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Human Naive CD8 T Cells Down-Regulate Expression of the WNT Pathway Transcription Factors Lymphoid Enhancer Binding Factor 1 and Transcription Factor 7 (T Cell Factor-1) following Antigen Encounter In Vitro and In Vivo

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The transcription factors lymphoid enhancer binding factor 1 (LEF1) and transcription factor 7 (TCF7) (T cell factor-1 (TCF-1)) are downstream effectors of the WNT signaling pathway, which is a critical regulator of T cell development in the thymus. In this study, we show that LEF1 and TCF7 (TCF-1) are not only expressed in thymocytes, but also in mature T cells. Our data demonstrate that Ag encounter in vivo and engagement of the TCR or IL-15 receptor in vitro leads to the down-regulation of LEF1 and TCF7 (TCF-1) expression in human naive CD8 T cells. We further show that resting T cells preferentially express inhibitory LEF1 and TCF7 (TCF-1) isoforms and that T cell activation changes the isoform balance in favor of stimulatory TCF7 (TCF-1) isoforms. Altogether, our study suggests that proteins involved in the WNT signaling pathway not only regulate T cell development, but also peripheral T cell differentiation.

The differentiation of T lymphocytes is mainly controlled by signals through their Ag-specific TCR. In the thymus, TCR signals regulate the development of a mature T cell repertoire via positive and negative selection. In the periphery, naive T cells (T_N) further differentiate into effector and memory T cells when recognizing foreign Ags derived from infectious pathogens via their TCR (1). Apart from TCR signals and cytokines, other factors contributing to peripheral T cell differentiation are not well characterized at present.

In addition to TCR signals, major developmental pathways such as the WNT, Notch, and Hedgehog signaling pathways influence T cell development (2–4). Interestingly, there is recent evidence that these major developmental pathways also control the differentiation of peripheral T cells. Thus, Notch regulates the decision of CD4 T cells between the Th1 vs the Th2 fate (5), while the Hedgehog pathway can influence the proliferation and cytotoxic production of human peripheral CD4 T cells (6, 7). In contrast, it is currently unknown whether the WNT pathway has any role in mature T cells.

The canonical WNT signaling pathway is a critical regulator of stem cell function, e.g., it controls the maintenance and self-renewal of hemopoietic stem cells (8–11). Furthermore, dysregulation of the WNT pathway commonly occurs in human cancers (11). In the absence of a WNT signal, cytoplasmic β-catenin is phosphorylated and targeted for degradation by the proteasome (3, 11). WNT signaling allows β-catenin to escape proteasomal degradation and to translocate to the nucleus. In the nucleus, β-catenin interacts with members of the lymphoid enhancer binding factor (LEF)/T cell factor (TCF) family of transcription factors (LEF1, TCF7 (TCF-1), TCF7L1 (TCF-3), and TCF7L2 (TCF-4)) to activate the transcription of WNT target genes such as c-myc and cyclin D1 (11). In the absence of WNT signaling, i.e., when not interacting with β-catenin, LEF/TCF family members act as transcriptional repressors by recruiting Groucho repressor proteins (12).

LEF1 and TCF7 (TCF-1) share common protein motifs, in particular, the C-terminal HMG domain of both proteins is responsible for DNA binding, while a β-catenin-binding domain at the N terminus mediates the interaction with β-catenin (12). Interestingly, there are multiple LEF1 and TCF7 (TCF-1) protein isoforms with distinct functional properties (13–17). In addition to the stimulatory full-length isoforms, there are N-terminally truncated isoforms that are without the β-catenin-binding domain (referred to as ΔCTNNB) but retain the ability to interact with Groucho repressors (12). Importantly, these truncated isoforms can function in a dominant-negative manner in the WNT signaling pathway as has been demonstrated for ΔCTNNB isoforms of LEF1 (17, 18), TCF7 (TCF-1) (19, 20), and the Xenopus homolog of LEF1/TCF7 (TCF-1) (21). Finally, there are also LEF1 and TCF7 (TCF-1) isoforms with alternative C-termini (termed tails) known as N- or B-tailed isoforms. Currently, very little is known about the different functional properties of these multiple LEF1 and TCF7 (TCF-1) isoforms in T cells.
Knockout and transgenic studies in mice have clearly shown a redundant and β-catenin-dependent role of LEF1 and TCF7 (TCF-1) in T cell development (3). TCF7 (TCF-1)−/− mice have impaired T cell development with a partial block at the intermediate single-positive to double-positive transition due to reduced thymocyte proliferation and survival (22–24). Although T cell development is normal in LEF1−/− mice, B cell development is impaired (25). Thymocytes from TCF7 (TCF-1)−/− LEF1−/− mice show a profound block at the intermediate single-positive stage with neither double-positive nor single-positive thymocytes present, and consequently no mature T cells in the periphery (26).

Taken together, the WNT-β-catenin-LEF1/TCF7 (TCF-1) axis plays a pivotal role in T cell development. However, it is unknown whether LEF1, TCF7 (TCF-1), and the WNT pathway have a specific function in peripheral T cells.

Therefore, we undertook a detailed analysis of the expression of LEF1 and TCF7 (TCF-1) in human peripheral T cells. We found that both LEF1 and TCF7 (TCF-1) are expressed in mature CD8+ T cells and that their expression is down-regulated following TCR or IL-15R engagement in vitro and Ag encounter in vivo. Furthermore, T cell activation changed the balance of stimulatory vs inhibitory LEF1 and TCF7 (TCF-1) isoforms. Our results suggest that the WNT pathway, in addition to its well-known role in T cell development, is likely to be involved in regulating peripheral T cell differentiation.

Materials and Methods

Isolation of CD8 T cell subsets

For microarray and quantitative RT-PCR (qRT-PCR) experiments, CD8 T cell subsets were isolated from healthy donors in accordance with institutional ethics approval as previously described (27). CD8 T cells were sorted using magnetic beads (Streptavidin Dynabeads, Dynal) and a surface marker (CD25+) and effector memory RA T cells (TEMRA) were sorted using the 7H3 mAb (Upstate Biotechnology) at a 1/1000 dilution, respectively. Briefly, 2 µg of primary mAb was added to 2 × 10^6 cells and incubated for 1 h at room temperature. After washing, this was followed by staining with PE-conjugated rabbit anti-mouse Ab (DakoCytomation) for 1 h at room temperature. Cells were subsequently washed in blocking buffer (PBS containing 2% mouse serum) before surface staining with directly conjugated mAbs specific for CD62 ligand (CD49d, CD45RA, and CD8 (all BD Biosciences)). CD62L was used instead of CCR7 as a surface marker because CCR7 staining was compromised following methanol permeabilization (27).

Stimulation of cord blood (CB) CD8 T cells in vitro

We obtained CB samples from the John Radcliffe Hospital maternity unit, upon written consent and approval by the local Medical Ethics Committee. CB CD8 T cells were isolated by immunomagnetic selection as described above. Phenotyping was conducted using mAbs specific for CCR7 (R&D Systems), CD45RA (BD Biosciences), CD8 (BD Biosciences), CD3, CD25, and HLA class II (all DakoCytomation). We stimulated CB CD8 T cells (1 × 10^6/ml) with either plate-bound anti-CD3 mAb OKT3 (1 µg/well), IL-15 (50 ng/ml), or TGFβ1 (3 ng/ml) in 24-well plates for the indicated time points. All cytokines were from obtained R&D Systems. Cells were cultured in complete medium (RPMI 1640 supplemented with 10% FCS, 1% sodium pyruvate, 2 mM l-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin) at 37°C in 5% CO₂. Alternatively, CD8 T cells (1 × 10^5) were cocultured with irradiated allogeneic EBV-transformed B cells at a 1:1 ratio. From day 3 onward, CD3⁺ cells represented >95% of live cells in these cocultures, i.e., the stimulator B cells had practically disappeared.

Cloning of LEF1 mRNA isoforms from primary CD8 T cells

Total RNA was extracted from primary CD8 T cells using TRI Reagent (Sigma-Aldrich) and cDNA was prepared as described above for the qRT-PCR experiments. RT-PCR was performed with the following primers:

- forward primers, CAGCGGAGCTGCTGATCAAGAG (full-length isoforms) and ACTCGAGCTTCCTGACTGATGAA (ΔCTNNB isoforms);
- reverse primers, CCTCGAATTCCTACATGCGAG (N-terminal isoform) and GTCGAAATTCCTACATATGCGAG (C-terminal isoform).

PCR products were cloned into the vector pRES2-EGFP (BD Clontech) followed by DNA sequencing.

Western blot analysis

We prepared protein lysates from ~3 to 5 × 10⁶ CD8 T cells by washing the cells in PBS and resuspending them in an equal volume of 2× sample buffer (100 mM Tris-HCl (pH 6.8), 4% SDS, 8% 2-ME, 0.2% bromphenol blue, and 20% glycerol). After sonication for 2 × 20 s and boiling for ~10 min, protein samples were separated by SDS-PAGE and gels blotted to nitrocellulose membranes. We performed immunodetection of LEF1 and TCF7 (TCF-1) with the REMB6 mAb (Exalpha) at a 1/500 dilution and with the TH3 mAb (Upstate Biotechnology) at a 1/1000 dilution, respectively. This was followed by incubation with secondary HRP-conjugated anti-mouse Ig (DakoCytomation) and signal detection with ECL reagent (BD Amersham). Blots were stripped by incubating the membrane at 50°C for 30 min in stripping buffer (62.5 mM Tris-HCl (pH 6.7), 2% SDS, and 100 mM 2-ME) and reprobed with anti-β-actin mAb AC-15 at a 1/5000 dilution (Sigma-Aldrich).

Statistical analysis

A two-sample, two-tailed t test assuming unequal variances was used to determine the statistical significance of differences in mRNA expression between the groups (α = 0.05). For multigroup comparisons, we applied one-way ANOVA with post hoc testing using Tukey’s significant difference test (α = 0.05).
Results

CD8\(^+\) T\(_N\) down-regulate expression of LEF1 and TCF7 (TCF-1) upon Ag encounter in vivo

We used microarray technology to screen for genes that are differentially expressed between human T\(_N\) and Ag-primed CD8 T cells. Our first, exploratory, microarray data set compared gene expression in purified CD8\(^+\) T\(_N\) (CCR7\(^+\)CD45RA\(^-\)) and CD8\(^+\) T\(_{EM/EMRA}\) (CCR7\(^-\)CD45RA\(^+\)) populations using RNA pooled from several donors. We also generated a more detailed second data set that analyzed the gene expression profiles of CD8\(^+\) T\(_N\) (CCR7\(^+\)CD45RA\(^-\)) in relation to that of CD8\(^+\) T\(_{CM}\) (CCR7\(^-\)CD45RA\(^-\)), T\(_{EM}\) (CCR7\(^-\)CD45RA\(^+\)), and T\(_{EMRA}\) (CCR7\(^-\)CD45RA\(^+\)) subsets. Technical advances allowed us to use RNA from individual donors instead of pooled RNA and to perform a greater number of replicate experiments (four instead of two) for the second data set. Furthermore, gene chips (HG-U133 Plus 2.0 instead of HG-U95Av2) with a greater number of probes and greater coverage of the human genome were available for this data set. Using this data set, we have previously investigated the molecular relationships between T\(_N\) and memory phenotype (Ag-primed) CD8 T cell subsets and the molecular basis for their different functional properties (27). We now aimed to examine selected differentially expressed genes in more detail to gain further insight into the molecular basis of CD8 T cell behavior.

We identified LEF1 as a gene highly expressed in T\(_N\), compared with Ag-primed CD8 T cell subsets. In both microarray data sets, LEF1 was found to be in the top three most differentially expressed genes when comparing the different mature CD8 T cell populations (Table I). Indeed, within data set 2, probe identifications for LEF1 accounted for two of the top three differentially expressed genes. LEF1 mRNA levels were 5- to 10-fold higher in T\(_N\) compared with the three Ag-experienced subsets, with T\(_{CM}\) expressing LEF1 more strongly than T\(_{EM}\) and T\(_{EMRA}\) (Fig. 1A). Interestingly, we found that TCF7 (TCF-1) was also expressed at higher levels in T\(_N\), compared with T\(_{CM}\), T\(_{EM}\), and T\(_{EMRA}\) (Fig. 1B).

qRT-PCR experiments confirmed the differential expression of LEF1 and TCF7 (TCF-1) in T\(_N\) and Ag-primed CD8 T cell subsets (Fig. 1). We also examined LEF1 and TCF7 (TCF-1) protein expression in peripheral CD8 T cell subsets by intracellular flow cytometry. Importantly, as shown in Fig. 2, T\(_N\) (and T\(_{EM}\)) expressed significantly higher levels of both LEF1 and TCF7 (TCF-1) protein than T\(_{EM}\) and T\(_{EMRA}\) (p < 0.05, one-way ANOVA/Tukey’s post hoc test). In summary, we found that the WNT pathway effectors LEF1 and TCF7 (TCF-1) are expressed in peripheral T cell subsets and that LEF1 is the most differentially expressed transcription factor between T\(_N\) and Ag-primed CD8 T cell subsets.

Expression of LEF1 and TCF7 (TCF-1) in CD8\(^+\) T\(_N\) is down-regulated by TCR signals and IL-15 in vitro

We studied the regulation of LEF1 mRNA expression in peripheral CD8 T cells in vitro by qRT-PCR. We observed down-regulation of LEF1 mRNA expression in CD8 T cells in response to TCR triggering (Fig. 3A). This down-regulation was rapid (within 12 h) and persisted for >48 h. Stimulation with homeostatic cytokines, such as IL-15, also led to a persistent decrease in LEF1 mRNA levels (Fig. 3B), with IL-2 having a similar effect (data not shown). In contrast, stimulation with TGF\(_{B}\) increased LEF1 expression in CD8 T cells, compared with the medium control (Fig. 3C).

To investigate the expression of LEF1 and TCF7 (TCF-1) in CD8\(^+\) T\(_N\), we used CD8 T cells isolated from CB. CB CD8 T cells have a predominant CCR7\(^-\)CD45RA\(^+\) phenotype similar to adult T\(_N\) (Fig. 4A) with some CCR7\(^+\)CD45RA\(^-\) cells also present. Importantly, it has been shown that CB T cells (including the CCR7\(^-\)CD45RA\(^+\) subset) are functionally naive (29). CB CD8 T cells showed strong up-regulation of activation markers such as CD25 (IL-2R\(_{\gamma}\); Fig. 4A) and HLA class II (data not shown) in response to TCR (allogenic) stimulation. At later time points, poststimulation CD8 T cells converted back to a resting state as shown by the absence of activation marker expression (Fig. 4A). Similar to bulk peripheral CD8 T cells, we observed down-regulation of LEF1 mRNA expression in CD8\(^+\) T\(_N\) (from CB) following TCR stimulation as measured by qRT-PCR (Fig. 4B). Importantly, at the time points examined (more than day 3) allogenic stimulator B cells had practically disappeared from the T cell-B cell cocultures. Interestingly, after the initial down-regulation, there was a progressive increase in LEF1 mRNA expression at

Table I. LEF1 is among the 10 most differentially expressed genes between T\(_N\) and Ag-primed CD8 T cell subsets

<table>
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<tr>
<th>No.</th>
<th>GenBank Identification</th>
<th>Gene Symbol</th>
<th>Description</th>
<th>T(_N)</th>
<th>T(_{EM/EMRA})</th>
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<td>65.3</td>
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* Listed are the 10 most differentially expressed genes between CD8 T cell subsets. Mean expression values (data set 1: n = 2; data set 2: n = 4) are shown.
later time points poststimulation, although it varied between donors. A further set of experiments corroborated these results: in addition to TCR triggering, homeostatic signals such as IL-15 also decreased LEF1 and TCF7 (TCF-1) mRNA expression in CD8 T cells primed with Ag in vivo (Fig. 1A). Thus, the signals that control mature CD8 T cell differentiation, i.e., TCR triggering and homeostatic cytokines, also regulate the expression of LEF1 and TCF7 (TCF-1) in CD8 T cells.

TCR and IL-15 stimulation of peripheral CD8 T cells lead to a relative decrease in inhibitory LEF1 and TCF7 (TCF-1) isoform expression

Several LEF1 and TCF7 (TCF-1) isoforms with different functional properties have been described (13–21), but which of these different isoforms are expressed in CD8 T cells is unknown. Therefore, we analyzed LEF1 and TCF7 (TCF-1) isoform expression in mature T cells. First, cloning of LEF1 mRNA isoforms by RT-PCR and DNA sequencing showed that peripheral CD8 T cells express both stimulatory full-length and inhibitory LEF1 mRNA isoforms (Fig. 5A). Furthermore, we found that CD8 T cells preferentially express N-tail LEF1 mRNA isoforms, although isoforms carrying a B-tail could also be detected (Fig. 5B). These mRNA isoforms were distinguished by the presence (N-tail isoforms) or absence (B-tail isoforms) of exon 11 (16). The predominance of N-tail isoforms applied to both full-length and CTNNB LEF1 mRNA isoforms (Fig. 5B).

Second, we investigated LEF1 and TCF7 (TCF-1) protein isoform expression by Western blot. Jurkat cells, a transformed immature T cell line with a high proliferative capacity, predominantly expressed stimulatory full-length LEF1 isoforms of 48–55 kDa (Fig. 6A) as previously reported (17). In contrast, primary resting CD8 T cells showed predominant expression of a 38-kDa LEF1 protein isoform, although isoforms carrying a B-tail could also be detected (Fig. 5B). These mRNA isoforms were distinguished by the presence (N-tail isoforms) or absence (B-tail isoforms) of exon 11 (16). The predominance of N-tail isoforms applied to both full-length and ΔCTNNB LEF1 mRNA isoforms (Fig. 5B).

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Similar to LEF1, we observed differences in the stimulatory vs inhibitory TCF7 (TCF-1) isoform balance between primary T cells and transformed Jurkat T cells. Jurkat cells showed predominant expression of stimulatory full-length TCF7 (TCF-1) isoforms of 42–48 kDa (Fig. 7A). In contrast, the ratio of stimulatory full-length (42–48 kDa)/inhibitory H9004 CTNNB (26–32 kDa) TCF7 (TCF-1) isoforms (14, 15) was about equal in resting (Fig. 7A) and CD8+ TN (from CB; Fig. 7B, lane 1). Clevers and colleagues (15) have previously demonstrated that the 26- to 32-kDa bands correspond to H9004 CTNNB TCF7 (TCF-1) isoforms that have a dominant-negative function (19, 20). Interestingly, TCR and IL-15 stimulation of CD8+ TN (from CB) lead to preferential down-regulation of the inhibitory TCF7 (TCF-1) isoforms (Fig. 7B, lanes 2 and 3). Thus, the TCF7 (TCF-1) protein isoform balance in CD8 T cells changed in favor of the stimulatory isoforms following activation. In conclusion, our data suggest that the negative effects of inhibitory LEF1 and TCF7 (TCF-1) isoforms prevail in resting CD8 T cells. In activated cells this negative effect seems to be relieved by down-regulation of LEF1 protein expression and by specific down-regulation of inhibitory TCF7 (TCF-1) isoform expression.

**Discussion**

Ag-experienced T cells are better able than TN to respond to Ag. This reflects both an increase in their frequency and a change in their state of differentiation. The molecular mechanisms that underpin the changes in cellular state and hence the development of T cell memory remain poorly understood. The capacity of transcription factors to modulate many different aspects of T cell function makes them an attractive candidate for study in this respect. In two separate microarray studies, we observed that the WNT pathway effector, LEF1, was expressed at higher levels in TN, compared with Ag-experienced CD8 T cells. Indeed, in both studies, it proved to be one of the most differentially expressed genes and the most differentially expressed transcription factor between these two populations of cells. A second effector of the same pathway, TCF7 (TCF-1), was also differentially expressed.

The differential expression of these molecules in the different CD8 T cell subsets proved to be consistent and robust; it was confirmed at the level of mRNA using qRT-PCR and at the level of protein using intracellular staining in many different donors.
The observed correlations between expression of LEF1 and TCF7 (TCF-1) and T cell naïveté and quiescence were not consistent with published data showing that LEF1 and TCF7 (TCF-1) are able to drive cellular proliferation. However, this paradox was resolved by additional experiments that analyzed the expression of the different LEF1 and TCF7 (TCF-1) isoforms. Our work shows that, compared with Jurkat cells, resting CD8 T cells express relatively more of the inhibitory LEF1 and TCF7 (TCF-1) protein isoforms. T cell stimulation results in the down-regulation of this inhibitory isoform. Importantly, it has previously been shown that although stimulatory full-length LEF1 and TCF7 (TCF-1) isoforms drive the proliferation of Jurkat T cells, dominant-negative ΔACTNNB isoforms inhibit proliferation (20). Furthermore, colon cancer cells predominantly express full-length ΔACTNNB isoforms while down-regulating the expression of ΔACTNNB isoforms (17). Finally, inhibitory ΔACTNNB isoforms are the most abundant TCF7 (TCF-1) isoforms in the intestine and TCF7 (TCF-1)-/- mice develop intestinal and mammary adenomas (19). Thus, it is been suggested that the balance between stimulatory and inhibitory

FIGURE 5. Expression of different LEF1 isoforms in primary CD8 T cells. A. Cloning of LEF1 isoforms from CD8 T cells. LEF1 mRNA isoforms were amplified from primary human CD8 T cells by RT-PCR using primers specific for either full-length or ΔACTNNB isoforms. Left panel. Gel electrophoresis of LEF1 PCR products. First lane on the left, DNA marker; and second lane, no template control. PCR products were ligated into the vector pIRES2-EGFP and analyzed by restriction digestion (right panel). First lane on left, DNA marker; second lane, undigested parental vector (pIRES2-EGFP); and third lane, digested parental vector; lanes 1–4, digested pIRES2-LEF1. B. CD8 T cells predominantly express N-tail LEF1 mRNA isoforms. DNA sequencing results for cloned LEF1 mRNA isoforms from A are displayed. The number of clones identical with a specific isoform among all of the clones sequenced is given in parentheses.

Furthermore, in a longitudinal study in an in vitro system, we were able to show that stimulation of TN, by alloantigen, led to a down-regulation/partial recovery of LEF1 expression upon activation. Interestingly, it has been shown that IL-15 and TCR stimulation induce very similar changes in gene expression in human CD8 T cells (30). This would suggest that IL-15 and TCR stimulation probably activate common signaling pathways, and this could also apply to the regulation of LEF1/TCF7 (TCF-1) expression in CD8 T cells. Furthermore, one recent study reported that another IL-2 family member, IL-7, can also inhibit LEF1 and TCF7 (TCF-1) expression (31). In contrast, we found that TGFβ1, which is known to inhibit T cell differentiation and maintain T cell quiescence (32), increased LEF1 expression. Interestingly, we noted a partial recovery of LEF1 expression in CD8 T cells in vitro when the cells converted back to a resting state following Ag stimulation. Similarly, one murine microarray study demonstrated initial down-regulation/partial recovery of LEF1 expression upon naive → effector → memory CD8 T cell differentiation in vivo (33).
Lef1 and Tcf7 (Tcf-1) is a checkpoint for cellular proliferation in the context of malignancy.

In a conceptually similar way, the experiments we describe lead us to formulate the hypothesis that the balance between stimulatory and inhibitory Lef1 and Tcf7 (Tcf-1) isoforms represent a checkpoint for the quiescence of peripheral T cells. Direct evidence for this will require future studies, in which expression of individual Lef1 and Tcf7 (Tcf-1) isoforms or combinations of individual isoforms is manipulated in primary mature T cells. The functional redundancy between Lef1 and Tcf7 (Tcf-1) and the presence of numerous isoforms will make such experiments difficult to design and perform. Consistent with this, in preliminary experiments, we did not find a clear phenotype when knocking down total Lef1 (i.e., all isoforms) by RNA interference in human peripheral T cells. Studies in knockout and transgenic mice are probably more suited to address the role of the Wnt pathway in mature T cells, although the conditional knockout of individual Lef1 and Tcf7 (Tcf-1) isoforms will be challenging.

In conclusion, our study identifies Lef1 as the most differentially expressed transcription factor between Tn and Ag-experienced CD8 T cells. It shows that, compared with a Jurkat cell line, CD8 T cells was examined by Western blotting. Two (of four) independent experiments are shown.

References


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Disclosures

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FIGURE 7. Activation of CD8 Tn changes the TCF7 (Tcf-1) isoform balance in favor of stimulatory isoforms. A. Expression of TCF7 (Tcf-1) protein isoforms in resting CD8 T cells. Expression of full-length and truncated (ACTNNB) TCF7 (Tcf-1) protein isoforms in the Jurkat T cell line and in primary human CD8 T cells was examined by Western blotting. B Preferential down-regulation of inhibitory TCF7 (Tcf-1) isoform expression in activated CD8 T cells. CD8 Tn (from CB) were either left unstimulated (lane 1) or stimulated with IL-15 at 50 ng/ml (lane 2) or alloantigen (lane 3). TCF7 (Tcf-1) isoform expression was analyzed by Western blotting. Two (of four) independent experiments are shown.


