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Interleukin-7 promotes the survival of human CD4$^+$ effector/memory T cells by up-regulating Bcl-2 proteins and activating the JAK/STAT signalling pathway

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Summary

Interleukin-7 (IL-7) is a crucial cytokine involved in T-cell survival and development but its signalling in human T cells, particularly in effector/memory T cells, is poorly documented. In this study, we found that IL-7 protects human CD4$^+$ effector/memory T cells from apoptosis induced upon the absence of stimulation and cytokines. We show that IL-7 up-regulates not only Bcl-2 but also Bcl-xL and Mcl-1 as well. Interleukin-7-induced activation of the janus kinase/signal transducer and activator of transcription (JAK/STAT) signalling pathway is sufficient for cell survival and up-regulation of Bcl-2 proteins. In contrast to previous studies with naive T cells, we found that IL-7 is a weak activator of the phosphatidylinositol 3 kinase (PI3K)/AKT (also referred as protein kinase B) pathway and IL-7-mediated cell survival occurs independently from the PI3K/AKT pathway as well as from activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway. Considering the contribution of both IL-7 and CD4$^+$ effector/memory T cells to the pathogenesis of autoimmune diseases such as rheumatoid arthritis and colitis, our study suggests that IL-7 can contribute to these diseases by promoting cell survival. A further understanding of the mechanisms of IL-7 signalling in effector/memory T cells associated with autoimmune inflammatory diseases may lead to potential new therapeutic avenues.

Keywords: Bcl-2; effector/memory T cells; interleukin-7; Janus kinase/signal transducer and activator of transcription; survival

Introduction

Apoptosis is a tightly regulated process that plays an important role in T-cell homeostasis and immune response. During activation, T cells undergo apoptosis triggered via T-cell receptor-mediated activation of the Fas/Fas ligand death pathway.[1–3] T cells can also be eliminated through a Fas-independent pathway in response to lack of stimulation or absence of cytokines.[2,3] This form of apoptosis, known as passive death or cytokine deprivation-induced apoptosis, occurs both in naive and memory T cells,[4,5] and involves the deregulation of members of the Bcl-2 family and activation of the mitochondrial death pathway.[2,6,7] Activation of pro-apoptotic Bcl-2 proteins and inactivation of Bcl-2 pro-survival proteins lead to mitochondrial damage, release of apoptogenic factors among which is cytochrome c, activation of caspase-9 and executioner caspases such as caspase-3 and subsequently to cell death. Protection of T cells from this form of apoptosis is believed to be essential for a productive immune response, generation of T-cell memory and persistence of inflammation.[4,5]

The survival of T cells at different stages of development is believed to be dependent on extrinsic signals delivered by cytokines. Interleukin-7 (IL-7), a member of the IL-2 γ-chain cytokines, has emerged as a crucial T-cell

Abbreviations: 7-AAD, 7-aminoactinomycin D; ERK, extracellular signal-regulated kinase; FACS, fluorescence-activated cell sorter; JAK, Janus kinase; mAb, monoclonal antibody; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol-3 kinase; RANKL, receptor activator of nuclear factor kappa B ligand; STAT, signal transducer and activator of transcription; Th, T helper.

survival factor.[8–10] Interleukin-7 is produced by stromal cells, and it is necessary for the development and maturation of lymphocytes in the thymus and their maintenance in the periphery.[11,12] It is also necessary for the development of memory T cells,[13–15] although some recent reports suggested that the expression levels of IL-7 receptor (IL-7R) may not necessarily identify memory T-cell precursors or that they are insufficient for the formation of memory T cells.[16,17.]

Several studies conducted with murine models using naive T cells and IL-7-dependent cell lines have associated IL-7 signalling with up-regulation of Bcl-2 pro-survival proteins. The up-regulation of Bcl-2 pro-survival proteins is thought to counteract the activation of the pro-apoptotic Bcl-2 proteins such as Bim and Bax, which are both essential for cytokine deprivation-induced apoptosis.[18–21] Cells that express low levels of IL-7R were shown to be susceptible to Bim-induced apoptosis *in vivo*, whereas those expressing higher levels of IL-7R are protected,[22,23] and Bax deficiency partially compensates IL-7R deficiency.[9] Furthermore, IL-7 has also been shown to activate cell survival signalling pathways including the Janus kinase/signal transducer and activator of transcription (JAK/STAT) and the phosphatidylinositol 3-kinase (PI3K)/AKT (also referred as protein kinase B) pathways.[24]

In contrast to mouse T cells, IL-7 signalling is still poorly documented in human T cells, particularly in effector/memory T cells. Therefore, we investigated this issue by examining the mechanisms of IL-7 signalling in the survival of human $CD4^+$ effector/memory T cells. We found that IL-7 protects human $CD4^+$ effector/memory T cells from apoptosis induced upon the absence of stimulation and cytokines and up-regulates not only Bcl-2 but also Bcl-xL and Mcl-1 as well. We further show that IL-7 activates only the JAK/STAT5 pathway, which is sufficient for IL-7-induced cell survival and up-regulation of Bcl-2 proteins. These studies bring additional insights into the mechanisms of IL-7 signalling, which could be important in understanding T-cell homeostasis and persistence during inflammation.

Materials and methods

Antibodies and reagents

Interleukin-7 and IL-2 were purchased from R&D Systems (Minneapolis, MN) and Sigma (St Louis, MO), respectively. The Pan-JAK inhibitor (JAK-Inhibitor I), the mitogen-activated protein kinase kinase 1/2 (MEK 1/2) inhibitor U0126 and the PI3K/AKT inhibitor LY294002 were purchased from Calbiochem (San Diego, CA). Anti-CD3/CD28 Dynabeads were from Invitrogen Dynal AS (Oslo, Norway). Anti-CD3 (OKT3), anti-CD28 (CD28·2), anti-CD127 (hIL-7R-M21), anti-CD45RO (UCLH1), isotype control antibodies and phycoerythrin (PE)-conjugated anti-Bcl-2 and its PE-conjugated isotypic control antibodies were from BD Biosciences (San Diego, CA). Alexa-fluor 488-conjugated anti-mouse antibody was from Invitrogen (Carlsbad, CA). Antibodies against phospho-STAT5 (Tyr-694), Bcl-xL, phospho-AKT (Ser-473), and AKT were from Cell Signaling Technologies (Beverly, MA); and antibodies against Caspase-3, Mcl-1, Bcl-2, phospho-p44/42 mitogen-activated protein kinase (MAPK; E-4), extracellular signal-regulated kinase (ERK2; C-14) and actin (C-2) were from Santa Cruz Biotechnology (Santa Cruz, CA).

Isolation of primary T cells and generation of effector/memory T cells

Peripheral blood mononuclear cells were isolated from healthy adult volunteers on Ficoll gradients. The participating volunteers signed a consent form and the study was approved by the ethical committee of Laval University. Primary human naive $CD4^+$ T cells were then purified by negative selection using magnetic beads from StemCell Technologies (Vancouver BC, Canada) according to the manufacturer's instructions. Staining with anti-CD3 and anti-CD4 monoclonal antibodies (mAbs) and fluorescence-activated cell sorting (FACS) flow cytometry analysis indicated that more than 97% of the isolated cells were CD3/CD4 double-positive T cells. To generate $CD4^+$ effector/memory T cells, freshly isolated T cells were activated with anti-CD3/CD28 coated beads for 24 hr in complete RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM/l glutamine and 100 units/ml penicillin and streptomycin. The cells were then washed, transferred to flasks and cultured for a further 7 days in the presence of 50 U/ml recombinant IL-2, which was added every 2 days. Because IL-2 can down-regulate the expression of IL-7R on activated/effector T cells [25] and to avoid any influence of IL-2 on IL-7 signalling, the cells at day 7 were then cultured for 1 day in fresh medium in the absence of IL-2 before being used in subsequent experiments. More than 95% of these cells express CD45RO, the marker of memory T cells, and will be referred to thereafter as effector/memory T cells. This model is suitable for cell signalling studies and has been previously used by different groups and by us.[26–28] Upon T-cell receptor restimulation, these cells produce interferon-γ, IL-2 and the osteoclastogenic cytokine receptor activator of nuclear factor-kappa B ligand (RANKL).[28]

Cell surface molecule expression

The expression of cell surface receptors was determined by immunostaining and flow cytometry analysis. The cells were incubated on ice with 10 μg/ml anti-CD127, anti-CD45RO or with control isotypic mAbs for 45 min in phosphate-buffered saline (PBS) containing 0·1% fetal bovine serum. The cells were washed and incubated with

Alexa-fluor-conjugated anti-mouse antibody for another 45 min. Cells were then washed in PBS and analysed by flow cytometry using FACSCalibur instrument (BD Biosciences).

Apoptosis assays

Apoptosis was determined using the Annexin V-PE/7-aminoactinomycin D (7AAD) detection kit from BD Biosciences as previously described.[29] After stimulation, the cells were washed in cold PBS and 10^5 cells were incubated in 1 × buffer containing 2·5 μl Annexin V-PE and 2·5 μl 7-AAD for 15 min at room temperature in the dark. The cells were then washed and analysed by flow cytometry using a FACSCalibur instrument (BD Biosciences) and apoptotic cells were identified as being Annexin V-positive.

Immunoblot analysis

After stimulation, the cells were harvested, washed in cold PBS and cell lysates were prepared in radioimmunoprecipitation assay buffer containing protease and phosphatase inhibitors as previously described.[29] Cell lysates were subjected to sodium dodecyl sulphate–polyacrylamide gel electrophoresis and analysed by immunoblot using specific antibodies. The blots were stripped and re-probed with control anti-actin mAb to ensure equal loading. Activation of caspase-3 was determined by immunoblot analysis using specific anti-caspase-3 mAb that recognizes both the inactive pro-caspase form (p32) and the cleaved active form (p17). Activation of STAT5, ERK1/2 and AKT was determined by immunoblot analysis using specific antibodies recognizing the phosphorylated forms of STAT5, ERK1/2 and AKT. The expression levels of Mcl-1, Bcl-xL and Bcl-2 were detected by immunoblot analysis using specific antibodies. In all experiments, immunoblots were visualized using a horseradish peroxidase-conjugated secondary antibody followed by enhanced chemiluminescence detection (Pierce, Rockford, IL).

Intracellular Bcl-2 staining

T cells were permeabilized for 30 min using the CytoFix/CytoPerm kit (BD Biosciences), washed and stained with PE-conjugated anti-Bcl-2 antibody or with its isotypic control antibody for 30 min at room temperature. The cells were washed and analysed by flow cytometry using a FACSCalibur instrument (BD Biosciences).

Statistical analysis

Statistical analysis was performed using paired Student's *t*-test. Results with $P < 0{\cdot}05$ were considered significant.

Results

Expression of CD45RO and IL-7R on effector/memory T cells

We used human effector/memory T cells that were generated by activating naive T cells with anti-CD3 + anti-CD28 followed by expanding them in IL-2 for 7 days. The cells were then rested for 1 day in complete medium without IL-2 before being used in subsequent experiments. In agreement with previous studies using a similar cell model,[26,30] we found that 98% of the generated effector/memory T cells expressed CD45RO (Fig. 1a). We then

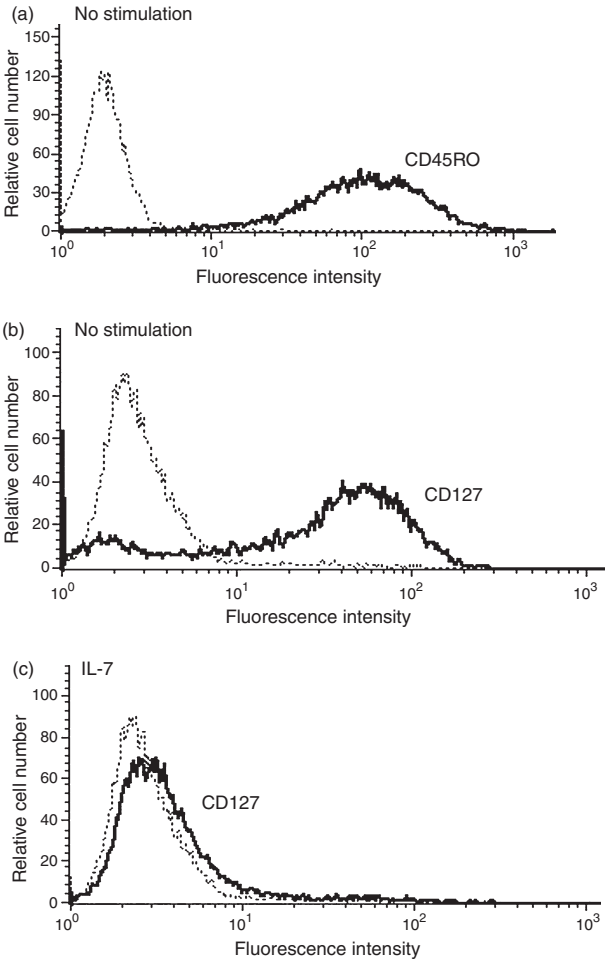

Figure 1. Human effector/memory T cells express CD45RO and the interleukin-7 receptor (IL-7R). The expression of IL-7R and CD45RO on the cell surface was determined by fluorescence-activated cell sorting analysis as described in the Materials and methods. (a) CD45RO expression on effector/memory T cells. (b) Expression of IL-7R on effector/memory T cells without stimulation and (c) upon stimulation with 2 ng/ml of IL-7 for 24 hr. Bold histograms (−) represent IL-7R or CD45RO staining and dashed histograms (…) represent cell staining with matched-control isotypic antibodies. The results are representative of five independent experiments performed with T cells from different blood donors.

determined the expression levels of IL-7R on human effector/memory T cells. As shown in Fig. 1(b), effector/memory T cells at day 8 expressed high levels of IL-7R, as determined by immunostaining of the cells for the α-chain (CD127) of the IL-7R. Expression of IL-7R is known to be down-modulated by IL-7 and other γ-chain survival cytokines in peripheral naive T cells.[31,32] Therefore, we looked into the regulation of CD127 expression on the cell surface of human effector/memory T cells and found that IL-7 treatment for 24 hr also down-regulated the levels of CD127 on these cells (Fig. 1c).

IL-7 protects effector/memory T cells from cytokine deprivation-induced apoptosis

Interleukin-7 is known to protect naive T cells from cytokine (IL-2) deprivation-induced apoptosis Therefore, we examined whether IL-7 can rescue effector/memory T cells subjected to lack of stimulation. To this end, the *in vitro*-generated effector/memory T cells at day 8, which exhibited a low rate of apoptosis (Fig. 2a; 0 hr time-point) were cultured in fresh medium under deprived conditions (without CD3/CD28 stimulation and in the absence of cytokines). Cytokine-deprived cells underwent significant apoptosis as 40% of the total cells became Annexin-V-positive within 24 hr of deprivation. The apoptotic cell population reached 55% and 65% after 48 and 72 hr of culture in deprived conditions (Fig. 2a). The observed apoptosis of cytokine-deprived effector/memory T cells was associated with significant activation of caspase-3, as shown by the reduction of pro-caspase-3 levels and the appearance of the p17 active form of caspase-3 (Fig. 2b). The addition of IL-7 resulted in approximately 50–60% reduction in apoptosis of effector/memory T cells (Fig. 2a), which was accompanied by a remarkable decrease of the p17 fragment indicative of reduced activation of caspase-3 (Fig. 2b). As a control, cells cultured in the presence of IL-2 were also protected from apoptosis and the protective effect of IL-7 was comparable to that of IL-2 (Fig. 2a). The use of the pan-caspase inhibitor carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]- fluoromethylketone (zVAD-FMK) strongly protected effector/memory T cells from apoptosis induced in the absence of cytokines (data not shown), indicating the implication of caspases. These results indicate that human effector/memory T cells undergo apoptosis when cultured in the absence of stimulation and are protected by IL-7, which inhibits activation of caspase-3.

IL-7 up-regulates Bcl-2 proteins Mcl-1, Bcl-xL and Bcl-2

Interleukin-7 signalling is associated with up-regulation of Bcl-2 anti-apoptotic proteins, which regulate cytokine deprivation-induced apoptosis.[2,6,7] We therefore examined

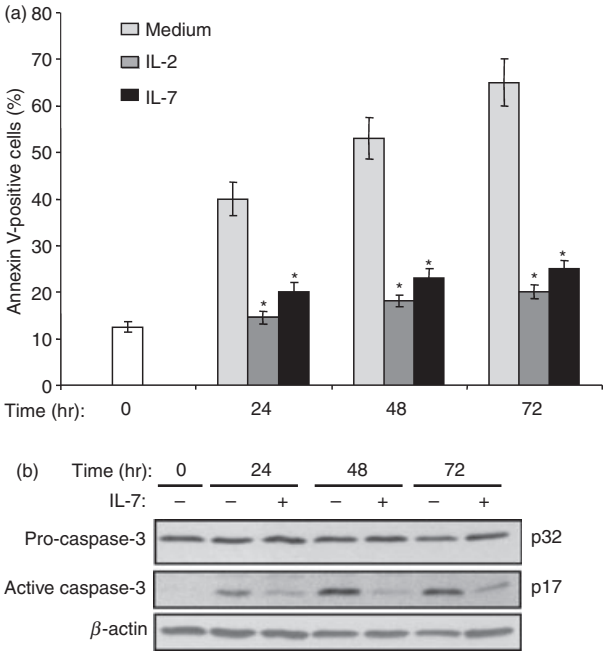

Figure 2. Interleukin-7 (IL-7) protects human effector/memory T cells from apoptosis and inhibits caspase-3 activation. (a) Cells at day 8 (0 hr time-point) were cultured under deprived conditions in the presence or absence of IL-7 (2 ng/ml) or IL-2 (50 U/ml) for different periods of time. Apoptosis was determined using Annexin V/7-aminoactinomycin D staining and flow cytometry analysis. The results are presented as mean percentages (± SE) of Annexin-V-positive cells from three independent experiments performed in triplicate with T cells isolated from different blood donors. $^*P < 0{\cdot}05$ between IL-7-treated or IL-2-treated samples and non-treated (medium) samples. (b) IL-7 inhibits caspase-3 activation. Effector/memory T cells were cultured under deprived conditions in the presence or absence of IL-7 for different periods of time. Cells at day 8 (0 hr time-point) were used as controls. Whole cell lysates were prepared and activation of caspase-3 was determined by immunoblot analysis using an anti-caspase-3 monoclonal antibody recognizing both pro-caspase-3 (p32) and the active form of caspase-3 (p17). The blot was stripped and re-probed with anti-β-actin monoclonal antibody to ensure equal loading. Results are representative of five independent experiments performed with T cells from different blood donors.

the expression levels of Bcl-2, Bcl-xL and Mcl-1 proteins in effector/memory T cells cultured under deprived conditions in the presence or absence of IL-7. As shown in Fig. 3(a), effector/memory T cells before starvation (0 hr time-point) expressed all three Bcl-2 proteins including Bcl-2, Mcl-1 and BcL-xL. However, culture of effector/memory T cells in deprived conditions led to a drop in the levels of all three Bcl-2 proteins, which was more pronounced for Mcl-1 and Bcl-xL. Addition of IL-7 to deprived cells up-regulated the levels of Mcl-1, Bcl-2 and Bcl-xL. The high levels of all three proteins induced by IL-7 were sustained up to 72 hr. The levels of β-actin were similar between non-treated cells and IL-7-treated cells. Densitometric analysis showed that IL-7 treatment

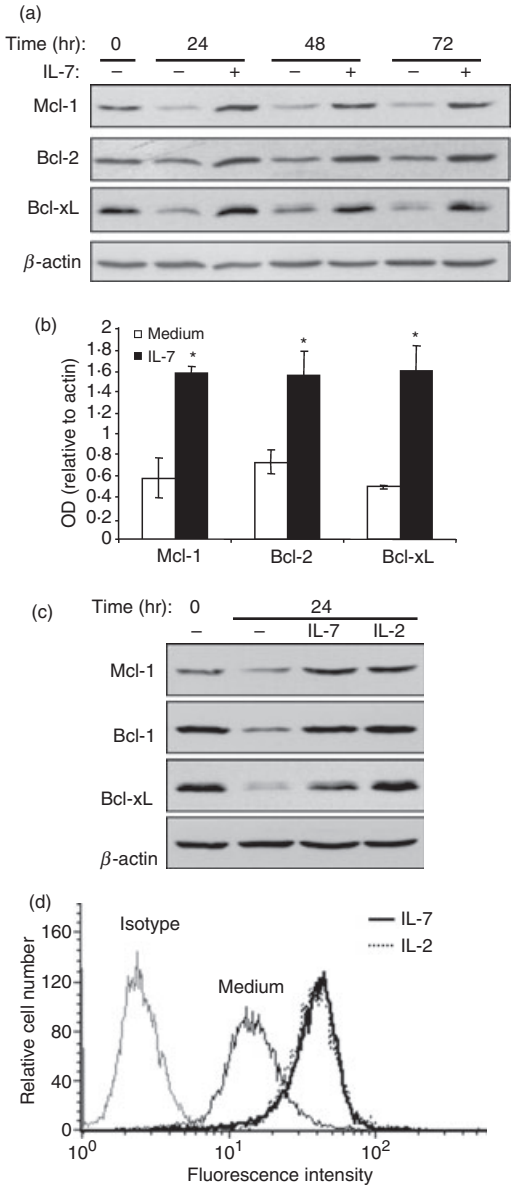

Figure 3. Interleukin-7 (IL-7) up-regulates Bcl-2 proteins in human effector/memory T cells. (a) Cells at day 8 (0 hr time-point) were cultured under deprived conditions in the presence or absence of IL-7 (2 ng/ml) for the indicated periods of time. Whole cell lysates were then prepared and subjected to immunoblot analysis with specific antibodies against Mcl-1, Bcl-2, or Bcl-xL. The blot was stripped and re-probed with anti-β-actin monoclonal antibody (mAb) to ensure equal loading. The results are representative of five independent experiments performed with T cells from different blood donors. (b) Densitometric quantification of relative increase in Bcl-2 proteins in cells cultured for 24 hr under deprived conditions in the absence (medium) or the presence of IL-7. The results represent mean values (± SE) from three independent experiments and are expressed as the ratio between Mcl-1, Bcl-2 or Bcl-xL values and β-actin values. $^*P < 0{\cdot}05$ between IL-7-treated samples and non-treated samples (medium). (c) The cells were cultured under deprived conditions in the presence or absence of IL-7 (2 ng/ml) or IL-2 (50 U/ml) for 24 hr. The expression of Mcl-1, Bcl-2 and Bcl-xL was determined by immunoblot analysis. The results are representative of three independent experiments performed with T cells from different blood donors. (d) The cells were left unstimulated (medium) or stimulated with IL-7, IL-2 for 24 hr. The cells were then washed, stained with phycoerythrin-conjugated anti-Bcl-2 or phycoerythrin-conjugated isotypic control antibodies and analysed by fluorescence-activated cell sorting analysis. The control isotypic staining is shown for unstimulated cells, which is similar to control isotypic staining of IL-2- and IL-7-stimulated cells (data not shown). The results are representative of three different experiments performed with T cells from different blood donors.

for 24 hr led to a significant twofold to threefold increase in the levels of Mcl-1, Bcl-2 and Bcl-xL (Fig. 3b). Similar results were also obtained in cells treated with IL-7 for 48 and 72 hr (data not shown). The capacity of IL-7 to up-regulate pro-survival Bcl-2 proteins after 24 hr of stimulation was comparable to that of IL-2, with the exception of Bcl-xL because IL-2 is slightly more potent that IL-7 (Fig. 3c). Similar results were also obtained when the cells were stimulated for 48 and 72 hr (data not shown).

To determine if the observed increase in the levels of Bcl-2 proteins also occurred at the cellular level, we measured the levels of intracellular Bcl-2 in cells stimulated or not with IL-7 or with IL-2. As shown in Fig. 3(d), IL-7 also up-regulated the levels of intracellular Bcl-2 protein and this effect was comparable with that of IL-2. Together, these results indicate that IL-7 up-regulates all three major Bcl-2 anti-apoptotic proteins in human effector/memory T cells.

IL-7 promotes cell survival and up-regulates Bcl-2 proteins via JAK/STAT signalling pathway

The JAK/STAT, PI3K/AKT and MAPK/ERK signalling molecules are major cell survival pathways, which can all be activated by IL-7. To provide insights into the signalling pathways contributing to IL-7-induced effector/memory T-cell survival, we have first assessed the effects of the JAK/STAT inhibitor Pan-Jak, the PI3K/AKT inhibitor LY294002 and the MEK1-2/ERK inhibitor U0126, on the ability of IL-7 to protect effector/memory T cells from apoptosis. Cells were cultured under deprived conditions with the different inhibitors or with IL-7 plus inhibitors, and their apoptosis was determined. The protective effect of IL-7 was only abrogated in the presence of the JAK/STAT inhibitor, but not in the presence of the MAPK/ERK or PI3K/AKT inhibitors (Fig. 4a). Interestingly, cells cultured under deprived conditions with LY294002 inhibitor led to a significant increase in cell apoptosis compared with cells cultured only in deprived conditions or with Pan-Jak or U0126 inhibitors (Fig. 4a), suggesting that the PI3K/AKT pathway can modulate the basal viability of effector/memory T cells. In addition, the presence

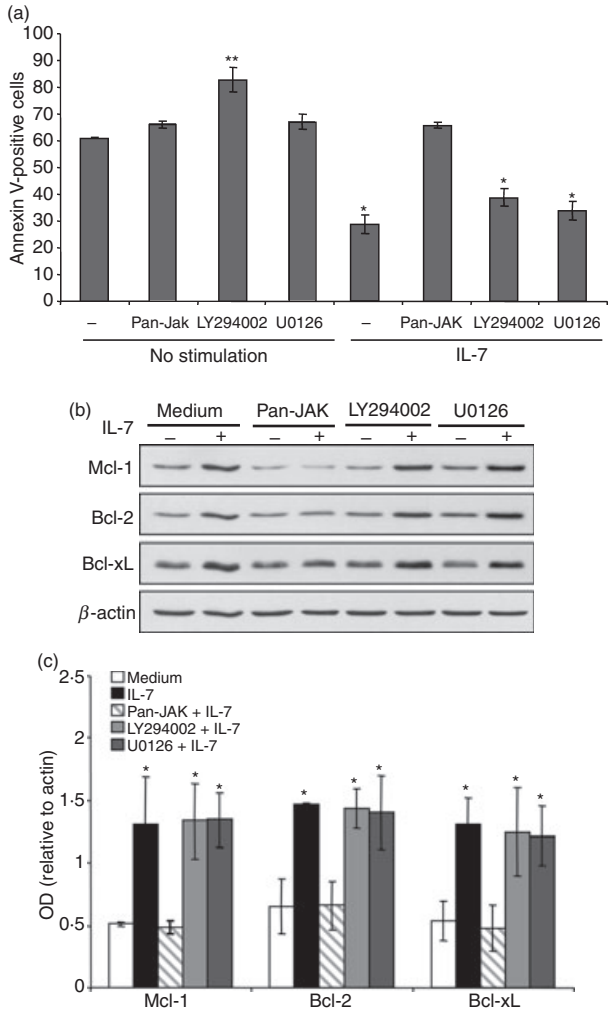

Figure 4. Interleukin-7 (IL-7) -mediated cell survival is abrogated by the Janus kinase/signal transducer and activator of transcription (JAK/STAT) inhibitor. (a) IL-7-mediated cell survival is reversed by the Pan-JAK inhibitor. Human effector/memory T cells were treated or not in the absence of cytokines with the Pan-JAK inhibitor (1 μM), the MEK-1 inhibitor U0126 (20 μM), or with the phosphatidylinositol 3-kinase (PI3K)/AKT inhibitor LY294002 (25 μM) for 1 hr. The cells were then stimulated or not with IL-7 (2 ng/ml) for 72 hr. Cells were collected and apoptosis was determined using Annexin V/7-aminoactinomycin D staining and flow cytometry analysis. The results are presented as mean percentages (± SE) of Annexin-V-positive cells from three independent experiments performed in triplicate with T cells isolated from different blood donors. $*P < 0{\cdot}05$ between IL-7- or IL-7 + LY294002- or IL-7 + U0126-treated samples and non-treated samples (no stimulation) or IL-7 + Pan-JAK-treated samples. $**P < 0{\cdot}05$ between LY294002-treated samples and non-treated or Pan-Jak- or U0126-treated samples. (b) IL-7-up-regulation of Bcl-2 proteins is abrogated by the Pan-JAK inhibitor. Cells were treated as described in (a) and the expression levels of Bcl-2 proteins were detected by immunoblot analysis. The results are representative of five independent experiments performed with T cells from different blood donors. (c) Densitometric quantification of Bcl-2 proteins in non-stimulated cells, versus cells stimulated with IL-7 and cells stimulated with IL-7 plus the different inhibitors as indicated. The results represent mean values (± SE) from three independent experiments and are expressed as the ratio between Mcl-1, Bcl-2 or Bcl-xL values and β-actin values. $*P < 0{\cdot}05$ between IL-7- or IL-7 + LY294002- or IL-7 + U0126-treated samples and -non-treated samples (medium) or IL-7 + Pan-JAK-treated samples.

of LY294002 also slightly reverses the protective effect of IL-7 (Fig. 4a) but statistical analysis indicated that the differences between IL-7 and IL-7 + LY294002 samples did not reach significance ($P = 0{\cdot}078$). Similarly and as shown by immunoblot and densitometric analysis, only the JAK/STAT inhibitor abrogated the capacity of IL-7 to up-regulate the pro-survival Bcl-2 proteins (Fig. 4b,c) suggesting that the IL-7 pro-survival function is mediated through the JAK/STAT pathway.

We then examined the ability of IL-7 to activate the JAK/STAT, PI3K/AKT and MAPK/ERK signalling pathways in effector/memory T cells. Interleukin-7 within 15 min of stimulation induced significant phosphorylation of STAT5, the major IL-7-activated STAT isoform, which remained detectable after 1 hr of stimulation with IL-7 (Fig. 5a). The presence of the Pan-Jak inhibitor completely abrogated the observed phosphorylation of STAT5 (Fig. 5a). The capacity of IL-7 to induce STAT5 phosphorylation is comparable to that of IL-2 (Fig. 5b). Conversely, we found that IL-7 is a weak activator of AKT and ERK phosphorylation in human effector/memory T cells (Fig. 5c). Extending the time of activation up to 6 hr or increasing the concentration of IL-7 for up to 10 ng/ml did not result in any further activation of AKT and ERK (data not shown). As a control, and in contrast to IL-7, cell stimulation with IL-2 induced significant phosphorylation of AKT and ERK1/2 kinases (Fig. 5c). Together these data indicate that the protective effect of IL-7 is dependent on the JAK/STAT5 pathway and is independent from the PI3K/AKT and MAPK/ERK signalling pathways in human effector/memory T cells.

Discussion

Elimination of activated T cells through apoptosis is a critical mechanism of immune homeostasis. Interleukin-7 is a crucial T-cell survival factor but its signalling in human T cells has been poorly addressed. In this study, we show that IL-7 protects human $CD4^+$ effector/memory T cells from apoptosis by activating the JAK/STAT signalling pathway and by up-regulating the levels of Bcl-xL, Bcl-2 and Mcl-1.

Apoptosis induced by lack of stimulation is regulated by Bcl-2 proteins and the mitochondrial death pathway.[2,6,7] Our results show that IL-7-induced effector/memory T-cell survival is associated with increased expression of anti-apoptotic proteins Bcl-2, Mcl-1 and

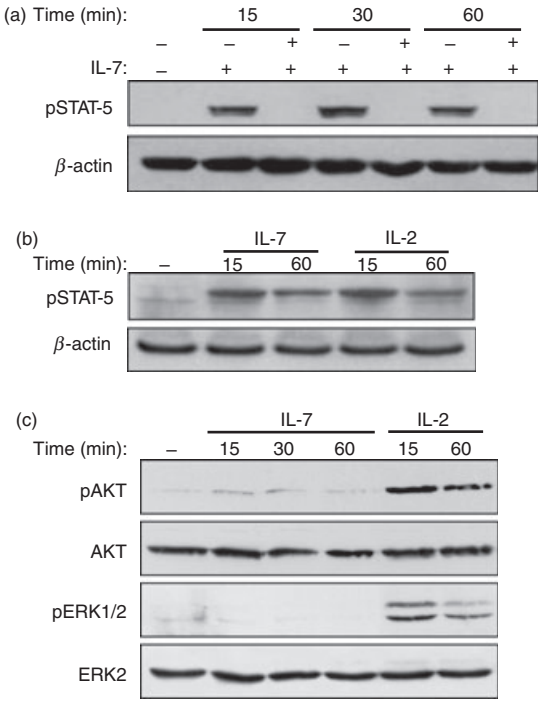

Figure 5. Activation of Janus kinase/signal transducer and activator of transcription (JAK/STAT), AKT and extracellular signal-regulated kinase (ERK) by interleukin-7 (IL-7) in human effector/memory T cells. (a) IL-7 activates the JAK/STAT signalling pathway. Human effector/memory T cells cultured under deprived conditions were stimulated or not with IL-7 (2 ng/ml) for different periods of time in the presence or absence of the Pan-JAK inhibitor. Non-stimulated cells (−) were maintained in medium alone for 1 hr. Activation of STAT5 was determined by immunoblot analysis using specific antibody that recognizes the phosphorylated form of STAT5. The blot was stripped and re-probed with anti-β-actin monoclonal antibody to ensure equal loading. (b) The cells were activated as in (a) with IL-7 or with IL-2 (50 U/ml) and STAT5 phosphorylation was determined by immunoblot analysis. (c) IL-7 is a weak activator of AKT and ERK in human effector/memory T cells. The cells were activated under deprived conditions with IL-7 for different periods of time. Non-stimulated cells (−) were maintained in medium alone for 1 hr. In some cases, the cells were also activated with IL-2 (50 U/ml) as indicated. AKT and ERK activation was determined by immunoblot analysis using antibodies that recognize the phosphorylated forms of AKT and ERK1/2. The blot was stripped and re-probed with anti-ERK2 and anti-AKT antibodies to ensure equal loading. The results in (a) and (c) and in (b) are respectively representative of five and three independent experiments performed with T cells from different blood donors.

Bcl-xL. Interleukin-7 has been reported to increase the expression of either Bcl-2 or Bcl-xL in human and murine naive T cells as well as in murine IL-7-dependent T-cell lines.[15,33–35] However, the up-regulation of Mcl-1 was observed only in a few studies.[36,37] Our study further extends the function of IL-7 to the regulation of all three major Bcl-2 proteins in human effector/memory T cells.

Our inhibition studies demonstrated that IL-7 protects effector/memory T cells from apoptosis by activating the JAK/STAT signalling pathway. Previous studies on IL-7 signalling in mouse naive T cells and IL-7-dependent T-cell clones indicated that IL-7 is connected to the JAK1/3-STAT5 and PI3K/AKT cell signalling pathways.[38–41] The IL-7-induced PI3K/AKT activation was implicated in cell survival mainly through the phosphorylation and inactivation of the Bcl-2 proapoptotic protein Bad [42,43] and through glucose uptake, which is necessary to maintain T-cell viability.[44] However, a recent study suggested that IL-7-induced survival of mouse naive T cells can rely solely on the up-regulation of Bcl-2 and can be independent from the PI3K/AKT pathway despite the fact that Bad is inactivated by AKT.[33] Our results showed that IL-7 is a weak activator of AKT in human effector/memory T cells and the use of specific inhibitors of the PI3K/AKT pathway did not abrogate the effect of IL-7 on cell survival and up-regulation of Bcl-2 pro-survival proteins. Hence, although activation of the PI3K/AKT pathway may contribute to IL-7 signalling and survival in certain mouse T-cell models, our study clearly showed that IL-7-induced survival of human effector/memory T cells is independent from the PI3K/AKT pathway. Interestingly, human effector/memory T cells express a basal level of phosphorylated AKT and treating them with the PI3K/AKT inhibitor slightly enhanced their apoptosis, suggesting that PI3K/AKT can be rather important in maintaining basal cell viability.

Interleukin-7 can also activate the MAPK/ERK signalling pathway as seen in immature thymocytes and acute T-cell leukaemia blasts.[41,45] However, our study indicates that IL-7 does not activate the MAPK/ERK in human effector/memory T cells and the use of the MEK-1/2 inhibitor did not abrogate the pro-survival effect of IL-7. Together these results indicate that IL-7 protects human effector/memory T cells from cytokine deprivation-induced apoptosis by activating the JAK/STAT signalling pathway independently from the PI3K/AKT and MAPK/ERK signalling pathways.

We have not addressed whether IL-7 regulates the function of pro-apoptotic proteins such as Bad and Bim, which are known to be inactivated through phosphorylation and subsequent degradation. Phosphorylation of Bim and Bad is a complex process involving multiple sites and different kinases. Recent evidence indicates that Bad can be phosphorylated by AKT, c-Raf/ERK kinases and other kinases as well,[46] whereas Bim seems to be inactivated mainly by MAPK/ERK.[47,48] In our cell model IL-7 does not activate the PI3K/AKT and MAPK/ERK pathways and IL-7-mediated cell survival is independent from these pathways; it is therefore unlikely that IL-7 regulates the phosphorylation of Bad or Bim through PI3K/AKT and MAPK/ERK. Notably, we found that Bad phosphorylation at serine-112, which has been reported to be regulated by

AKT,[43] c-Raf [49] or pim kinase [50] is not affected by IL-7 (data not shown). Whether IL-7 regulates Bim or Bad phosphorylation and function through other kinases and/or other mechanisms is unknown and warrants further investigation.

In addition to promoting the development and maintenance of memory T cells during protective immunity, IL-7 is also associated with inflammation and autoimmunity. High levels of IL-7 have been associated with T-cell activation and immunopathogenesis of rheumatoid arthritis and inflammatory bowel disease.[51–53] Given that $CD4^+$ effector/memory T cells are the cells that are associated with tissue damage in autoimmune diseases, it is conceivable that by enhancing cell survival, IL-7 contributes to the persistence of effector/memory T cells in the inflammatory sites, which will further exacerbate tissue damage and inflammation. The cells used in this study produce two major inflammatory cytokines including interferon-γ and the osteoclastogenic cytokine RANKL [28] and both cytokines are known to induce tissue inflammation and bone erosion in diseases like rheumatoid arthritis and inflammatory bowel disease. Therefore, our study suggests that targeting the JAK/STAT pathway and anti-apoptotic Bcl-2 proteins can be beneficial for these inflammatory diseases. A further understanding of IL-7 signalling in effector/memory T cells associated with autoimmunity may lead to the development of novel therapeutic strategies.

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Disclosures

Authors have no conflicts of interest.

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