits HTLV-1 Tax-induced NF-κB activation. (A) The competent knockdown of the endogenous Bcl-3 and p65 by shRNA plasmids was checked by western blot analysis. (B) Jurkat cells were transfected using pNF-κB-luc and indicated amounts of Bcl-3 shRNA plasmids with or without co-transfection of pCMV-Bam-Tax plasmids. The cell lysates were extracted for the luciferase activity assay at 48 h post-ransfection. (C) TaxN, TaxP and MT2 cells were co-transfected using pNF-κB-luc with control or Bcl-3 shRNA or p65 shRNA plasmids. The cell lysates were extracted for the luciferase activity assay after 48 h of transfection. The results from three independent experiments are shown as means ± SD. Single, double and triple asterisks indicate $p<0.05$, $p<0.01$ and $p<0.001$, respectively.

and canonical pathways (29-32). We investigated the effect on NF-κB activation in TaxP and MT2 cells after the knockdown of p65 (Fig. 3A), which is a central member of the canonical NF-κB pathway, to characterize the role of canonical NF-κB pathway in Tax-induced NF-κB activation. The analysis of the luciferase activity indicated that p65 knockdown greatly inhibits the Tax-induced NF-κB activation (Fig. 3C). These results demonstrate the important positive role of Bcl-3 in NF-κB pathway and confirm that Tax promotes NF-κB activity through both non-canonical and canonical pathways.

Bcl-3 overexpression dramatically inhibits the Tax-induced NF-κB activation. Tax-induced Bcl-3 promotes the NF-κB activation because the knockdown of Bcl-3 suppresses the NF-κB activity. We analyzed the effect of Bcl-3 overexpression on NF-κB activation in Tax positive cells to determine whether this condition further enhances the Tax-induced NF-κB activation. Unexpectedly, Tax-induced NF-κB activation was significantly repressed by Bcl-3 overexpression in Jurkat cells (Fig. 4B). The redundant Bcl-3 depressed the NF-κB activity in the absence of Tax and inhibited the NF-κB activity in a dose-dependent manner in Jurkat cells in its presence. Excess Bcl-3 led to the inhibition of NF-κB activation when co-transfected with more Tax plasmids (Fig. 4C). The NF-κB activity in TaxP and MT2 cells was dramatically decreased by excess Bcl-3 compared with that in TaxN cells (Fig. 4D). Similarly, the expression vectors for Bcl-3 without tag of Flag (pcDNA3-Bcl-3) led to the same inhibitory action (Fig. 4D). These data indicate that the exogenous Bcl-3 regulates negatively the NF-κB activity and can specifically inhibit Tax-induced NF-κB activation.

Excess Bcl-3 stabilizes p50 and reduces NF-κB/DNA binding activity in MT2 cells. We examined the production of NF-κB factors, p50 and p52, which were associated with Bcl-3 by western blot analysis to investigate the molecular mechanism through which excess Bcl-3 inhibited the Tax-induced NF-κB activity. The production of p50 was elevated significantly in the presence of exogenous Bcl-3 in MT2 cells, whereas its precursor, p105, was not significantly changed (Fig. 5A). By contrast, the p100 and p52 proteins slightly increased in the Bcl-3-transfected MT2 cells. Cyclin D1 expression was weakly inhibited in MT2 cells, which expressed high levels of exogenous Bcl-3. The redundant Bcl-3 almost did not influence the p65 expression (Fig. 5A). These data indicate that excessive levels of Bcl-3, while stabilizing p50, may play a negative role in the regulation of cyclin D1.

We performed EMSA to clarify the mechanisms of NF-κB inhibition induced by excess Bcl-3 to assess the effect of exogenous Bcl-3 on NF-κB/DNA binding activity in MT2 cells. The Bcl-3-transfected MT2 cells displayed lower NF-κB/DNA binding compared with the control MT2 cells (Fig. 5B, compare lanes 2 and 3), suggesting that overexpression of Bcl-3 reduces the binding of NF-κB with DNA to inhibit the NF-κB transcriptional activity.

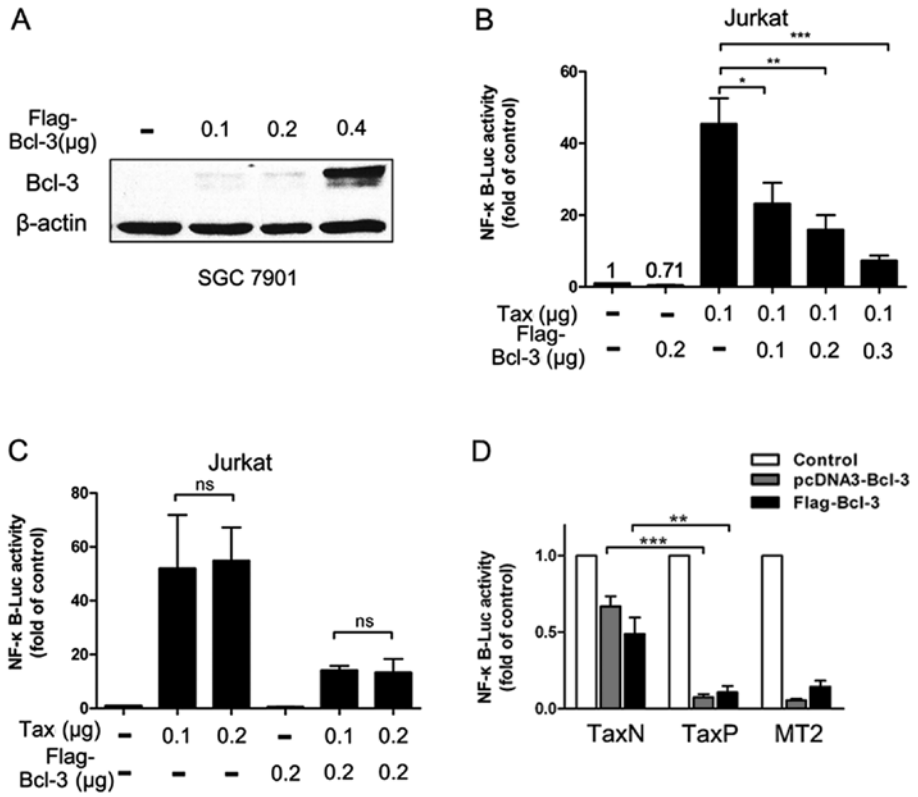

Figure 4. Excess Bcl-3 significantly suppresses HTLV-1 Tax-induced NF-κB activation. (A) The expression of exogenous Bcl-3 was checked by western blot analysis. (B) Jurkat cells were transfected using pNF-κB-luc and the indicated amounts of Flag-Bcl-3 plasmids with or without the co-transfection of pCMV-Bam-Tax plasmids. The cell lysates were extracted for the luciferase activity assay at 48 h post-transfection. (C) Jurkat cells were transfected using pNF-κB-luc and the indicated amounts of pCMV-Bam-Tax plasmids with or without the co-transfection of Flag-Bcl-3 plasmids. Cell lysates were extracted for the luciferase activity assay after 48 h of transfection. (D) TaxN, TaxP and MT2 cells were co-transfected using pNF-κB-luc with control or pcDNA3-Bcl-3 or Flag-Bcl-3 plasmids. The cell lysates were extracted for the luciferase activity assay. The results from three independent experiments are shown as means ± SD. Single, double and triple asterisks indicate $p<0.05$, $p<0.01$ and $p<0.001$, respectively.

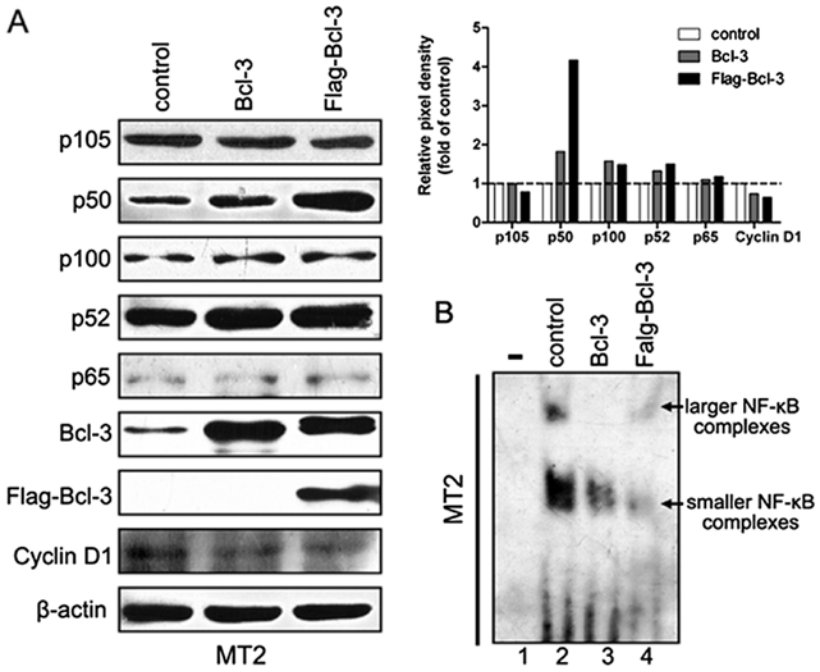

Figure 5. Effect of Bcl-3 overexpression on the expression of various proteins and NF-κB/DNA binding activity in MT2 cells. (A) The effect of excess Bcl-3 was measured using western blot analysis. Whole cell lysates were extracted after transfection and assessed by western blot analysis for Bcl-3, Flag-Bcl-3, p100/p52, p105/p50 and cyclin D1. Analysis of β-actin protein was included as a loading control. (B) Overexpression of Bcl-3 decreases NF-κB/DNA binding activity. The nuclear proteins were extracted from MT2 cells transfected with 500 ng empty vectors (control), or pcDNA3-Bcl-3 (Bcl-3), or Flag-Bcl-3 plasmids and examined for NF-κB/DNA binding activity by EMSA with a biotin-labeled specific probe. '-' indicates the blank control. The top band indicates the larger NF-κB complex that mainly contains the p65/p50 heterodimer and the bottom band indicates the smaller NF-κB complexes that mainly contain the p52 and p50 homodimer.

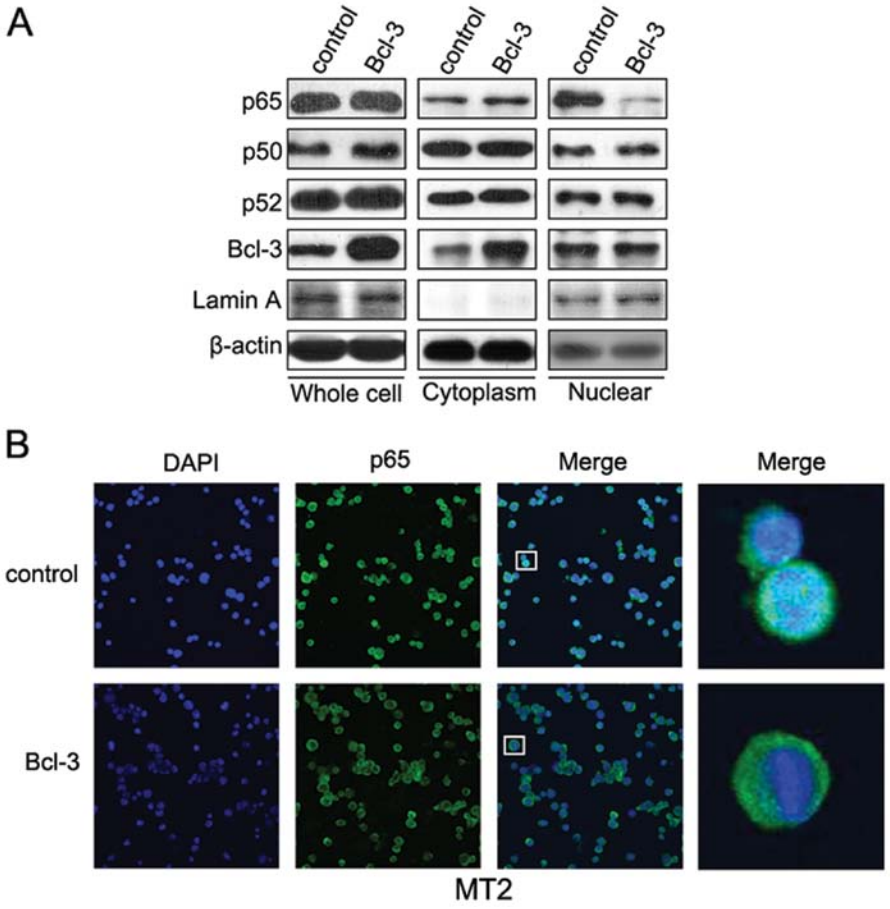

Figure 6. Effect of excess Bcl-3 on the translocation of NF-κB factors. (A) Whole cell, cytoplasm and nuclear proteins were extracted from MT2 cells transfected with empty vectors (control) or pcDNA3-Bcl-3 (Bcl-3) plasmids and examined by western blot analysis for p50, p52, p65 and Bcl-3. β-actin analysis was included as a loading control of the whole cell and cytoplasmic extracts and lamin A protein was included as a loading control of the nuclear extracts. (B) pcDNA3.0 (control) or pcDNA3-Bcl-3 (Bcl-3) plasmids were transfected into MT2 cells. The subcellular localizations of p65 (green) were visualized with anti-p65 and FITC-conjugated anti-rabbit IgG. The nuclei were stained with DAPI (blue). The higher magnification of the 'Merge' is shown in the right panels.

Bcl-3 expression promotes p65 translocation from nucleus to cytoplasm. NF-κB activation is always related to the nuclear translocation of NF-κB factors. Considering that Bcl-3 overexpression inhibits Tax-induced NF-κB activation, we analyzed the distribution of NF-κB factors in the cytoplasm and nucleus by western blot analysis after transfection (Fig. 6A). Bcl-3 was detected in both nuclear and cytoplasmic extracts. However, compared with the control, excess Bcl-3 was located in the cytoplasm, suggesting that the redundant Bcl-3 plays a negative role on NF-κB activity in the cytoplasm. Cytoplasmic p50 and p52 levels were enhanced slightly after the Bcl-3 transfection, whereas the p50 and p52 levels in the nucleus were not altered. We then examined the p65 nuclear and cytoplasmic levels. Strikingly, the nuclear p65 was significantly reduced in the presence of excessive Bcl-3 levels. By contrast, the p65 expression in whole cell extracts was not significantly changed after the transfection of pcDNA3-Bcl-3, suggesting that the excess Bcl-3-induced inhibition of NF-κB activation is due to the blockage of p65 nuclear translocation.

We immunostained MT2 cells transfected with Bcl-3 or empty vectors to confirm that p65 nuclear translocation is inhibited by excess Bcl-3 (Fig. 6B). p65 was mainly located in the nucleus in the control MT2 cells, but p65 was observed mainly in the cytoplasm after Bcl-3 transfection. These data strongly indicate that Bcl-3 overexpression inhibits p65 translocation in HTLV-1-infected cells.

Discussion

HTLV-1 Tax protein exerts an essential role in cell transformation and Tax-induced activation of NF-κB is critical for the immortalization and survival of HTLV-1-infected T cells. Tax activates the NF-κB pathway mainly through direct recruitment of p65 from the cytoplasm to the nucleus (7,29). As an IκB family member and a co-activator, Bcl-3 has been reported to either promote or inhibit the NF-κB activation with p50 and p52 homodimers. Elevated levels of Bcl-3 have been detected in many HTLV-1-infected cell lines (28) and are mainly regulated by the Tax-NF-κB pathway. The protein levels of p100, p105 and cyclin D1 were found to be upregulated by Tax alone, in contrast to their slight decrease in HTLV-1-positive cells. Bcl-3 has several intermediate phosphoforms in different cell lines and migrates in SDS-PAGE as several bands (14). Bcl-3 exists mainly in one form in MT2 cells and two forms were found in TaxN and TaxP cells, indicating that Bcl-3 plays multiple roles in different forms in different cells. Bay 11-7082 has been reported to inhibit specifically the Tax-induced NF-κB activation and expression of cyclin D1 (33), a cell cycle regulator,

which can be regulated by Bcl-3 and p52. Bay 11-7082 decreases the expression of cyclin D1 and Bcl-3 in the TaxP and MT2 cells. Therefore, Tax-induced Bcl-3 is regulated by the NF-κB pathway and the downregulated cyclin D1 is at least partially attributable to the inhibition of Bcl-3.

The knockdown of Bcl-3 was found to inhibit NF-κB activity in the Tax-expressing cells and HTLV-1-positive cells, suggesting that Bcl-3 plays a positive role in the Tax-induced NF-κB activation and a Bcl-3 positive feedback is confirmed in the Tax-positive cells. The positive regulation of Bcl-3 in the NF-κB pathway may be caused by its co-activator function with p50 and p52. Bcl-3 overexpression was found to inhibit enormously the NF-κB activation in the Tax-positive and virus-positive cells. Both knockdown and overexpression of Bcl-3 inhibit the Tax-induced NF-κB activation, suggesting the pleiotropic roles of Bcl-3 in the presence of Tax. Tax-induced p65 nuclear translocation has been reported to act as a major cause in the increased NF-κB activity (34,35). Reduced p65 nuclear translocation was observed after the forced Bcl-3 expression in HTLV-1-transformed cells and the p65 nuclear distribution is dramatically decreased by the excess Bcl-3. The ankyrin repeat domain of Bcl-3 can interacts with Tax (24) and excessive Bcl-3 were located in the cytoplasm. With respect to the importance of Tax in p65 nuclear translocation, Bcl-3 possibly interacts with Tax in the cytoplasm and blocks Tax nuclear translocation. Thus, p65 nuclear translocation pathway is blocked by Bcl-3.

Hence, the downregulation and upregulation of Bcl-3 inhibit Tax-induced NF-κB activation. Exogenous Bcl-3 inhibits Tax-induced NF-κB activation by reducing the NF-κB/DNA binding activity and blocking the Tax-induced nuclear translocation of p65. Tax induces Bcl-3 overexpression by activating the NF-κB pathway and the elevated Bcl-3 leads to increased cell proliferation, cell survival and malignant potential. Tax-induced NF-κB activation is critical in the HTLV-1 malignant progression. We found that Bcl-3 modulates Tax-induced NF-κB activation both positively and negatively. Positive regulation of NF-κB activation by Tax-induced Bcl-3 would promote cell proliferation and inhibit apoptosis. Excessive Bcl-3 would inhibit the Tax-induced NF-κB activation to modulate other NF-κB target gene transcription. Thus, Tax-induced Bcl-3 may contribute to the pathogenic process of ATL through the regulation of NF-κB activation. The present findings contributed to a better understanding of the interaction between Bcl-3 and Tax-induced NF-κB activation. The data also highlight the pleiotropic functions of Bcl-3 in the Tax-induced NF-κB activation. A balance in the aberrant Bcl-3 expression may be established by Tax to act as a modulator of the NF-κB pathway and to play an important role in the maintenance of proliferation, as well as anti-apoptosis, in HTLV-1-infected and ATL cells. Further studies should explore the potential stabilization of NF-κB factors by Bcl-3 in HTLV-1-infected and ATL cells. The other functions of Bcl-3 in the pathologic process of ATL should be investigated further to improve the understanding of the oncogenic potential of this protein with Tax and other NF-κB factors.

Acknowledgements

We are most grateful to Haojiang Luan and Shoji Yamaoka for the plasmids as well as to Ms. Wen Zhang, Mr. Jiqiang Guo and Xiaofei Zhu for the technical assistance. We thank all laboratory staff members for the critical reading of the manuscript. We would like to acknowledge Dr Tomas Ondrejcak, School of Medicine, Trinity College Dublin, for the English language improvement. The present study was supported by a grant from the National Natural Science Foundation of China (grant no. 30972755).

References

1. Gessain A, Barin F, Vernant JC, *et al*: Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. Lancet 2: 407-410, 1985.
2. Yoshida M, Miyoshi I and Hinuma Y: Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. Proc Natl Acad Sci USA 79: 2031-2035, 1982.
3. Osame M, Usuku K, Izumo S, *et al*: HTLV-I associated myelopathy, a new clinical entity. Lancet 1: 1031-1032, 1986.
4. Harhaj E and Harhaj N: Mechanisms of Persistent NF-κB Activation by HTLV-I Tax. Int Union Biochem Mol Biol: Life 57: 83-91, 2005.
5. Shvarzbeyn J and Huleihel M: Effect of propolis and caffeic acid phenethyl ester (CAPE) on NFκB activation by HTLV-1 Tax. Antiviral Res 90: 108-115, 2011.
6. Yasunaga J, Lin FC, Lu X and Jeang KT: Ubiquitin-specific peptidase 20 targets TRAF6 and human T cell leukemia virus type 1 tax to negatively regulate NF-κB signaling. J Virol 85: 6212-6219, 2011.
7. Azran I, Jeang K-T and Aboud M: High levels of cytoplasmic HTLV-1 Tax mutant proteins retain a Tax-NF-κB-CBP ternary complex in the cytoplasm. Oncogene 24: 4521-4530, 2005.
8. Hayden MS, West AP and Ghosh S: SnapShot: NF-κB signaling Pathways. Cell 127: 1286-1287, 2006.
9. Hironaka N, Mochida K, Mori N, Maeda M, Yamamoto N and Yamaoka S: Tax-independent constitutive IκB kinase activation in adult T-cell leukemia cells. Neoplasia 6: 266-278, 2004.
10. Michel F, Soler-Lopez M, Petosa C, Cramer P, Siebenlist U and Muller CW: Crystal structure of the ankyrin repeat domain of Bcl-3: a unique member of the IkappaB protein family. EMBO J 20: 6180-6190, 2001.
11. Courtois G and Gilmore TD: Mutations in the NF-kappaB signaling pathway: implications for human disease. Oncogene 25: 6831-6843, 2006.
12. Palmer S and Chen YH: Bcl-3, a multifaceted modulator of NF-κB-mediated gene transcription. Immunol Res 42: 210-218, 2008.
13. McKeithan TW, Takimoto GS, Ohno H, *et al*: BCL3 rearrangements and t(14;19) in chronic lymphocytic leukemia and other B-cell malignancies: a molecular and cytogenetic study. Genes Chromosomes Cancer 20: 64-72, 1997.
14. Bundy DL and McKeithan TW: Diverse effects of BCL3 phosphorylation on its modulation of NF-kappaB p52 homodimer binding to DNA. J Biol Chem 272: 33132-33139, 1997.
15. Fujita T, Nolan GP, Liou HC, Scott ML and Baltimore D: The candidate proto-oncogene bcl-3 encodes a transcriptional coactivator that activates through NF-kappa B p50 homodimers. Genes Dev 7: 1354-1363, 1993.
16. Franzoso G, Bours V, Azarenko V, *et al*: The oncoprotein Bcl-3 can facilitate NF-kappa B-mediated transactivation by removing inhibiting p50 homodimers from select kappa B sites. EMBO J 12: 3893-3901, 1993.
17. Bours V, Franzoso G, Azarenko V, *et al*: The oncoprotein Bcl-3 directly transactivates through kappa B motifs via association with DNA-binding p50B homodimers. Cell 72: 729-739, 1993.
18. Franzoso G, Bours V, Park S, Tomita-Yamaguchi M, Kelly K and Siebenlist U: The candidate oncoprotein Bcl-3 is an antagonist of p50/NF-kappa B-mediated inhibition. Nature 359: 339-342, 1992.
19. Inoue J, Takahara T, Akizawa T and Hino O: Bcl-3, a member of the I kappa B proteins, has distinct specificity towards the Rel family of proteins. Oncogene 8: 2067-2073, 1993.
20. Nolan GP, Fujita T, Bhatia K, *et al*: The bcl-3 proto-oncogene encodes a nuclear I kappa B-like molecule that preferentially interacts with NF-kappa B p50 and p52 in a phosphorylation-dependent manner. Mol Cell Biol 13: 3557-3566, 1993.
21. Caamano JH, Perez P, Lira SA and Bravo R: Constitutive expression of Bc1-3 in thymocytes increases the DNA binding of NF-kappaB1 (p50) homodimers in vivo. Mol Cell Biol 16: 1342-1348, 1996.

22. Kabuta T, Hakuno F, Cho Y, *et al*: Insulin receptor substrate-3, interacting with Bcl-3, enhances p50 NF-κB activity. Biochem Biophys Res Commun 394: 697-702, 2010.
23. Brocke-Heidrich K, Ge B, Cvijic H, *et al*: BCL3 is induced by IL-6 via Stat3 binding to intronic enhancer HS4 and represses its own transcription. Oncogene 25: 7297-7304, 2006.
24. Kim YM, Sharma N and Nyborg JK: The proto-oncogene Bcl3, induced by Tax, represses Tax-mediated transcription via p300 displacement from the human T-cell leukemia virus type 1 promoter. J Virol 82: 11939-11947, 2008.
25. Saito K, Saito M, Taniura N, Okuwa T and Ohara Y: Activation of the PI3K-Akt pathway by human T cell leukemia virus type 1 (HTLV-1) oncoprotein Tax increases Bcl3 expression, which is associated with enhanced growth of HTLV-1-infected T cells. Virology 403: 173-180, 2010.
26. Xia Y SY, Gao Y, Guo JQ, Song XF and Wang H: Establishment of the tax gene expression cell lines and their biological activity. Zhongguo Shi Yan Xue Ye Xue Za Zhi 16: 1425-1429, 2008.
27. Kress AK, Schneider G, Pichler K, Kalmer M, Fleckenstein B and Grassmann R: Elevated cyclic AMP levels in T lymphocytes transformed by human T-cell lymphotropic virus type 1. J Virol 84: 8732-8742, 2010.
28. Ishikawa C, Nakachi S, Senba M, Sugai M and Mori N: Activation of AID by human T-cell leukemia virus Tax oncoprotein and the possible role of its constitutive expression in ATL genesis. Carcinogenesis 32: 110-119, 2011.
29. Boxus M, Twizere J-C, Legros S, Dewulf J-F, Kettmann R and Willems L: The HTLV-1 Tax interactome. Retrovirology 5: 76, 2008.
30. Sun SC and Ballard DW: Persistent activation of NF-kappaB by the tax transforming protein of HTLV-1: hijacking cellular IkappaB kinases. Oncogene 18: 6948-6958, 1999.
31. Li XH and Gaynor RB: Mechanisms of NF-kappaB activation by the HTLV type 1 tax protein. AIDS Res Hum Retroviruses 16: 1583-1590, 2000.
32. Kfoury Y, Nasr R, Hermine O, de The H and Bazarbachi A: Proapoptotic regimes for HTLV-I-transformed cells: targeting Tax and the NF-kappaB pathway. Cell Death Differ 12 (Suppl 1): 871-877, 2005.
33. Mori N, Yamada Y, Ikeda S, *et al*: Bay 11-7082 inhibits transcription factor NF-kappaB and induces apoptosis of HTLV-I-infected T-cell lines and primary adult T-cell leukemia cells. Blood 100: 1828-1834, 2002.
34. O'Mahony AM: Human T-cell lymphotropic virus type 1 Tax induction of biologically active NF-κB requires IκB kinase-1-mediated phosphorylation of RelA/p65. J Biol Chem 279: 18137-18145, 2004.
35. Sun SC, Elwood J, Beraud C and Greene WC: Human T-cell leukemia virus type I Tax activation of NF-kappa B/Rel involves phosphorylation and degradation of I kappa B alpha and RelA (p65)-mediated induction of the c-rel gene. Mol Cell Biol 14: 7377-7384, 1994.