Expression of Tcf-1 mRNA and surface TCR–CD3 complexes are reduced during apoptosis of T cells


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Abstract

When a T cell hybridoma, 70.7, was treated with a Ca\(^{2+}\) ionophore (A23187), apoptotic cell death was induced. Interestingly, we observed that the expression of Tcf-1, a T cell-specific transcription factor, mRNA was reduced by ~5-fold in the A23187-treated apoptotic cells compared to an ethanol-treated control. The hybridoma cells, however, did not display such a reduced expression of Tcf-1 mRNA upon treatment with buthionine sulfoxide, which is known to induce a necrosis-like cell death. When another T cell hybridoma, KMls-8.3.5, was treated with A23187 and phorbol myristate acetate, which leads to activation-induced apoptosis, Tcf-1 expression was again greatly reduced. However, a mutant line (KCIT1-8.5) derived from KMls-8.3.5, which produces IL-2 upon activation and is resistant to apoptosis, did not show such reduction in Tcf-1 expression. We also showed that the reduced expression level of CD3\(\varepsilon\) mRNA and surface TCR–CD3 complex in apoptotic T cells is caused by the reduced expression of Tcf-1. When 70.7 cells were transfected with a plasmid DNA pSVtcf-1, in which Tcf-1 gene expression is driven by the SV40 promoter, such reduction of the Tcf-1 mRNA and the surface expression of the TCR–CD3 complex were not observed upon apoptosis induction. Our results suggest that the reduced expression of Tcf-1 is specific for the apoptotic, but not for the activating, process of T cells and is also responsible for the reduced surface expression of the TCR–CD3 in apoptotic T cells.

Introduction

The Tcf-1 gene encodes a T lymphocyte-specific transcription factor which binds to the specific sequences in enhancers of various T lymphocyte-specific genes. The Tcf-1 gene was originally cloned through its binding affinity to the AACAAAG motif of the human CD3\(\varepsilon\) enhancer (1). It was later found that it could also bind to a moderately degenerate heptamer motif A/TA/TCAAAG in enhancer regions of other T cell-specific genes such as TCR \(\alpha\), \(\beta\), \(\delta\) and CD4 (2–4). The Tcf-1 gene is unique in that its expression is only detected in the T lineage cells (5,6). The pattern of Tcf-1 expression in different stages of thymocyte differentiation has been thoroughly characterized (7). Tcf-1 expression level is very low at the early stage of thymocyte development in double-negative (DN) thymocytes when CD3 and TCR gene expressions are also low. However, in immature single-positive (ISP) thymocytes, the Tcf-1 expression is at its highest level and declines thereafter, resulting in a slightly lower level in double-positive (DP) thymocytes. Later in development at the mature single-positive (MSP) stage, its expression is further reduced and the mature T cells in the periphery seem to have much lower levels of Tcf-1 mRNA (7). The results from the experiment with Tcf-1 knockout mice also suggest that Tcf-1 may play a role in T cell development (7). Thymocytes from these mice showed very scarce single-positive (SP) and DP thymocyte population. Instead, the majority of thymocytes from Tcf-1 knockout mice are at ISP and DN stages. Recently, it was reported that the Tcf-1 family proteins form a functional complex with \(\beta\)-catenin in normal embryonic cells or colon.
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carcinoma cells, suggesting that it may have a role in cell differentiation, proliferation and blocking of apoptosis (8–10).

The mechanism of positive/negative selections in the thymus has been a subject of intense studies. The positive selection of immature thymocytes seems to be related to the protection offered by Bcl-2 during apoptotic assault generated by the high concentration of glucocorticoid hormone (11). The negative selection in the thymus seems to result from the activation by ligation TCR, which eventually leads to apoptosis of immature thymocytes (12). In addition, mature T cells also go through cell death in the periphery as a way of regulating immune responses (13–15). Increase of intracellular Ca\(^{2+}\) concentration with various agents, including Ca\(^{2+}\) ionophores, has also been shown to cause apoptosis in immature thymocytes. Such a Ca\(^{2+}\)-dependent apoptosis seem to be mediated either by glucocorticoids (GC) or by a TCR-mediated pathway (16–19).

T cell hybridomas have been used as a valuable model system to study apoptosis in vitro, because the GC- or TCR-mediated apoptosis can be artificially induced in these cells (20–23). Activation-induced apoptosis in T cell hybridomas has been especially useful as a model for negative selection in the thymus and for extrathymic deletion of T cells in the periphery (20,21). From the studies utilizing this system, it now seems clear that the activation-induced apoptosis in the periphery is mediated by the up-regulation of Fas and Fas ligand (24), and involves an increase of intracellular Ca\(^{2+}\) level (17), which leads to the activation of endonuclease (12). The activation-induced apoptosis seems to be an important mechanism in terminating an immune response to a nominal antigen, thus maintaining cellular homeostasis. However, the detailed cellular processes in apoptotic T cells have not been thoroughly investigated. For example, it is not yet known whether a T cell can be reactivated by the same activating agent even after it enters the process of the activation-induced apoptosis. In addition, the state of the surface expression of the TCR–CD3 complexes in apoptotic T cells is not known. Often, the T cell unresponsiveness is related to the down-modulation of its surface receptor level (25–27) for the TCR gene or 1.2 kb EcoRI fragment of the CD3ε gene as a probe. The Tcf-1 clone, DNA sequencing and computer analysis

Genes expressed preferentially in thymus were isolated from the thymic cDNA library by subtractive hybridization as described elsewhere (28). The nucleotide sequence of the cloned genes were determined by dideoxy chain termination method (29) using Sequenase 2.0 kit (United States Biochemical, Cleveland, OH). Comparisons of the nucleotide sequence with sequences in GenBank were performed at the National Center for Biotechnology Information, using the BLAST network service (30).

A full-length cDNA clone containing the Tcf-1 coding sequence was selected from the cDNA library. A 2.6 kb EcoRI fragment containing the full-length coding sequence of the Tcf-1 gene was inserted into the pZeoSV vector (Invitrogen, San Diego, CA) and designated as pSVtcf-1. The plasmid construct was transfected into the 70.7 cells by electroporation. Zeocin-resistant cells were selected and maintained with 500 µg/ml Zeocin (Invitrogen) in RPMI-1640 supplemented with 10% FBS.

Induction and measurement of apoptosis

Apoptosis of T cell hybridomas and T lymphoma was induced as follows. The 70.7 T hybridoma cells (10⁵ cells/ml) were treated with A23187 to a final concentration of 1 µM for 24 h or cultured on anti-CD3 antibody (145-2C11)-coated plates for 48 h. KMls-8.3.5, KCit1-8.5 and EL4 cells were treated with either 12 nM PMA plus 500 nM A23187 or 10 µM hydrocortisone dissolved in DMSO. Apoptosis was induced by incubating the cells with these reagents for 24–72 h. To induce necrosis-like cell death, cells were treated with 100 µM BSO for 24 h. The extent of apoptosis was estimated by measuring the degree of DNA fragmentation as described by Nicoletti et al. (31).

Northern blot analysis

Total cellular RNA was extracted by the method described by Chomczynski and Sacchi (32). The isolated RNA (10 µg) was separated on 1.2% agarose gel, transferred onto nitrocellulose and hybridized with the 32P-labeled 1.3 kb XhoI fragment of Tcf-1 gene or 1.2 kb EcoRI fragment of the CD3ε gene as a probe.

Flow cytometry

To measure the surface expression levels of the TCR complex on T cell hybridomas and T lymphoma, cells were stained with PE-conjugated anti-εTCR antibody (H57-597). The labeled
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Results

Reduced expression of Tcf-1 in apoptotic T cell hybridoma

To determine the effect of apoptosis induction on the expression of Tcf-1, we first analyzed mRNA level of Tcf-1 in T cell hybridomas undergoing apoptosis. The T cell hybridoma, 70.7, was treated with a Ca2+ ionophore, A23187, to induce apoptosis. As shown in Fig. 1(A), A23187 treatment of the 70.7 cells resulted in extensive DNA ladder formation, suggesting that the cells are going through an active apoptotic process. Northern blot analysis with total RNA isolated from these cells showed that the level of Tcf-1 mRNA was reduced by ~5-fold in A23187-treated 70.7 cells compared to the control group treated with ethanol alone (Fig. 1B). We were also able to induce apoptosis by treating the 70.7 cells with anti-CD3 antibody (145-2C11) and have observed a similar reduction in Tcf-1 expression (data not shown). A time-course analysis of the DNA fragmentation and Tcf-1 mRNA reduction is shown in Fig. 2. DNA fragmentation ladders were easily observed as early as 6 h after the A23187 treatment (Fig. 2A), and the Tcf-1 mRNA level was also reduced to 60–70% of the control at this time point and to the level of ~35% of the control by 18 h after the treatment (Fig. 2B). These results suggest that the reduction of Tcf-1 mRNA is a rather early event in the apoptotic process of the 70.7 cells. The reduction of Tcf-1 mRNA was not due to the non-specific degradation of RNA in dying cells because of the following reasons. Firstly, it was shown that the β-actin mRNA was shown to be reduced only to ~75% of the control by 24 h after the treatment when the same blot was re-probed with its 32P-labeled cDNA fragment (Fig. 2B). Secondly, the TATA binding protein (TBP) mRNA level was increased to ~3-fold after the apoptosis induction when the same blot was hybridized with 32P-labeled cDNA probe (Fig. 2B). Therefore, we concluded that the reduction in Tcf-1 mRNA level in apoptotic T cell hybridoma is not due to a non-specific degradation but a specific phenomenon occurring at a relatively early time point of apoptotic process.

Reduced level of the Tcf-1 mRNA level is specific to apoptosis

Even though the reduction of the Tcf-1 mRNA observed during apoptotic cell death was not due to the non-specific degradation of RNA in dying cells, it was shown that the β-actin mRNA was shown to be reduced only to ~75% of the control by 24 h after the treatment when the same blot was re-probed with its 32P-labeled cDNA fragment (Fig. 2B). Secondly, the TATA binding protein (TBP) mRNA level was increased to ~3-fold after the apoptosis induction when the same blot was hybridized with 32P-labeled cDNA probe (Fig. 2B). Therefore, we concluded that the reduction in Tcf-1 mRNA level in apoptotic T cell hybridoma is not due to a non-specific degradation but a specific phenomenon occurring at a relatively early time point of apoptotic process.
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degradation of RNA, it is questionable whether the reduction of the Tcf-1 messages can also be induced by a necrosis-like cell death. To test this possibility, the T cell hybridoma cells were treated with BSO, a chemical known to induce a necrosis-like cell death in neuronal cells (33). In contrast to the A23187-treated cells, BSO-treated cells do not show any sign of DNA fragmentation, even though the results from the Trypan blue exclusion assay suggested that >50% of the BSO-treated cells were dead at the same time point (data not shown). When the mRNA levels of the treated cells were compared, the BSO-treated cells showed only a slight reduction in the Tcf-1 mRNA level (Fig. 2B). Furthermore, the mRNA levels of Tcf-1, β-actin and TBP genes in the BSO-treated cells all showed a similar degree of reduction (77, 85 and 83% of ethanol-treated controls respectively). These are in sharp contrast to the results obtained from the A23187-treated cells, in which the Tcf-1 and TBP mRNA levels changed dramatically. These data strongly suggest that the reduction of Tcf-1 mRNA and the increase of TBP mRNA are specific to apoptotic T cells.

Since we used a Ca²⁺ ionophore, A23187, to induce apoptosis, it is possible that the reduction in Tcf-1 mRNA levels in A23187-treated cells may be due to the indirect effects of the increase in intracellular Ca²⁺ concentration, rather than due to the apoptotic process itself. It may also be the activation process, but not the apoptotic process of T cell hybridoma that causes the changes of the mRNA levels of the Tcf-1 genes. To test these possibilities, a pair of T cell hybridomas, KMls-8.3.5 and KCIT1-8.5 (34), was employed. The KMls-8.3.5 cells enter into the apoptotic pathway after treatment with PMA plus Ca²⁺ ionophore (A23187). The KCIT1-8.5 cell, a mutant line derived from KMls-8.3.5, is resistant to such an activation-induced apoptosis when treated with PMA and Ca²⁺ ionophore, even though it is able to produce IL-2 and proliferate. It has been shown that the KCIT1-8.5 cell line has a normal signaling pathway for activation; however, a pathway to induce apoptosis after activation is blocked (34). The KCIT1-8.5 cell, on the other hand, is more sensitive to GC-induced apoptosis than the KMls-8.3.5 cell (34). After PMA and A23187 treatment, the expression of Tcf-1 mRNA was not significantly changed in KCIT1-8.5 cells, in which apoptosis was not induced by this treatment (Fig. 3). In contrast, its parental line, KMls-8.3.5, showed massive DNA fragmentation by the same treatment (data not shown) and, more importantly, the expression level of the Tcf-1 mRNA was significantly reduced. With GC treatment, the expression of Tcf-1 mRNA was reduced in both cell lines. However, the KCIT1-8.5 cell line, which is known to be more sensitive to GC-induced apoptosis than the KMls-8.3.5 cell (34), expressed much less Tcf-1 mRNA than its parental cell line (Fig. 3). These results suggest that the reduced expression of the Tcf-1 mRNA in T cell hybridomas treated with A23187 and PMA is not due to the indirect effect of Ca²⁺ or the activation pathway itself, but specifically related to the apoptotic pathway.

Down-regulation of the surface TCR after A23187 treatment

The CD3ε protein is a component of the multiprotein complex of TCR–CD3 on the T cell surface. Tcf-1 is known to bind to the enhancers of various T cell specific genes, including the CD3ε gene. Therefore, it is expected that the reduced expression of the Tcf-1 gene following apoptotic stimuli affects the expression of CD3ε in these cells, resulting in the reduced surface expression of the TCR-CD3 complex. To test this possibility, the expression level of the CD3ε messages in apoptotic cells was measured by Northern blot analysis. In A23187-treated 70.7 cells, the expression of the CD3ε mRNA was reduced to ~60% of normal cells, as was Tcf-1 mRNA (Fig. 4). We then examined the surface levels of the CD3ε protein in apoptotic cells. As expected from the Northern blot analysis, the apoptotic cells induced by either A23187 or anti-CD3 antibody treatment express lower levels (~60% of normal level) of surface TCR–CD3 complex compared to the normal ones (data not shown). In addition, the surface level of the CD4 protein was also reduced on apoptotic cells (data not shown). The expression patterns of the TCR-CD3 complex

Fig. 3. Down-regulation of the expression of the Tcf-1 mRNA is specific to apoptosis. KMls-8.3.5 and the KCIT1-8.5 cells were treated with 12 nM PMA plus 500 nM A23187 (P + I) or 10 μM hydrocortisone alone (GC). The results show that the combined treatment with PMA and A23187 reduced the Tcf-1 mRNA expression to 12% of the non-treated control in KMls-8.3.5. However, the expression of the Tcf-1 mRNA was changed much less in KCIT1-8.5 cells (72%) compared to its parental cell line. When GC was treated, KCIT1-8.5 cells expressed much less Tcf-1 mRNA (25%) than its parental cell line, KMls-8.3.5 (51%). Quantitated results were obtained from three independent experiments of Northern blot analysis.

Fig. 4. Down-regulation of CD3ε mRNA expression in apoptotic T cell hybridomas. Northern hybridization was performed using the 1.2 kb EcoRI fragment of the murine CD3ε gene as a probe. Each lane was loaded with the same amount of total RNA (10 μg). Lane 1, normal 70.7 cells; lane 2, apoptotic cells treated with A23187.
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Fig. 5. The expression of the TCR–CD3 complex in KMls-8.3.5 (A) and KCIT1-8.5 cells (B). The level of surface expression of the αβ TCR on KCIT1-8.5 cells (B, dashed line) is similar to the parental cell line KMls-8.3.5 (A, dashed line). The surface level of TCR in KCIT1-8.5 cells was not changed by apoptosis induction (B, solid line). In contrast, the surface expression level of TCR in KMls-8.3.5 cells was significantly reduced after apoptosis induction (A, solid line). Total cells were stained with PE-conjugated anti-αβ TCR mAb, H57-597, and analyzed using a FACStar.

Discussion

Programmed cell death (PCD) is defined as a cell suicide process characterized by a specific series of intracellular events leading to DNA fragmentation in multiples of 200 bp, which is commonly exploited as an indicator of the process. PCD is an active process since it requires protein synthesis as well as membrane and organelle integrity. PCD seems to play a central role in developmental processes. Most thymocytes die by apoptosis during development due to negative selection or lack of positive selection. In the periphery, immune homeostasis is maintained by a balance between the generation of new lymphocytes, antigen-induced lymphocyte proliferation and differentiation, and apoptotic cell death. Lymphocytes are known to undergo apoptotic cell death either due to deficiencies of growth factors or as a consequence of repeated stimulation (35–37). In particular, T

on KMls-8.3.5 and KCIT1-8.5 cells were also investigated after treatment with PMA and A23187 (Fig. 5). The reduction of surface expression of the TCR–CD3 complex on KMls-8.3.5 cells was apparent after the treatment (Fig. 5A). In contrast, the surface expression of αβ TCR on KCIT1-8.5 cells, which express similar levels of surface TCR as the parental cell line (34), was not changed by apoptosis induction (B, solid line). Total cells were stained with PE-conjugated anti-αβ TCR mAb, H57-597, and analyzed using a FACStar.

These data clearly suggest that only those cells undergoing the apoptotic process express the reduced level of surface TCR–CD3 complexes. However, it was still uncertain if mature T lymphocytes would also display similar reduction in the surface expression of TCR–CD3 complexes upon apoptosis induction since the T cell hybridomas we used were obtained by fusing peripheral mature T cells with thymoma cells. For this, we have tested whether the expression of Tcf-1 and surface TCR-CD3 levels are also reduced in a mature T cell lymphoma, EL4, upon apoptosis induction (Fig. 6). When apoptosis is induced in EL4 cells by treatment with PMA and A23187, the expression of the Tcf-1 mRNA was significantly reduced and the surface level of TCR–CD3 was also down-regulated. Therefore, it seems that reduced expression of Tcf-1 and the surface TCR–CD3 complex upon apoptosis induction does occur in mature peripheral T lymphocytes.

It is quite likely that the reduced expression of Tcf-1 upon apoptosis induction results in the reduced level of expression of CD3ε (and other proteins such as CD4 and TCR α, etc.), which eventually lead to the reduced surface expression of the TCR–CD3 complex. To directly test this possibility, a plasmid DNA pSVtcf-1, in which the expression of the Tcf-1 gene is driven by the SV40 promoter, was constructed and transfected into the 70.7 cells. After treatment with A23187, the level of Tcf-1 mRNA was not significantly changed in the pSVtcf-1 transfectants (Fig. 7A), even though they showed profound DNA fragmentation indicating that these cells were in the apoptotic process (data not shown). To test whether the surface expression of the TCR–CD3 complex is affected by the expression level of Tcf-1, the pSVtcf-1 transfected and non-transfected cells were analyzed for surface TCR–CD3 expression after apoptosis was induced. The surface expression of αβ TCR in pSVtcf-1 transfectants was not changed, while that of parental 70.7 cells was dramatically reduced by the induction of apoptosis (Fig. 7B and C). To exclude the possibility that the results were due to a clonal variation, we have also performed the same experiment using transiently transfected cells with pSVTcf-1. The results were essentially similar to those obtained from the long term-transfected cells. Transient transfected cells seemed to show slight variations among cells in the level of surface TCR, probably due to the differences in transfection efficiency. However, the surface TCR on the transiently transfected cells were maintained at similar levels to non-treated controls but significantly higher levels than non-transfected controls (Fig. 7D). The results strongly suggest that the reduced surface expression of the TCR–CD3 complex during apoptosis is primarily caused by the reduction in the expression level of the Tcf-1 gene.
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Fig. 6. Expression of Tcf-1 mRNA and surface TCR level are reduced during apoptosis of T cell lymphoma, EL4. The cells were treated with 12 nM PMA plus 500 nM A23187 for 24 h. (A) After treatment with PMA and A23187 for 24 h, the total RNA of EL4 cells was isolated and analyzed by Northern blot analysis using the 32P-labeled 1.3 kb XhoI fragment of the Tcf-1 gene as a probe. The same blot was re-probed with the β-actin cDNA fragment as a control. Lane 1, no treatment; lane 2, PMA and A23187-treated cells. (B) Expression of surface TCR on normal (dashed line) and apoptotic cells (solid line). Total cells were stained with PE-conjugated anti-αβ TCR mAb, H57-597, and analyzed using a FACStar.

cells undergo activation-induced cell death as a result of repeated antigenic stimulation. This phenomenon has been regarded as a means to terminate an immune response (38,39). Therefore, apoptosis is important not only for the shaping the immune system, but also for controlling the size of antigen-stimulated clones.

T cell hybridomas undergo apoptosis after activation with various stimuli, including specific antigens, mitogens and antibodies against the TCR–CD3 complex, or a combined treatment of phorbol ester and calcium ionophore (24,33,34,40). The system has been widely used to investigate the mechanism for clonal deletion in the immune system, in which self-reactive T cells are eliminated by apoptosis after activation, either in the thymus or in the periphery. This activation-induced cell death, which also maintains cellular homeostasis after an immune response to an antigen, requires de novo synthesis of RNA and proteins. For example, CD4 T cells are known to express Fas ligand, a death-gene product, during the process of activation-induced cell death (24,41,42).

In this paper, we have shown that the expression of Tcf-1 and its target gene CD3ε was significantly reduced by apoptosis induction in T cells. It seems likely that the expression levels of TCR α and β chains are also reduced in these apoptotic T cells. However, cells undergoing a necrosis-like cell death after treatment with BSO did not show such a dramatic reduction in the expression of Tcf-1. Furthermore, a mutant hybridoma cell line KClT1-8.5, which can be activated to produce IL-2 and proliferate by anti-TCR mAb or PMA plus Ca2+ ionophore treatment but is resistant to apoptosis following the activation, did not show any significant decrease in expression of the Tcf-1 mRNA. Therefore, the decrease in the Tcf-1 expression is specific to the apoptotic process but not to necrosis-like cell death and the activation process itself. Interestingly, in contrast to the Tcf-1 gene, the expression of TBP is increased by the induction of apoptosis with A23187. It is not clear why TBP mRNA expression increases in apoptotic cells.

One important consequence of reduced expression of Tcf-1 during apoptosis appeared to be the reduced surface expression of the TCR–CD3 complex. This is supported by our results showing that such a reduced expression of surface TCR was not evident in apoptotic T hybridoma cells transfected with pSVtcf-1, in which the expression of Tcf-1 cDNA is driven by the SV40 promoter and, therefore, the Tcf-1 expression level remained unchanged by apoptosis induction. It has been known that surface TCR is down-modulated by activating T cells due to the internalization of the receptor complex (43). Ligand-induced TCR–CD3 complex internalization was shown to happen at a relatively early stage of T cell activation and to be mediated by endocytosis via clathrin coated pits (44,45). The TCR internalization is mediated by protein kinase C (45) and requires a p56lck activity (46). We think that the down-regulated expression of TCR–CD3 in apoptotic T cells occurs by a different mechanism from the one observed during the activation process. Our results strongly suggest, for the first time, that surface TCR–CD3 is down-expressed in apoptotic T cells and that it is due to the reduced expression of Tcf-1, a major regulator for the transcription of CD3ε, TCR α and β. However, it has been reported that the surface expression of TCR–CD3 was up-regulated during thymocyte apoptosis (47). The reason for the discrepancy between the two results is not clear, but it may be due to the fact that cells at different stages of differentiation were used in each experiment. Kishimoto et al. (47) used CD4+CD8+ thymocytes expressing low levels of the surface TCR–CD3. They have shown that only a part, not all, of the apoptotic thymocytes expressed higher levels of surface TCR–CD3. Therefore, it seems possible that cells in different stages of differentiation may respond differently to
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Fig. 7. Surface expression of the TCR during the apoptotic process of the T cell hybridoma is affected by Tcf-1 expression. The 2.6 kb EcoRI fragment of the Tcf-1 gene was inserted into the pZeoSV vector (Invitrogen) for the expression of Tcf-1 under the control of the SV40 promoter (pSVtcf-1) and transfected into the 70.7 cells by electroporation. The transient transfected (D) or the selected clone (C) by resistance to 500 µg/ml Zeocin were induced to apoptose with A23187 treatment, stained with PE-conjugated anti-αβ TCR mAb, H57-597, and analyzed using a FACStar. (A). Northern blot analysis of Tcf-1 expression of 70.7 (lanes 1 and 2) and pSVtcf-1 transfectants (lanes 3 and 4). Lanes 1 and 3, control (no treatment); lanes 2 and 4, A23187. (B–D) Surface expression of TCR on 70.7 (B) and pSVtcf-1 cells (C and D). In contrast to the 70.7 cells (B), the pSVtcf-1 transfectants (C and D) did not show any significant reduction of surface TCR-CD3 proteins. Dashed lines indicate control (no treatment) and the solid lines indicate TCR-CD3 expression levels of the cells treated with A23187.

The reduced expression of Tcf-1 may be a fortuitous phenomenon in apoptotic T cells. However, the active process of down-regulation of Tcf-1 may confer an important nature to apoptotic T cells, especially by lowering the level of surface TCR-CD3 complexes. The reduced expression of surface TCR is known to affect the effectiveness of antigenic stimulation. It was reported that the mAb-induced surface modulation of the TCR-CD3 complex causes T cell unresponsiveness by inhibiting the early metabolic events that follow receptor-ligand interactions (48). It was also reported that the density of TCR on T cells is an important parameter in driving T cell activation together with the ligand concentration on the surface of antigen-presenting cells by contributing to the TCR–APC avidity (26,27). There also have been reports that anergic T lymphocytes in the periphery express lower levels of surface TCR than the normal T lymphocytes (25,49). All these results indicate that the level of surface TCR is a critical parameter in driving T cell activation and T cells become unresponsive or anergic to an antigen by down-regulating the surface level of TCR. Our results showed that TCR-CD3 complex expression on a mature T lymphoma, EL4, as well as on T cell hybridomas was down-regulated by apoptosis induction. This may suggest that once antigen-activated T cells in the periphery enter into the apoptotic process, the cells express...
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A reduced level of surface TCR due to the down-regulation of the Tcf-1 mRNA and become less sensitive to the same antigen. Since the death of activated T cells is known to be an important way of regulating immune responses, down-regulation of Tcf-1 may be a supporting mechanism for regulating immune responses by rendering the apoptotic T cells unresponsive to re-stimulation by the antigen.

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