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Modulation of TCR β Surface Expression During TCR Revision

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Abstract

TCR revision is a tolerance mechanism by which self-reactive TCRs expressed by mature CD4⁺ peripheral T cells are replaced by receptors encoded by genes generated by post-thymic DNA rearrangement. The down modulation of surface TCR expression initiates TCR revision, and serves as a likely trigger for the induction of the recombinase machinery. We show here in a V β 5 transgenic mouse model system that downregulation of the self-reactive transgene-encoded TCR is not maintained by transgene loss or diminished transcription or translation. The downregulation of surface TCR expression likely occurs in two stages, only one of which requires tolerogen expression.

Keywords

receptor revision; peripheral T cell tolerance; TCR expression

1. Introduction

The immune system is charged with the difficult task of protecting the individual from invading pathogens while maintaining self-tolerance. Within the T cell arm of the adaptive immune response, tolerance is primarily induced by the deletion of overtly autoreactive T cells as they develop within the thymus (reviewed in [1]). However, alternate means are required to induce tolerance to antigens whose expression is developmentally regulated or restricted to extrathymic sites. One such mechanism is TCR revision, the process by which at least one chain of an autoreactive TCR expressed by mature peripheral T cells is replaced by the product of a newly rearranged gene (reviewed in [2, 3]). The resulting “post-revision” T cells express a diverse, extrathymically generated TCR repertoire [4, 5], and are both self tolerant and fully functional, responding appropriately to homeostatic signals and to bacterial infection [6].

The sequential steps along the pathway of TCR revision have been delineated in a mouse model system, in which V β 5⁺ CD4⁺ T cells from V β 5 transgenic (Tg) C57BL/6 (B6) mice

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encounter B cells [7] expressing a superantigen encoded by a defective retrovirus, mammary tumor virus (Mtv)-8 [3, 4]. Revision begins with the downregulation of surface TCR β (sTCR β) expression and the re-expression of the lymphocyte-specific recombinase machinery, including RAG1, RAG2, and TdT [8, 9]. Using GFP to report RAG expression, revising CD4 T cells have been characterized as large, metabolically active but nondividing CD44^{hi} cells localized to germinal centers [10]. RAG-mediated DNA rearrangement in revising cells occurs at endogenous TCR β loci, enabling the expression of endogenous TCR β chains (TCR β^e), which upon pairing with TCR α , traffic to the cell surface. TCR revision intermediates, defined by their ability to complete revision in adoptive hosts, are characterized by the surface expression of both V β 5 and an endogenous TCR β [11]. In V β 5 Tg mice, post-revision CD4⁺ T cells are Mtv-8 nonresponsive and maintain a stable sTCR β^+ sV β 5⁻ phenotype [6].

The mechanism by which self-reactive sTCR levels are modulated during TCR revision is unclear, but the importance of this regulation is underscored by the fact that in both B and T cells, loss of antigen receptor surface expression and consequent interruption of tonic signaling may be instrumental in triggering RAG expression [12-14]. RAG re-induction in mature lymphocytes may therefore mimic the link between the absence of TCR-mediated signaling and RAG expression in developing thymocytes [15-17]. Understanding how TCR expression levels are downregulated during TCR revision is complicated by the complexity and diversity of the model systems used to investigate this process. Although often studied in the V β 5 Tg model system, TCR revision does not depend on TCR transgenesis, but also occurs in both TCR nonTg mice [9, 18, 19] and in mice carrying rearranged TCR alleles knocked into the appropriate TCR loci [20, 21]. Furthermore, TCR revision has been reported in normal human donors [22-24] and in patients with defective DNA repair pathways [24-26].

The initial downregulation of sTCR β expression and eventual replacement of the autoreactive TCR β with a newly rearranged TCR β^e is particularly unexpected in the case of a transgene-encoded receptor. The ectopic location of the transgene prevents its deletion during revision-associated recombination events. The studies reported here were aimed at exploring the modulation of sTCR expression during TCR revision in a V β 5 Tg model system. Our results show that the downregulation and eventual loss of sTCR β expression during TCR revision is not dependent upon transgene loss or impaired transgene expression, and appears to proceed through two stages, separable by their relative Mtv-8 dependence.

2. Materials and methods

2.1. Mice

V β 5 Tg mice on the B6 background [27] were bred as heterozygotes under specific pathogen free conditions at the University of Washington. B6 Ly5.2⁺, B6.SJL (B6.SJL-*Ptprca^dPepc^b*/BoyJ) Ly5.1⁺, CD4^{-/-} [28], and I-A β ^{-/-} [29] mice were originally purchased from The Jackson Laboratory (Bar Harbor, ME), or bred in house, and crossed to the V β 5 Tg B6 background. B6 mice carry the endogenous proviral integrants Mtv-8, -9, -17, and -30. Mtv⁻ V β 5 Tg and single-integrand-carrying Mtv-8⁺ V β 5 Tg mouse lines were derived by intercross/backcross breeding of B6 V β 5 Tg mice with an Mtv⁻ wild-derived male WLC-0 mouse [4] originally provided by Dr. D. Morris (University of California at Irvine, CA). All experiments were performed in compliance with the University of Washington Institutional Animal Care and Use Committee.

2.2. Cell preparation, flow cytometry, and sorting

Single-cell suspensions were prepared from spleen and LNs and RBCs were removed from the splenocytes by water lysis. For flow cytometry, Fc receptors were blocked with anti-CD16/32 (2.4G2; BD Biosciences, San Diego, CA). Cell surface staining was performed in HBSS containing 1% BSA using fluorochrome-conjugated antibodies recognizing CD4 (RM4-5), CD44 (IM7), panTCR β (H57-597.12), V β 5 (MR9-4), V β 8 (F23.1), V β 3 (KJ25), CD45.1 (A20), CD45.2 (104), CD11b (M1/70), CD11c (N418), or B220 (RA3-6B2), according to standard procedures. All antibodies were purchased from either BD Biosciences or eBioscience (San Diego, CA). Flow cytometry data were collected using a FACSCanto (Becton Dickinson, Mountain View, CA) and analyzed with FlowJo software (Treestar, Ashland, OR). For cell sorting, CD4⁺ T cells were enriched using an EasySep Negative Selection Mouse CD4⁺ T cell Enrichment Kit (StemCell Technologies, Vancouver, BC). Enriched cells were surface stained and sorted using a FACSaria (Becton Dickinson).

2.3. Quantitative RT-PCR

Total mRNA was extracted from purified cells using the RNeasy mini kit (Qiagen, Valencia, CA) and first-strand cDNA synthesis was performed with either oligo(dT) primers or random hexamer primers (Invitrogen, Carlsbad, CA) using SuperScript III (Invitrogen), according to the manufacturer's protocol. The cDNA was subjected to quantitative (q)PCR, performed on an ABI 7300 Real Time PCR System (Applied Biosystems, Carlsbad, CA) with primer pairs, using Power SYBR Green PCR Master Mix (Applied Biosystems). PCRs were performed in 96-Well Optical Reaction Plates (Applied Biosystems) and consisted of a 10 min 95°C denaturation step, followed by 40 cycles of 15 s at 95°C, 60 s at 55°C, and 30 s at 72°C. The following intron-spanning primers were used: V β 5-specific sense primer, 5'-GAAGACTGTCTAACACTGTCC-3'; TCR C β 2 antisense primer, 5'-ACTGTGCGACCTCCTTGCCATTCA-3'; Hypoxanthine guanine phosphoribosyltransferase (HPRT) sense primer, 5'-GTTGGATACAGGCCAGACTTTGTTG-3'; and HPRT antisense primer, 5'-GAGGGTAGGCTGGCCTATAGGCT-3'. The expected product sizes are ~600 bp and 352 bp respectively. Reactions were run in triplicate and samples were normalized to the internal HPRT control.

Genomic DNA was extracted from purified cells using Gentra's Puregene DNA Purification Kit and DNA was subjected to qPCR using the system and reagents described above. PCRs consisted of a 10 min 95°C denaturation step, followed by 40 cycles of 30 s at 94°C, 30 s at 61°C, and 30 s at 72°C. The following primers were used to amplify the V β 5 transgene: V β 5 sense primer, 5'-ACGTGTATTCCCATCTCTGG-3'; antisense primer spanning the transgene V β 5/D β 2/J β 2.6 junctional sequences, 5'-CTGTTCATAATTGCCCCGA-3'; β -actin sense primer, 5'-AGCCATGTACGTAGCCATCC-3'; and β -actin antisense primer, 5'-CTCTCAGCTGTGGTGGTGAA-3'. The expected product sizes are 238 bp and 228 bp, respectively. Reactions were run in triplicate and samples were normalized to the internal β -actin control.

2.4. Western blot analysis

Because the only available anti-V β 5 monoclonal antibody (MR9-4) does not recognize intracellular V β 5 protein, V β 5 peptide-specific antisera were generated for use in Western blots. V β 5 peptide, N-CLIQHYEKVERDKGFLPS-C, located in the CDR2 region of the V β 5 TCR, was obtained through Quality Controlled Biochemicals, Inc (now Biosource International Inc, Carlsbad, CA). The peptide was conjugated to keyhole limpet hemocyanin for immunization of rabbits and antisera were collected. For purification, V β 5-CDR2 peptide was solubilized in 0.1M Na₂CO₃ (pH 8.9) for thiol coupling to epoxy agarose beads (Pierce, Rockford, IL). Antisera were purified over V β 5 peptide-coupled columns, eluted

with 0.1M glycine (pH 2.5), neutralized with Tris (pH 11), dialyzed, and resuspended in PBS. Cells were lysed in the presence of 1% Triton X-100, 20mM Tris, 150mM NaCl, and protease inhibitors (pH 7.4) for 20 min with rocking. The lysates were passaged 10 times through 22-gauge needles and centrifuged at 20,000g for 10 min. Lysates were immunoprecipitated with pan-specific anti-TCR β antibodies, resolved by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes, blocked in 3% nonfat dry milk in 2% BSA + Tris-buffered saline + 0.1% Tween 20 (TBST) for one hour at room temperature, and immunoblotted overnight at 4°C with V β 5 peptide-specific antisera diluted with 3% nonfat dry milk/2% BSA/TBST. The membranes were then washed 3-5 times in TBST, incubated with secondary anti-rabbit IgG-HRP for one hour at room temperature, and then washed 3-5 times in TBST. The membranes were developed with enhanced chemiluminescence reagents.

2.5. T cell activation

Splenocytes and LN cells were incubated at 1×10^8 /ml with 5 μ M CFSE (Molecular Probes/Invitrogen) for 10 min at 37°C and then washed twice in ice-cold complete RPMI 1640 medium (containing 10% FBS, 10mM HEPES, 4mM L-glutamine, and 50 μ M 2-mercaptoethanol) before culture. In 48-well plates, 0.5×10^6 CFSE-labeled cells were cultured with IL-2 at 20 U/ml alone or 1:1 with α CD3/ α CD28 Dynabeads (Invitrogen) at 37°C with 7% CO₂. On day 2.5, the cells were harvested and separated from beads, surface stained, and analyzed as described above.

2.6. Adoptive transfer

CD4⁺ TCR β ⁺ V β 5^{hi}, V β 5^{lo}, and V β 5⁻ sorted T cell populations were obtained from V β 5 Tg Ly5.1⁺ B6 donor mice, and 0.3 - 1.2×10^6 cells were injected in the lateral tail vein of sublethally irradiated (600 rad) B6 Ly5.2⁺ mice. Recipients included CD4^{-/-} and I-A β ^{-/-} B6 mice. Both sets of recipients are lymphopenic for CD4 T cells, but only the former mice can present endogenous Mtv. The absence of class II molecules renders I-A β ^{-/-} B6 mice Mtv⁻ [30].

3. Results

3.1. V β 5 Tg mice are characterized by the age- and Mtv-8-dependent appearance of V β 5⁻CD4⁺ T cells through the process of TCR revision

In V β 5 Tg B6 mice, CD4⁺ peripheral T cells encounter an endogenous superantigen encoded by Mtv-8 and are either deleted or become tolerized to this pseudo self antigen through TCR revision. The end result of this latter tolerance pathway is the appearance and age-dependent accumulation of V β 5⁻ post-revision CD4⁺ T cells (Figure 1A). These cellular products of the TCR revision pathway express rearranged endogenous TCR β^e chains and are uniformly CD44^{hi}, bearing the mark of their chronic encounter with superantigen-expressing APCs (Figure 1B). In the absence of Mtv-8, CD4⁺ T cells from V β 5 Tg mice are neither deleted nor revised, and like CD8⁺ T cells even from Mtv-8⁺ V β 5 Tg mice [31], remain >95% V β 5⁺.

3.2. V β 5⁻ post-revision T cells from V β 5 Tg mice retain TCR transgenes and V β 5-specific RNA and protein

V β 5⁺CD4⁺ T cells from V β 5 Tg Mtv-8⁺ mice begin the TCR revision process by downregulating sV β 5 expression, prior to the complete loss of V β 5 surface expression that characterizes post-revision T cells. To determine whether these distinct sV β 5 expression levels result from transgene loss or diminished RNA or protein levels, sV β 5^{hi}, sV β 5^{lo}, and sV β 5⁻ CD4 T cells were sorted from the spleens and LNs of middle aged V β 5 Tg B6 mice

and the resulting DNA analyzed for transgene content, RNA analyzed for V β 5-specific sequences, and total cell lysates analyzed for V β 5 protein (Figure 2A). Whether sV β 5^{hi} or sV β 5⁻, CD4 T cells from V β 5 Tg mice contain >30x the amount of V β 5.2/J β 2.6 rearranged DNA as do V β 5⁺CD4⁺ T cells from nonTg mice (Figure 2B), consistent with earlier estimates of ~10 head to tail transgene integrants in V β 5 Tg founder mice [27]. That these transgenes remain transcriptionally active is apparent from qRT-PCR analyses, showing that sV β 5⁻ CD4⁺ T cells contain ~3.5-fold the amount of V β 5-specific RNA found in V β 5⁺ CD4⁺ T cells from nonTg mice (Figure 2C). Even more remarkable, Western blot analyses of cell lysates revealed that sV β 5⁺ CD4⁺ T cells from V β 5 Tg mice contain only ~3-fold more V β 5 protein than do sV β 5⁻ T cells (Figure 2D). Thus, the sV β 5⁻ status of post-revision CD4⁺ T cells from V β 5 Tg mice is not driven or maintained by transgene loss or solely by impaired transgene transcription or translation.

3.3. In TCR nonTg mice, Mtv-8 triggers the downregulation of sTCR β specifically on V β 5⁺ CD4⁺ T cells

Knowing that TCR revision also targets V β 5⁺CD4⁺ T cells from TCR nonTg Mtv-8⁺ mice [9, 18, 19], we set about to determine if loss of sV β 5 expression also characterizes revising nonTg T cells. For these experiments, it was important to measure sTCR β expression on V β 5⁺CD4⁺ T cells in comparison to those expressing other TCR V β alleles. We therefore counterstained cells stained for V β 5, V β 3, and V β 8 expression with a non-crossblocking antibody that detects all TCR β s. Using this scheme, we found that in the CD4⁺ population, sTCR β expression is lower in V β 5⁺ than in V β 3⁺ or V β 8⁺ T cells from nonTg mice (Figure 3A and B). Furthermore, this downregulation of sTCR β expression is Mtv-8-dependent in TCR nonTg mice (Figure 3C), as it is in V β 5 Tg mice (Figure 3D). Therefore, whatever mechanism regulates the modulation of V β 5 surface expression during the process of TCR revision, it does not appear to depend on the TCR being transgene-encoded.

3.4. V β 5⁻ post-revision CD4⁺ T cells do not regain sV β 5 in the absence of Mtv-8, but V β 5^{lo} cells do

To assess the stability of the downregulation of sV β 5 expression in revision-prone CD4⁺ T cells, we sorted CD4⁺TCR β ⁺ T cells into populations of sV β 5^{hi}, sV β 5^{lo} revision intermediate, and sV β 5⁻ post-revision T cells. Each population was labeled with CFSE and cultured in the presence of IL-2 alone or IL-2 plus anti-CD3/CD28 coated beads (Figure 4A). The absence of MHC class II⁺ cells ensures that Mtv-8 was not presented to the T cells during this culture period [30]. By analyzing sV β 5 expression levels and CFSE dilution 2.5 days later, we assessed the stability of sV β 5 expression levels in the absence of Mtv-8. Aside from the expected activation-induced partial downmodulation of sTCR expression, sV β 5⁻ and sV β 5^{hi} CD4⁺ T cells maintained their respective V β 5 surface expression levels under all the conditions analyzed. In contrast, sV β 5^{lo} T cells upregulated sV β 5 expression levels, and analysis of CFSE dilution indicated that this upregulation did not require cell proliferation (Figures 4A and B).

To assess the stability of the sV β 5 phenotype in vivo in the absence of Mtv-8, we transferred sV β 5⁻, sV β 5^{lo}, and sV β 5^{hi} T cells into sublethally irradiated CD4^{-/-} or I-A β ^{-/-} congenic B6 hosts. Both types of hosts are CD4 T cell lymphopenic, but because presentation of Mtv-8 requires MHC class II expression, only the former mice are capable of Mtv expression. Both sV β 5⁻ and sV β 5^{hi} CD4⁺ T cells maintain their respective phenotype 8 days after transfer into both types of hosts, while sV β 5^{lo} T cells upregulate V β 5 surface expression in the Mtv⁻ hosts (Figure 4C). Therefore, these in vivo transfer studies recapitulate our in vitro results and show that the loss of V β 5 surface expression in sV β 5⁻ post-revision CD4 T cells is stably maintained even in the absence of Mtv-8, while the

sV β 5^{lo} status of revision intermediates is dynamically maintained and requires the expression of Mtv-8.

4. Discussion

TCR revision, the replacement of an autoreactive TCR through RAG-mediated DNA rearrangement in peripheral T cells, appears at first glance to be a tolerance mechanism fraught with danger [32]. Confining the process to germinal centers [10], coupled with the likely imposition of post-revision cellular selection that is influenced by both Fas- [7] and Bim- [11] mediated death pathways may mitigate some of the risk inherent in the extrathymic rearrangement of TCR loci. Furthermore, the reinduction of RAG expression that initiates TCR revision may tap into a tried and true regulatory pathway that operates during T cell development, in which TCR mediated signaling extinguishes RAG expression, while the absence of this signaling prompts its maintenance [15-17]. As such, the modulation of sTCR expression levels represents a key control point for TCR revision, and sTCR downregulation is a hallmark of this process in humans [22, 24-26] and both TCR Tg [4, 8, 10, 33] and nonTg (reference [9] and Figure 3) mice.

Our work shows that the loss of sTCR expression in revising and post-revision T cells in a V β 5 Tg model system is due neither to transgene loss nor solely to diminished transcription or translation (Figure 2). Furthermore, while the loss of sV β 5 expression by post-revision cells is stable in the absence of the Mtv-8 tolerogen, the sV β 5^{lo} status of revision intermediates is maintained dynamically, in an Mtv-8-dependent manner (Figure 4). Our data are compatible with a two-step model for the regulation of sTCR expression during TCR revision (Figure 5). We hypothesize that ligand-mediated TCR internalization initiates the first step, and drives the appearance of sTCR^{lo} TCR revision intermediates. This step is reversible, such that sTCR levels return to baseline upon removal of the ligand (the superantigen Mtv-8 in the case of V β 5⁺ CD4⁺ T cells). Diminished sTCR expression levels eventually trigger RAG induction, rearrangement at endogenous TCR β loci, and expression of newly generated TCR β chains. In contrast, the 2nd stage of sTCR modulation during TCR revision is stable and ligand independent (Figure 5).

We suggest that the steps leading to the generation of sTCR^{lo} revision intermediates and the consequent induction of RAG expression are similar, whether the starting T cell population is of human or mouse origin, and if the latter, whether or not it is TCR Tg. In contrast, divergent means may exist to render post-revision T cells incapable of expressing the initial, autoreactive TCR. In the case of TCR nonTg T cells, loss of this receptor could be rendered permanent (and tolerogen-independent) if the secondary rearrangement deletes the rearranged allele encoding the self-reactive chain. However, in the case of TCR Tg T cells, such leap frogging rearrangement would not take place, and the ectopic TCR transgenes would not be deleted.

Given that post-revision sV β 5⁻ T cells still carry transgene-encoded message and protein, what maintains the loss of sV β 5 expression in TCR Tg T cells? Although mouse T cells are MHC class II negative and therefore do not present Mtv-8 on the cell surface, it is possible that Mtv-8 protein is present inside the cell and acts to chaperone the V β 5 chain into the lysosomal degradation pathway, bypassing the plasma membrane [34, 35]. Delineating such an intracellular trafficking pattern would require use of Mtv-8-specific antibodies, and anti-Mtv reagents have been notoriously difficult to generate [36], although the apparent preference for superantigens in triggering TCR revision [20] is suggestive. Preferences in TCR α and TCR β pairing have been well described [37-41], and it is possible that V β 5 pairs poorly with the available TCR α chains, although the diversity of the TCR α repertoire in CD4⁺ T cells from V β 5 Tg mice [4] renders this possibility unlikely. Furthermore, while

steric incompatibility caused by long CDR3 loops in the TCR β chain is known to impair association with TCR α [42], the CDR3 loop of the transgene-encoded V β 5 is relatively short [27]. Perhaps the fact that transgene-encoded RNA and protein are both somewhat diminished in post-revision T cells (Figure 2), in combination with protein pairing preferences, enforce the sV β 5[−] status of post-revision T cells. Regardless, the importance of regulating sTCR expression at many levels in revising and post-revision T cells is underscored by the existence of elaborate intrathymic mechanisms to enforce allelic exclusion (reviewed in reference [43]) and the apparent perils imposed by the 1-10% of dual TCR expressing T cells that slip through this net [44-46]. The induction of tolerance through TCR revision is tasked not only with the extrathymic generation of new TCR rearrangements, but with modulating sTCR expression, by first downregulating and then eliminating surface expression of the self-reactive TCR.

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Abbreviations used

B6	C57BL/6
Mtv-8	mouse mammary tumor virus-8
Tg	transgenic
nonTg	nontransgenic
TBST	Tris-buffered saline + 0.1% Tween 20
s	surface
e	endogenous
HPRT	Hypoxanthine guanine phosphoribosyltransferase

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Highlights

- Through TCR revision, autoreactive TCRs are replaced by those generated through post-thymic DNA rearrangement
- Downmodulation of surface TCR expression initiates RAG re-expression and TCR revision
- In V β 5 transgenic mice, loss of surface TCR is not mediated by transgene loss or diminished expression
- Downregulation of surface TCR expression occurs in two stages, only one of which requires tolerogen expression

Figure 1.

TCR revision is characterized by the Mtv-8-driven appearance of Vβ5⁻ CD4⁺ T cells. A) Splenocytes from Mtv-8⁺ and Mtv⁻ Vβ5 Tg mice of the indicated age ranges (5-16 mice per age group) were surface stained for CD4 and Vβ5. In Mtv-8⁺ but not Mtv⁻ Vβ5 Tg mice, sVβ5⁻CD4⁺ T cells accumulate over time. B) Post-revision cells are Vβ5⁻, TCRβ⁺, and CD44^{hi}. Representative flow cytometry plots showing CD4⁺ splenocytes from a 40-week-old Mtv⁺ Vβ5 Tg mouse surface stained for Vβ5, TCRβ, and CD44. Numbers represent the percentage of cells falling within each quadrant.

Figure 2.

Post-revision Vβ5⁻ CD4⁺ T cells have intact TCRβ transgenes and continue to express Vβ5-specific RNA and protein. Splenocytes from 20-30 week old TCR nonTg and Vβ5 Tg B6 mice were surface stained and sorted to >98% purity into CD4⁺Vβ5^{hi}, CD4⁺Vβ5^{lo}, and CD4⁺Vβ5⁻ populations from Vβ5 Tg donors, and into CD4⁺Vβ5⁻ and CD4⁺Vβ5⁺ populations from TCR nonTg donors. DNA, RNA, and protein were isolated from sorted cells. A) Representative example of Vβ5 Tg pre- and post-sort populations; numbers indicate post-sort purity. Gray gate delineates the Vβ5^{lo} population. B) qPCR was used to amplify and quantify Vβ5 transgenes and endogenous Vβ5.2-DJβ2.6 rearrangements from the indicated cell populations. Data are normalized to β-actin, and are representative of two DNA samples, each extracted from sorted cells pooled from 10 mice and analyzed 2-3 times. Bars represent means ± SD. C) cDNA was synthesized from the RNA of cells sorted from 8-10 mice, quantified using qPCR and normalized to HPRT. qPCR data are normalized to the Vβ5⁺ TCR nonTg population, and are representative of 2 biological samples, each analyzed multiple times. D) Lysates from the indicated number of cells sorted from Vβ5 Tg donors were immunoprecipitated with anti-TCRβ antibodies, electrophoresed, and blotted with Vβ5 peptide- or actin-specific antibodies. The number of cell equivalents loaded from each lysate is noted above the lane. No bands of the relevant size were visible from sVβ5⁻ CD4⁺ T cells from TCR nonTg mice.

Figure 3.

In TCR nonTg mice, surface TCRβ expression is downregulated in an Mtv-8-dependent manner specifically on CD4⁺Vβ5⁺ T cells. Splenocytes from 21-32 week old mice were surface stained for CD4, TCRβ, and CD44 expression, and for Vβ5, Vβ3 or Vβ8. A) Histogram overlays showing surface pan TCRβ expression of CD4⁺ T cells gated as Vβ5⁺, Vβ3⁺ or Vβ8⁺. B) TCRβ MFI of Vβ5⁺ relative to Vβ5⁻, Vβ3⁺ relative to Vβ3⁻, and Vβ8⁺ relative to Vβ8⁻ cells. All cell populations were gated as CD4⁺CD44^{hi}. Asterisks indicate p < 0.0001 as calculated using a 2-tailed unpaired Student's *t*-test. C, D) Histogram overlays showing sVβ5 expression on CD4⁺Vβ5⁺ T cells from Mtv⁺, Mtv-8⁺, and Mtv⁻ C) TCR nonTg and D) Vβ5 Tg mice.

Figure 4.

Post-revision T cells do not regain sVβ5 in the absence of Mtv, but Vβ5^{lo} cells do. A) Splenocytes and mesenteric LN cells were harvested from ten 27-35 week old Mtv⁺ Vβ5 Tg mice, and enriched for CD4⁺ T cells by negative selection. The cells were then surfaced stained and sorted to >96% purity as CD4⁺ TCRβ⁺, and either sVβ5⁻, sVβ5^{lo} or sVβ5^{hi}. Each of the sorted populations was CFSE labeled, and then cultured for 2.5 days with IL-2 +/- αCD3/αCD28-coated beads, after which they were stained for CD4, TCRβ, and Vβ5. Numbers indicate the percentage of cells falling within each quadrant. B) Histogram overlays show sVβ5 expression of Vβ5⁻, Vβ5^{lo}, and Vβ5^{hi} populations on d0 (post sort) or on d2.5 of culture. Numbers indicate the MFI of Vβ5 staining for each of the peaks. C) Splenocytes from 25-29 week old Ly5.1⁺ Vβ5 Tg mice were enriched for CD4⁺ T cells as in A) and stained for Vβ5, B220, CD11b, CD11c, and CD8 expression. Lineage negative cells (B220⁻, CD11b⁻, CD11c⁻, CD8⁻) were sorted to >96% purity into 3 populations: Vβ5⁻, Vβ5^{lo}, and Vβ5^{hi}. Each of the sorted populations was transferred into irradiated, Ly5.2⁺, CD4^{-/-} (Mtv⁺) or I-Aβ^{-/-} (Mtv⁻) recipient mice. After 8 days, splenocytes of recipient mice were stained for CD4, TCRβ, and Vβ5. Histogram overlays show sVβ5 expression on d0 (post sort) and on d8 after transfer.

Figure 5. Model for the two-stage loss of Vβ5 surface expression during and after TCR revision. During the first stage, loss of sVβ5 expression is maintained by the presence of Mtv. 1. Mtv on an MHC class II⁺ B cell interacts with sVβ5 on a CD4⁺ T cell. 2. sVβ5 is then internalized and degraded. 3. The loss of tonic signaling through the TCR induces transcription of RAG genes. Throughout this process, the Vβ5 transgene is expressed, and the newly translated TCRβ^e chain pairs with the available TCRα chain for expression on the surface. This dynamic process maintains Vβ5 surface expression at low levels. The second hypothesized stage is Mtv-independent. 4. RAG-mediated rearrangements of endogenous TCRβ loci occur and the resulting TCRβ^e protein pairs with the available TCRα and is expressed on the surface. 5. Tonic signaling through this TCR is initiated and RAG genes are turned off. 6. While the Vβ5 transgene is still transcribed and translated, for unknown reasons, only TCRβ^e successfully pairs with available TCRα for cell surface expression. Thus in the second stage, sVβ5 expression is permanently lost. A summary of the key properties of revising (1st stage) and post-revision (2nd stage) cells is tabulated beneath the diagram.