Abnormal V(D)J recombination of T cell receptor β locus in SMAR1-transgenic mice

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Scaffold/Matrix Associated Region-1 binding protein (SMAR1) specifically interacts with MARβ sequence, which is located 400 bp upstream of the murine TCRβ enhancer and is highly expressed during DP stage of thymocyte development. To further analyze the functions of SMAR1, transgenic mice were generated that express SMAR1 in a tissue-independent manner. SMAR1 overexpressing mice exhibit severely altered frequency of the T cells expressing commonly used Vβs (Vβ5.1/5.2 and Vβ8.1/8.2/8.3). The rearrangements of Vβ5.1/5.2, Vβ8.1/8.2/8.3 loci are also reduced in SMAR1 transgenic mice. The T cells in SMAR1-transgenic mice exhibit a mild perturbation at the early DN stage. SMAR1 transgenic mice exhibit hypercellular lymph nodes and spleen accompanied with prominent architectural defects in these organs. These results indicate that SMAR1 plays an important role in the regulation of T cell development as well as V(D)J recombination besides maintaining the architecture of the lymphoid organs.

INTRODUCTION

The immunoglobulin (Ig) and T cell receptor (TCR) genes are assembled by a complex process of V(D)J recombination to generate antigen receptor diversity. In this process, the antigen binding domains of Ig and TCR chains that are encoded by germline variable (V), diversity (D), and joining (J) gene segments are rearranged to produce functional B or T cell receptors (1-3). For IgH and TCRβ-chains, V, D and J gene segments are involved whereas for IgL and TCRα-chains, V and J gene segments take part in the recombination event (4-6). V(D)J recombination is known to be stage
specific within a given lymphoid lineage and the antigen receptors are expressed from only one of the two alleles, the phenomenon called as allelic exclusion (7, 8). At the TCRβ locus, the recombination occurs during DN (CD4⁻ CD8⁻ CD44⁻ CD25⁺) stage of thymocyte development (9). V(D)J recombination is mediated by lymphoid and non-lymphoid-specific factors as well as cis-regulatory elements (10). The lymphoid-specific factors of the recombination machinery include Recombination Activating Genes (RAG) 1 and 2, and terminal deoxynucleotidyltransferase (TdT) (6, 10 and 11). Besides lymphoid-specific factors, non-lymphoid restricted factors have also been implicated in the regulation of recombination process. Among these proteins that are involved in DNA double-strand break repair also play a critical role the recombination events. These include Ku-80, XRCC4 protein, the catalytic subunit of DNA-dependent protein kinase (DNA-PK), poly (ADP-ribose) polymerase (PARP) (12-14).

In addition to activating transcription of germline gene segments, cis-regulatory elements also participate in the recombination (8, 15). Recently, it was reported that deletion of the germline promoter PDβ1, upstream of the Dβ1 gene segment in murine TCRβ locus (16, 17), not only resulted into reduction of Dβ1 germline transcription but also reduced the rearrangement of Dβ1 (15, 18). Interestingly, the germline transcription and rearrangement of Dβ2, Jβ2 and Vβ gene segments remain unaffected by the deletion of the PDβ1 promoter. Similarly, the enhancer at TCRα locus, Eα, is essential for rearrangement of all Jα gene segments whereas for proximal Jα gene segments T early α promoter is required (19). The transcriptional enhancers such as Eα, Eβ, Eμ and Eκ are also shown to be essential for recombination of their respective genomic loci as well as minilocus recombination substrates (20, 21).
Besides promoters and enhancers, MARs (Matrix Associated Regions) are the *cis* regulatory elements that contribute in the regulation of TCRβ gene rearrangement and transcription. MARs, though being distinct from promoters and enhancers, are often closely associated with these regulatory elements (22, 23). MARs are commonly located at the boundaries of transcription units often flanking enhancer-like regulatory sequences (24), particularly those lying in the antigen receptor gene loci such as Igκ light chain gene locus, IgH heavy chain gene locus (25), TCRβ gene locus (26), TCR α/δ gene locus (27) and CD8α gene locus (28). MARs help the cell type-specific expression of genes by residing close to enhancers as well as by synergistically acting with them (29), thus maintaining some chromatin domains into condensed inactive structures and others into decondensed transcriptionally active structures. MARs flanking the enhancers in the immunoglobulin or T cell receptor genes are known to function in association with their specific MAR binding proteins such as Cux (30) and SATB1 (31). Earlier, we reported a novel MAR binding protein, SMAR1, interacting with MARβ (32), a MAR flanking 5′ end of TCRβ enhancer (Eβ) (33). SMAR1 is the first known MAR binding protein that functions as a candidate tumor suppressor by interacting with and activating p53, thereby regulating cell cycle (34).

To investigate the role of SMAR1 at the TCRβ locus, we have generated SMAR1 transgenic mice. Here, we show that overexpression of SMAR1 severely reduces the frequency of cells expressing the commonly used Vβs (Vβ5.1/5.2, Vβ8.1/8.2 and Vβ8.3) including Vβ13. Accompanied with reduced transcription, there is a significant impairment in V(D)J gene rearrangement of these specific Vβs. In vitro results demonstrate that SMAR1 strongly reduces transcription mediated by Eβ enhancer. A mild perturbation in the thymocyte development is observed at the early DN stage in
SMAR1 transgenic mice. Taken together, the data indicates that SMAR1 regulates V(D)J recombination by modulating the function of TCRβ enhancer.

**EXPERIMENTAL PROCEDURES**

*Generation of SMAR1 transgenic mice*

For generating SMAR1<sup>S</sup> transgenic mice, SMAR1 expressing vector, pBK-CMV-SMAR1<sup>S</sup> was used where SMAR1 is under the control of CMV promoter (Fig. 1B). The construct was first linearized with *Mlu*I and *Apa*LI, giving rise to a 3.8 kb fragment that contains CMV-promoter along with 1.8 kb full-length alternatively spliced form of SMAR1 (SMAR1<sup>S</sup>). The insert was purified on agarose gel, followed by DNA extraction with phenol-chloroform and ethanol precipitation. The DNA pellet was resuspended at a final concentration of 4 µg/ml. DNA was microinjected into fertilized pronucleus of fertilized eggs derived from F-2 generation of (C57BL/6 X SJL) mice at 4 ng/µl concentration using Nikon micromanipulator. After injection, the eggs were transferred into the oviduct of 0.5-d pseudopregnant foster CD-1 female mice.

*Analysis of smar1 transgene by Southern hybridization*

To identify the presence of SMAR1, tail DNA was prepared from mice by following standard protocol. Briefly, a 2-mm tail biopsy was incubated in high salt digestion buffer containing 50 mM Tris-HCl, 100 mM EDTA, 0.5% SDS and 0.5 mg/ml Proteinase K for 16 h at 55°C. The lysates were processed for DNA isolation by phenol/chloroform extraction followed by ethanol precipitation. For Southern analysis, 15-20 µg of DNA was subjected to restriction digestion with *Bam*HI-*Hind*III or *Eco*RI-*Hind*III enzymes. The digested DNAs were fractionated on a 0.9% agarose gel,
followed by denaturation and neutralization. DNA was transferred to Zeta-probe filters (Bio-Rad) under vacuum (BioRad 785 Model vacuum blotter). The filters were pre-hybridized for 4 h, followed by overnight hybridization with the CMV-probe. Filters were washed twice for 10 min in 2x SSC and 0.1% SDS at 65°C. Hybridization signals were detected by phosphorimaging and autoradiography.

RT-PCR analysis

The smar1 transgene was detected by performing RT-PCR on total cell lysates from thymus, spleen and lymph node of transgenic mice as well as the littermate control. The primers used were:

SMAR1-7: 5' GCATTGAGGCAAAGCTGCAAGCTC;
SMAR1-8: 5' CGGAGTTCAGGGTGATGAGTGTGAC;
m-β-actin-a: 5' TACCACGTGGCATCGTGATGGACT;
m-β-actin-b: 5' TTTCTGCATCCTGTCGGCAAT.

RT-PCR assays were done as described previously (34) except that the cDNA was amplified for 27 to 30 cycles (94°C for 1 min, 65°C for 1 min, 72°C for 1 min). RT-PCR products were then separated on a 1.2 % agarose gel and visualized by staining with ethidium bromide. The band intensities corresponding to the RT-PCR products were quantified using a phosphorimager (BioRad) and normalized with respect to the β-actin product.

Probes and primers

The DNA probe (CMV probe) used to screen the transgenic mice was obtained by restriction digestion of pBK-CMV-SMAR1S plasmid with BamHI-NdeI that generated a 0.6 kb fragment specific for CMV promoter only. For TCRβ gene rearrangement
PCRs, the probes used were: TCRβ1 probe corresponding to the Jβ1.6 fragment, obtained by the PCR amplification with the Dβ1-Jβ1 primers; TCRβ2 probe corresponding to Jβ2.6 fragment resulting from the PCR amplification with the Dβ2-Jβ2 primers and Thy1 probe generated by isolating the PCR fragment obtained from amplification with Thy1 primers. The PCR amplification of the indicated probes was followed by their gel purification (Qiagen gel extraction kit). All the probes were labeled using the Random Primed Labeling Kit (Roche, Germany) and [α-32P] dCTP (BARC, India). The PCR primers used were as follows:

- **Dβ1**: 5’-AGCTTATCTGGTGGTTTCTTCCAGC;
- **Dβ2**: 5’-GTAGGCACCTGTGGGGAAGAAACT;
- **Jβ1**: 3’-CTGAAGAAAGGCATTCTGTGTCCAG;
- **Jβ2**: 3’-GTAGAGCTGTCTCCTACTATCGATT;
- **Thy1-F**: 5’-CCATCCAGCATGAGTTCAGC;
- **Thy1-R**: 3’-GCATCCAGGATGTGTTCTGA;
- **Vβ4**: 5’-GAAGCCTCTAGAGTTCATGT;
- **Vβ6**: 5’-GTATCCCTGGATGAGCTGTATCAGCA;
- **Vβ5.1**: 5’-GTCCAACAGTTGTGACTATCAC;
- **Vβ5.2**: 5’-CAGATTCTGGGTTGTCCAGTCTCAA;
- **Vβ6**: 5’-GTATCCCTGGATGAGCTGTATCAGCA;
- **Vβ8.1**: 5’-GTGACATTGAGCTGTACCAGACT;
- **Vβ8.2**: 5’-CCTCATTCTGGAGTTGGCTACCC;
- **Vβ8.3**: 5’-AACACATGGAGGCTGCAGTCACCCAAA;
**PCR amplification for detection of TCRβ rearrangement**

PCRs for TCRβ rearrangements along with Thy1 as a control were performed using genomic DNA of thymus from control and transgenic mice. The primers and PCR condition used as described in Experimental Procedures (34). A brief initial denaturation at 95°C for 2 min was followed by 25 cycles with following steps: 94°C for 1 min, annealing at 56°C for 2 min, extended at 68°C for 3 min. PCR products were separated on a 1.2% agarose gel, blotted onto Zeta-probe membrane (Bio-Rad) followed by Southern analysis. The PCR blots were first hybridized with either TCRβ1 or TCRβ2 probe, then stripped and rehybridized with Thy1 probe. The blots were exposed as described (35, 36) that is, for 30 min (DJβ), 2 h (VDJβ), and 1 h (Thy 1).

**Flow Cytometry and Antibodies**

Single cell suspensions were prepared in RPMI medium from thymus, spleen, lymph nodes of transgenic mice as well as their age-matched control. A total of 5 X 10^5 cells were spun at 1200 rpm at 4°C for 5 min, followed by washing twice with FACS buffer [1x PBS (phosphate buffered saline) containing 2% FBS (fetal bovine serum) and 0.05% sodium azide]. Non-specific binding of antibodies to the cells was prevented by pre-incubating the cells with 30 µg/ml of normal goat serum (Bangalore Genei, Bangalore, India) for 30 min on ice with intermittent shaking. This was followed by staining the cells with appropriate combinations of FITC-or PE-conjugated antibodies for 45 min on ice with intermittent tapping. The monoclonal antibodies used for FACS staining were purchased from BD PharMingen (San Diego, CA). These were as follows: anti-CD3ε-PE (145-2C11), mouse Vβ TCR screening panel (BD PharMingen) containing monoclonal antibodies which recognize mouse Vβ2, 3, 4, 5.1 and 5.2, 6, 7,
8.1 and 8.2, 8.3, 9, 10b, 11, 12, 13, 14, and 17a T-cell receptors, anti-CD4-PE (GK1.5), anti-CD8-PE (53-6.7), anti-CD44-PE (IM7), anti-CD25-PE (PC61), anti-CD8-FITC (53-6.7) and anti-CD25-FITC (7D4). The isotype control antibodies, either FITC- or PE-conjugated, used were: α-mouse, α-rat, or α-hamster. To analyze DN thymocytes, BD™ IMAG anti-mouse CD4 and anti-mouse CD8 particles (BD Biosciences) were used to deplete CD4 and CD8 positive cells from the total thymocytes population. Dead cells and debris were removed by appropriate gating of FSC and SSC. After surface staining, the cells were washed twice with FACS buffer and were fixed with 1% p-formaldehyde. Around 10,000 live cells (propidium iodide negative) were collected for each sample using a FACS Vantage flow cytometer (BD Biosciences, Mountain View, CA) and data was analyzed using CellQuest software (BD Biosciences).

Purification of Double-Negative thymocytes

The total thymocytes were washed with DMEM containing 10% FCS. The cells were spun at 1000 rpm for 5 min at 4°C and the supernatant was discarded. The blocking of cells was performed with rat serum followed by incubation on ice for 20 min. The contaminating double positive thymocytes were eliminated by magnetic depletion with BD™ IMAG anti-mouse CD4 or CD8 antibodies (BD Biosciences). The purified DN (lineage-negative) thymocytes were then stained with CD44-FITC (BD PharMingen) and CD25-PE. The cells were washed with FACS buffer followed by fixation with 1% p-formaldehyde. The cells were then FACS analyzed as mentioned before.
**Northern blot hybridization**

Total cellular RNA was isolated from thymi of transgenic as well as control mice by one-step acid guanidine isothiocyanate-phenol method using Trizol reagent (GIBCO-BRL, USA), precipitated with ethanol and quantitated by spectrophotometry. Twenty micogram of RNA was electrophoresed on 1% formaldehyde denaturing gel, blotted onto Zeta-probe membrane (Bio-Rad) followed by Northern analysis. The blots were then probed with Vβ4, Vβ5.1, Vβ8.1, Vβ8.2, Vβ8.3 and β-actin probes. The probes for all the Vβ's were isolated from respective plasmids clones by *EcoRI-BamHI* digestions.

**Luciferase reporter assays**

Transient transfection assays were performed in T cell line (4980) using luciferase reporter constructs Vβ13-Eβ, Vβ13- MARβ, Vβ13-MARβ-Eβ that was driven by Vβ13 promoter (53). Vβ13-Eβ contains 550-bp core Eβ enhancer whereas Vβ13-MARβ contains 170-mer sequence of MARβ upstream of the reporter gene. In Vβ13-MARβ–Eβ construct, there is an additional one-kilobase fragment within which MARβ and Eβ spans for about 170 and 550 bp respectively (33). For Vβ14 clones, a 1.2 kb BglII-BglII fragment that spans the Vβ14 promoter and was isolated and blunt ended using klenow enzyme. The fragment was then cloned either in the vector alone or upstream of either Eβ enhancer or MARβ-Eβ. The cells were transfected by lipofectamine, employing variable amounts of expression plasmids having luciferase reporter gene. Two micrograms of pRL-CMV (*Renilla* luciferase reporter DNA) was included in all transfections and used to normalize the transfection efficiency. Luciferase activity was assessed using the dual luciferase assay reporter kit (Promega), according to the manufacturer’s instructions and the luciferase activity was measured.
by using Fluoroskan Ascent Luminometer (Labsystems). For all the luciferase assays, the data shown are the mean ± SD of three independent experiments.

RESULTS

Analysis of transgene in mice expressing SMAR1

Previously, we have shown that within a 100 kb region of the TCRβ locus, there are 11 hypersensitive sites (Fig. 1A) (26). Among these sites, HS1/MARβ is a major DNase I hypersensitive site that gets strongly induced during CD4⁻CD8⁻ (DN) to CD4⁺CD8⁺ (DP) stage of thymocyte differentiation (26, 33). MARβ, a matrix associated region located 400 bp upstream of the TCRβ enhancer (Eβ), associates with two MAR-binding proteins SMAR1 and Cux (Fig. 1A; 32, 33). During transition from DN to DP stage of thymocyte development, there is a halt in TCRβ gene rearrangement at DP stage during which MARβ is strongly induced and a parallel upregulation in SMAR1 expression was observed (26, 32). To investigate the role of SMAR1 in V(D)J recombination and T cell development, we generated SMAR1 transgenic mice using a eukaryotic expression vector carrying SMAR1 cDNA as described in Experimental procedures (Fig. 1B). SMAR1 transgene incorporation into the genome was confirmed by Southern blot analysis (Fig. 1C). Upon hybridization with CMV probe, one major band of 3.8-4.0 kb was generated. Among number of positive lines, two independent SMAR1 transgenic lines named T-12 and T-15 were used for breeding purposes (Fig. 1C, lanes 2 and 6). Since most of the results were similar in both the transgenic lines, we have presented the data obtained from T-12 line only. Although thymus of the transgenic mice was slightly smaller than the littermate mice, both spleen and lymph nodes were comparatively much larger in the transgenic mice. To verify the expression
of SMAR1 at mRNA level, total RNA was isolated from thymus, spleen and lymph nodes of both transgenic and littermate control mice and processed for RT-PCR analysis (Fig. 1D). Densitometric analysis demonstrated that compared to the littermate normal, SMAR1 transgenic mice exhibited a three-five fold increase in the smar1s transcript in thymus, spleen and lymph nodes (Fig. 1D) indicating higher expression of SMAR1 in transgenic mice. As a control for equal template concentration used in these two samples, cDNA from both transgenic and normal littermate were processed for RT-PCR using Thy-1 expression. There was no apparent difference in the expression of Thy-1 between these mice (Fig. 1E). Some other tissues form these mice were also checked for SMAR1 expression. Among the other organs checked, higher expression was observed both in kidney and testis (data not shown).

**SMAR1 transgenic mice exhibit organomegaly**

To observe the phenotypic effect of overexpression of SMAR1 in mice, we analyzed transgenic mice of various age groups. At birth and until three months of age, wild type and transgenic mice were indistinguishable. The survival of transgenic mice appeared similar to that of their normal counterparts. After 6 to 8 weeks, the transgenic mice displayed splenomegaly and marked lymphadenopathy but the average body weight remained same as that of wild type. By 8 weeks, all the lymph nodes isolated from the transgenic mice were 3 to 5 folds larger than the control mice. Particularly, the lymph nodes isolated from the inguinal region of SMAR1 transgenic mice (1 cm) were five times bigger than that of the control (2 mm) (Fig. 2A). Compared to control, the size of the spleen was also increased by 1.5-2.5 folds in transgenic mice (Fig. 2E and G). There was no appreciable size difference in other organs of transgenic and control mice.
indicating that overexpression of SMAR1 might be deleterious for the normal development of lymph nodes and spleen.

The histological sections of lymph node depicted severe hyperplasia and infiltration of lymphocytes (Fig. 2B-D). Moreover, in the lymph nodes, the normal distribution of the lymphoid cells within the germinal center was altered. The overall network within the medullar region in transgenic mice was irregular and more compact compared to normal mice thus exhibiting a distinct loss in normal nodal architecture (Fig. 2B-D). Interestingly, lymph node sections in transgenic mice exhibited more number of lymphoid cells in the form of round patches. Compared to the littermate control, spleen in transgenic mice also showed high infiltration of lymphocytes, a characteristic of hyperplasia (Fig. 2F and H). Thus, overexpression of SMAR1 results in architectural alteration in spleen as well as lymph nodes accompanied with their significant enlargement.

**SMAR1 overexpression mildly perturbs T cell development at early DN stage**

Earlier, we have shown that SMAR1 expression is higher during DP stage compared to either DN or SP stage T cells (32). We reasoned that overexpression of SMAR1 in transgenic mice may further elucidate its role in the T cell development. To assess the development of T cells in the thymus of transgenic mice, thymocytes were stained for the surface expression of CD4/CD8 and analyzed by flow cytometry. Compared to the littermate control, in SMAR1 transgenic mice the percentage of cells in the DP (CD4⁺CD8⁺) compartment remained unchanged (~73%) (Fig. 3A-C). There was no significant difference in the population of T cells expressing CD4⁺ SP or CD8⁺ SP cells in transgenic mice (Fig. 3A-C). Interestingly, in transgenic mice both CD4⁺ and CD8⁺ single positive T cells were reduced by 1.5 and 1.8 folds respectively (Fig. 3D and E).
Thus, overexpression of SMAR1 perturbed maturation of either CD4$^+$ or CD8$^+$ T cells in thymus. Since V(D)J recombination of TCR$\beta$ locus occurs in DN subset of thymocyte population (CD4$^-$CD8$^-$CD44$^-$CD25$^+$) (12), the expression profile of CD44 and CD25 was studied. The DN cells were purified as described in Experimental procedures. CD44 versus CD25 FACS profiles of purified DN thymocytes revealed about 2-fold increase in the population of CD44$^+$CD25$^+$ cells in SMAR1 transgenic mice compared to the control mice (Fig. 3F-H). Accumulation of such a significant population of CD44$^+$CD25$^+$ cells in the thymus suggests that overexpression of SMAR1 moderately perturbs the T cell development at an early DN stage.

**SMAR1 affects T cell maturation**

Since SMAR1 transgenic mice exhibit a defect in the T cell development at an early DN stage, it is possible that its overexpression may affect the maturation of T cells. The expression of early T cell markers was checked by FACS analysis of T cells isolated from lymph nodes of either transgenic or littermate control mice. Interestingly, there is a significant increase in the frequency of T cells expressing CD4$^+$CD62L$^+$ (7.3-folds) (Fig. 4A-C), CD4$^+$CD45RB$^+$ (3.4-fold) (Fig. 4D-F) and CD4$^+$CD44$^+$ (2.5-fold) markers (Fig. 4G-I). An equivalent increase in the population of CD4$^+$CD45RB$^-$ and CD4$^+$CD44$^+$ T cells as well suggesting that there is an increase in the number of CD4$^+$ T cells in transgenic mice compared to the littermate control. In other words, even though there is a selective increase in the population of T cells expressing the early markers suggestive of a mild perturbation in the T cell maturation, the increased CD4$^+$ T cell number is consistent with the increased hypercellularity observed in lymph node and spleen of transgenic mice.
Altered frequency of T cells expressing specific V\(\beta\)s in transgenic mice

SMAR1 is highly expressed in DP thymocytes (32) and thus, it is possible that its binding to MAR\(\beta\) (induced during DN to DP transition) might control the V(D)J recombination at the TCR\(\beta\) locus in a stage specific manner. Since, overexpression of SMAR1 results in decreased population of mature T cells, T lymphocytes from thymus and lymph nodes were subjected to surface staining with a panel of V\(\beta\) specific antibodies using mouse V\(\beta\) TCR screening panel (BD PharMingen). Analysis of V\(\beta\) profile in thymus from transgenic mice reveal that there was a substantial decrease in the frequencies of T cells expressing V\(\beta\)5.1/5.2 [3.7-folds], V\(\beta\)8.1/8.2 [4.4-folds], V\(\beta\)8.3 [5.7-folds] and V\(\beta\)13 (3.7-folds) compared to the control (Fig. 5A-D). A similar decrease in the frequency of T cells expressing V\(\beta\)5.1/5.2 [3.0-folds], V\(\beta\)8.1/8.2 [3.6-folds], V\(\beta\)8.3 [7.5-folds] and V\(\beta\)13 (3.4-folds) was detected in lymph nodes from transgenic mice than that of littermate control (Fig. 5E-H). In addition, there was a marginal decrease in majority of the V\(\beta\) specific T cells that include V\(\beta\)s 9, 10\(^b\), 11 and 12 (data not shown). No significant decrease in the number of T cells expressing V\(\beta\)2, V\(\beta\)3, V\(\beta\)14 and V\(\beta\)17\(^a\) was observed in thymus as well as in lymph nodes of transgenic mice (data not shown).

These results suggest that upon overexpression of SMAR1, there is a severe perturbation in the frequency of T cells expressing the V\(\beta\)s that are frequently used in TCR\(\beta\) gene recombination. The reduction in the number of commonly used V\(\beta\) T cells was more prominent in lymph nodes than that of thymus.
Impaired V(D)J rearrangement in SMAR1 transgenic mice

Overexpression of SMAR1 leads to decrease in the number of T cells expressing frequently used Vβs both in thymus as well as lymph nodes. To find out the effect of SMAR1 on TCRβ gene rearrangement and to quantitatively test the differences in the recombination of V, D and J gene segments, PCR assays were carried out. Genomic DNA from thymus of transgenic mice as well as the littermate controls was used as templates. The primers used for PCR amplification are depicted in Fig. 5A and are described in Experimental Procedures. The PCR products were gel fractionated followed by Southern blot analysis. Hybridizations were performed either with Dβ1-Jβ1 (TCRβ1) or Dβ2-Jβ2 (TCRβ2) probes (Fig. 6A) to analyze the effect of SMAR1 on either V-Dβ1-Jβ1 or V-Dβ2-Jβ2 rearrangements respectively. SMAR1 transgenic mice exhibited no change in the rearranged products corresponding to all possible Dβ1 to Jβ1.1-1.5 (Fig. 6B) as well as Dβ2 to Jβ2.1-Jβ2.6 in thymus (Fig. 6C). Consistent with our FACS analysis of T cells from thymus, the V to DJβ rearrangement of frequently used Vβ5.1, 5.2, 8.1, 8.2 and 8.3 genes are significantly reduced compared to the wild type control mice. Depending on the specific Vβ’s mentioned above, there was about 10-20 folds lower amount of recombined products in SMAR-Tg mice samples (Fig. 6B and C). Interestingly, there was no significant difference in the rearrangement pattern of other Vβs such as Vβ4 and Vβ6 (Fig. 6B and C, leftmost two panels). Together, these findings demonstrated that SMAR1 overexpression selectively impaired V(D)J recombination of commonly used Vβs. As an internal control Thy-1 primers were used under identical PCR conditions. To obtain a linear relationship between the amounts of input genomic DNA and the PCR product, the PCR assay was performed with serially diluted template DNA from thymus of both the mice (LM and
Tg) with a limited number of PCR cycles (30) (Fig. 6D). This allowed semi-quantitative comparison of Thy-1 levels found in normal littermate and the SMAR1 transgenic. The level of Thy-1 as a marker of T cells was the same for both normal littermate and SMAR1 transgenic (Fig. 6D), indicating that there was significant decrease in the levels of rearrangement between LM and Tg for the Vβ 5.1, Vβ 5.2, Vβ 8.1, Vβ8.2 and Vβ 8.3 genes.

**SMAR1 acts as a transcriptional repressor**

The locus accessibility generally correlates with the transcription of the particular genes. Decreased V(D)Jβ rearrangement in SMAR1 transgenic mice could be due to poor accessibility of the TCRβ locus to the recombination machinery. Since there was a significant decrease in the frequency as well as rearrangement of T cells expressing the commonly used Vβs, as shown by FACS and genomic PCR analysis, (Fig. 5 and 6), it is possible that the defect could be at the level of poor transcription at the locus. To check the levels of transcription for the specific Vβ’s, the expression levels of transcripts of Vβ4, Vβ5.1, Vβ8.2, Vβ8.3 and Cβ1/ Cβ2 were verified by Northern blot analysis. Total RNA was isolated from thymocytes of littermate control and SMAR1-Tg mice and the respective probes were used to detect the transcripts. Compared to the littermate control there was no significant difference in the level of the transcripts for Vβ4 (Fig. 7, leftmost panel). As expected, there was a strong reduction in the transcript levels for Vβ5.2 (3.2 folds), Vβ8.2 (5.3 folds) and Vβ8.3 (3.1 folds) (Fig. 7). Thus, the constitutive expression of SMAR1 reduced the transcription of specific Vβ’s that are used frequently. On analyzing the expression of Cβ1 and Cβ2 transcripts in transgenic mice compared to the control mice (Fig. 7, rightmost two panels) no apparent
difference could be detected between the two. The reason for this unaltered expression of Cβ1/ Cβ2 transcripts in SMAR1 transgenic mice could be due to compensatory effects introduced by those Vβs that are not affected in the SMAR1 transgenic mice. β-actin PCR was done for normalization of the templates used in the northern analysis. Thus, the data suggest that diminished rearrangement of the commonly used Vβs correlates with their reduced transcription as well as frequency of the T cells expressing the specific Vβs.

**SMAR1 represses transcription through MARβ**

Earlier, we have shown that MARβ, the binding site of SMAR1, functions as a silencer of Eβ mediated transcription (33). The factors contributing to this silencing function were unknown. Since SMAR1 binds to MARβ, it is possible that binding of SMAR1 to MARβ might result in transcriptional repression of Eβ enhancer. To find out the specific role of SMAR1 in transcription, transient transfection assays were performed in T cell line (4980) using luciferase reporter constructs pGL2 vector, pGL2-MARβ, pGL2-Eβ, and pGL2- MARβ-Eβ that are driven either by Vβ13 promoter (33) (Fig. 8A) or by Vβ14 promoter (Fig. 8B). The detail of Vβ14 constructs is mentioned in the Experimental procedures. MARβ alone contained only 170 mer AT rich sequence that binds to SMAR1 (32). No appreciable transcriptional activity was observed when the cells were transfected with either the control pGL2 vector or pGL2-MARβ either in absence or in presence of SMAR1 (Fig. 8C, lanes a-d). In the presence of increasing amount of SMAR1, there was no change in the transcriptional activity. In the presence of Eβ enhancer alone there was about 5.5 folds higher transcriptional activity (Fig. 8C, lane e). On increasing the amount of SMAR1, we could see a very small reduction in
the transcriptional activity (Fig. 8C, lanes f and g) indicating that presence of SMAR1 did not change the transcriptional activity in the presence of enhancer alone. When the pGL2-MARβ-Eβ containing plasmid driven by Vβ13 promoter was transfected, transcriptional activity was about 6.5 folds higher than that of basal promoter (Fig. 8D, lanes a and b). Importantly, on cotransfecting with an increasing amount of SMAR1 reporter plasmid along with pGL2-MARβ-Eβ, a drastic reduction in the transcriptional activity was observed (Fig. 8D, lanes c-f). In the presence of highest SMAR1 concentration, the transcriptional activity came down almost near to basal level of transcription. Thus the effect of SMAR1 on Vβ13 promoter correlates with the decreased transcription along with reduced frequency of Vβ13 expressing T cells in the transgenic mice. Since the frequency of Vβ14 increases in transgenic mice, the effect of SMAR1 was then analyzed on Vβ14 promoter in the presence of Eβ enhancer. In the presence of Vβ14-Eβ enhancer alone, there was about seven-fold higher transcriptional activity (Fig. 8E, lane b). On increasing the amount of SMAR1, we could see a very minor reduction in the transcriptional activity (Fig. 8E, lanes c and d) compared to Vβ13 promoter. At the highest concentration of SMAR1, there was only 1.2 fold of decrease in the transcription indicating that presence of SMAR1 did not appreciably change the transcriptional activity in the presence of enhancer alone. As expected, there was a minor effect on the Eβ transcription when SMAR1 was cotransfected alongwith Vβ14-MARβ-Eβ (Fig. 8E, lanes e-g). Thus, the transcription silencing activity of specific Vβs corresponded with their decreased frequency in transgenic mice of respective T cells. These data suggest that the repressor activity of SMAR1 depends on the presence of MARβ upstream of the Eβ enhancer through which it controls the Eβ transcription, which in turn regulates the transcription of
specific Vβ promoters. Since SMAR1 is overexpressed during the DP stage of thymocyte development (32), it is possible that during this stage when HS1/MARβ is induced (32), SMAR1 is directly recruited at the site in turn inhibiting Eβ enhancer mediated transcription.

DISCUSSION

The *cis* regulatory elements have been known to control the recombination and transcription of antigen receptor genes (8, 37). T cell receptor beta (TCRβ) enhancer, Eβ, is one such *cis* element that has been extensively studied with respect to its role in the regulation of V(D)J recombination and transcription by both deletion and transgenic mouse studies. The role of enhancer in the maintenance of local accessibility is critical and in its absence recombination is halted resulting into blockage of lymphoid cell division (38-40). The function of any enhancer is controlled by various positive and negative regulatory elements (acting either in *cis* or in *trans*) that may either promote or inhibit its activity. MARs are one of the *cis* elements that closely associate with promoters and enhancers and are often found to flank antigen receptor genes (27). MARs either promote (41) or inhibit transcription (33) depending on the context. Earlier, we have reported identification of a new MAR (MARβ), a 170 bp AT rich sequence, present 400 bp proximal to the Eβ enhancer (26, 33). MARβ is one of the 11 DNaseI hypersensitive sites that get induced during DP stage of T cell development (26). Our report showed that MARβ provides docking site for a novel MARBP, SMAR1 (32) along with two other well-known MARBPs - Cux/CDP and SATB1. SMAR1 is abundantly expressed in the DP thymocytes that commensurate with high accessibility of MARβ as well as halting of V(D)J recombination at the DP
stage thereby suggesting a role of SMAR1 in the early T cell development (32, 33). In order to delineate specific role of SMAR1 in the T cell development as well as in the regulation of V(D)J recombination, transgenic mice were generated that express SMAR1 in a tissue independent manner.

Our data show for the first time that the MAR\(\beta\) binding protein, SMAR1, is directly associated with the recombination of antigen receptor genes in the T cells. The results from our analysis of the stage specific thymocyte population indicate that overexpression of SMAR1 perturbs the T cell development during transition from DN to DP stage. Thus, SMAR1 overexpression results in generation of T cells that tend to remain in the early stage. Even though SMAR1 transgenic mice exhibit a selective increase in the population of T cells expressing early markers such as CD4/CD62-L, CD4/CD45RB and CD4/CD44 but on analyzing the CD4\(^+\)CD45RB\(^-\) and CD4\(^+\)CD44\(^-\) population of T cells, it appears that indeed there is an increase in the number of CD4\(^+\) T cells. The phenotype of SMAR1 transgenic mice exhibit marked organomegaly of lymph nodes and spleen accompanied by follicular hyperplasia, suggestive of a hyperresponsive immune system. This increased cellularity of the lymphoid organs can be attributed to the increase in the CD4\(^+\) T cell population observed in these organs.

MAR sequences are distributed every 10 kb of human genome and MAR binding proteins upon interaction to these MAR sequences alter the chromatin structure. Earlier it was shown that the accessibility of the receptor loci alters the V(D)J recombination (16). MARs and other cis elements have been implicated in maintaining the locus accessibility (26, 33 and 42). Since SMAR1 is expressed more at the DP stage of T cell development, we analyzed the effect of SMAR1 transgene on the antigen receptor gene loci in terms of its usage and recombination. The most critical feature exhibited by SMAR1 transgenic mice is the decreased frequency of T cells
expressing commonly used Vβs, particularly, Vβ5.1, 5.2, 8.1, 8.2 and 8.3 in thymus and lymph nodes. Interestingly, these Vβs are present more than one-third of the entire population of peripheral T cells in normal mice. In addition, SMAR1 also significantly reduces the frequency of a few other Vβs including Vβ9, Vβ10β, Vβ11, Vβ12 and Vβ13. Importantly, the decreased frequency of T cells expressing the commonly used Vβs correlated with their diminished somatic gene rearrangement in the transgenic mice compared to the littermate control. There was no gross change in the recombination frequency of Dβ to Jβ indicating that the overexpression of SMAR1 affects recombination only from V to DJ but not from D to J locus. Since transcriptional promoters have been implicated as essential regulators of V(D)J recombination (43), it is possible that SMAR1 either directly or indirectly (by recruiting other factors) might control the enhancer function that in turn regulates the promoter activity of individual Vβs, thereby controlling recombination as well as transcription at the locus. The impairment in the rearrangement of the frequently used Vβs was reflected by their reduced transcription at mRNA level. This implies that the reduced transcription observed is due to less number of T cell expressing the specific Vβs in transgenic mice. Thus after the incorporation of SMAR1 transgene, the frequency as well as transcription of the commonly utilized Vβs are affected thereby resulting into their reduced rearrangement.

Earlier, it was demonstrated in reporter gene assays that MARβ silences the TCRβ enhancer mediated transcription (33) suggesting, therefore, involvement of associated trans factors that may interact with enhancer and repress transcription. SMAR1 exhibits significant sequence homology with the MAR-binding domain of SATB1, Cut repeats of Cux and SATB1 and with tetramerization domain of Bright
SATB1 is a more T cell specific protein; Bright being specific for B cells whereas Cux exhibits ubiquitous expression. Cux and SATB1 have been known to function as strong transcriptional repressors (30, 31) whereas Bright functions as a transcriptional activator (44). Both Cux and SATB1 proteins play significant role in various processes such as chromatin remodeling, tissue-specific gene regulation, and cell cycle progression, specifying cell fates during cell development and differentiation as well as tumor-specific metabolism (45-48). Since, SMAR1 shares homology with transcriptional repressors Cux and SATB1 and all of them interact with MARβ, it is possible that SMAR1 might function in a coordinated manner through interaction with these proteins at MARβ thereby inducing the silencing function of MARβ. To decipher the role of SMAR1 with respect to Eβ-mediated transcription, transient transfection assays were performed on DN T cell line using Vβ13 or Vβ14 driven luciferase reporter constructs containing either Eβ or MARβ or MARβ-Eβ. Interestingly, these results show that SMAR1 upon interaction with MARβ reduces the Eβ-mediated transcription of the promoter of that TCR Vβ whose frequency as well as rearrangement is decreased in transgenic mice. These results can be extrapolated to those frequently used Vβs that were significantly reduced in SMAR1 transgenic mice. On the other hand, there is no significant effect on the transcription of that Vβ whose frequency is increased in SMAR1 transgenic mice. Overall the data points out to the critical role played by SMAR1 in regulating Eβ-mediated transcription of specific Vβs.

Our results regarding the transcriptional repressor role played by SMAR1 through interaction with MARβ is supported by our recent observation wherein we show that SMAR1 physically associates with negative regulator Cux, especially through its Arginine-Serine rich (RS) domain, and both the proteins synergistically
function to repress Eβ mediated transcription (42). Overexpression of both SMAR1 and Cux results in chromatin modulation of MARβ thereby increasing its accessibility to DNaseI both in T as well as non-T cells (42). Both the proteins form a ternary complex with MARβ and negatively regulate the transcription mediated by Eβ enhancer. Previously, it was shown that at DP stage of T cell development, V(D)J recombination is halted and during this stage MARβ gets induced. Moreover, at DP stage SMAR1 is abundantly expressed (32) and recently it was reported that expression of CDP/Cux p75, a spliced variant, was at a higher level in the thymus in CD4+/CD8+ T cells (49). Interestingly, both SMAR1 and Cux were shown to interact with MARβ in DP T cells (42). Therefore, in a time specific window of T cell development, the cellular machinery recruits negative regulators in the form of SMAR1 and Cux at MARβ that may either independently or in a concerted manner regulate T cell development through maintaining locus accessibility of the particular gene segments and in effect control the V(D)J recombination via Eβ regulation.

The mechanistic model for SMAR1 mediated perturbation of V(D)J recombination can be hypothesized in two independent ways. One, SMAR1 might function as a potent repressor controlling the Eβ enhancer (Fig. 7) function together with negative regulator Cux/CDP protein through recruitment of HDACs. In fact, we have recently found that SMAR1 recruits HDAC1 at cyclin D1 promoter resulting into strong repression of the promoter (Rampalli et al., unpublished data). It was found that in various breast cancer cell lines where SMAR1 is less expressed, cyclin D1 expression is induced. Thus, SMAR1 might function as a repressor for other genes as well and function through recruitment of HDAC1. The second model could be that SMAR1 works through the MARs present next to V gene segments as shown in Figure
10. In support of this hypothesis, recently it was reported that MAR sequences are distributed more frequently in the V regions (48), particularly in the 5’ region of commonly used Vβs such as Vβ8.1, Vβ8.2 and Vβ5.1. In the mouse genome, Vβ5.1, Vβ8.2 and Vβ8.3 gene segments exist in close proximity within a region of 6.0 kb. Interestingly, these MARs are shown to be putative sites for Cux/CDP binding. Since, SMAR1 and Cux are recruited together through their direct association with MAR sequences, it is possible that upon overexpression of SMAR1 in transgenic mice, these two MAR binding proteins together alter chromatin at these MARs and thus perturb the recombination events at this loci. Thus, SMAR1 may either directly or indirectly in the form of a complex with enhancer cross talk with the MAR sites close to the specific Vβ sequences mentioned. Such a combinatorial effects may thus perturb the recombination of the specific Vβs that has frequent usage during recombination (Fig. 9).

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FIGURE LEGENDS

FIG. 1. Expression of smar1 in transgenic mice. (A) TCRβ locus showing various gene segments and DNaseI hypersensitive sites. Total of eleven sites distributed with the 100 kb region of TCRβ locus. The HS1/MARβ site present upstream of Eβ enhancer is shown by solid circle while hollow circles show other hypersensitive sites. Both SMAR1 and Cux were shown earlier to be bound at MARβ. (B) Plasmid map of pBK-CMV-SMAR1 showing the 3.7 kb SMAR1 and 0.6 kb CMV promoter. DNA fragment containing CMV-SMAR1 was injected into the pronucleus of fertilized mouse eggs to generate SMAR1 transgenic mice as described in Methods. A 0.6 kb BamHI-NdeI probe corresponding to the CMV-promoter was used as a probe for screening transgenic mice. (C) Southern blot analysis of tail DNA for identifying smar1 transgene upon digestion of genomic DNA with EcoRI plus HindIII and hybridization with CMV probe. The blot shows many transgenic lines including T-12 and T-15 those were used for all experimental purposes. The CMV-probe hybridized with a major band of about 3.8 kb confirming the presence of CMV promoter alongwith the smar1 transgene. D. RT-PCR analysis of smar1-specific mRNA (upper panels) in thymus, lymph nodes and spleen from SMAR1 non-transgenic littermate (LM) or transgenic (Tg) mice. SMAR1 specific primers were used as discussed in Experimental Procedure. Lower band corresponds to RT-PCR product of β-actin used as loading control. (E) RT-PCR analysis of Thy-1 specific mRNA in thymus, lymph nodes and spleen from SMAR1 non-transgenic littermate (LM) or transgenic (Tg) mice.
FIG. 2. Architectural defect in lymph node and spleen sections. (A) Lymph node size of non-transgenic littermate (LM) and transgenic (Tg) mice are shown at the same scale. The lymph node of the transgenic mice was 5 times bigger than the control mice (B) Histological analyses of lymph node from control littermate and at 10X and 40X magnification are displayed. (C) and (D) Lymph node sections of transgenic mice at 10X and 40X magnification respectively. The round patches with dark stain shows higher infiltration of lymphoid cells indicating hyperplasia. (E) Direct photograph of spleen from control mice. (F) Histological sections at 10X and 40X magnification upon staining with HE. (G) Photograph of spleen from SMAR1 transgenic mice. (H) Histological sections of spleen SMAR1 transgenic mice at 10X and 40X magnifications.

FIG. 3. Defective T cell maturation in SMAR1 transgenic mice. (A) and (B) CD4/CD8 profile of thymi from non-transgenic littermate (LM) and transgenic (Tg) mice. (C) Plot showing the number of CD4^+ /CD8 (DP) cells, (D) CD4^+ SP and (E) CD8^+ SP thymocytes, respectively. Numbers in the quadrant showing the percentage of specific for CD4 and CD8 markers. (F) and (G) Thymocytes isolated from thymus of either normal (LM) or transgenic (Tg) were processed for staining with early markers CD25 and CD44. The percentage of cells and percentage are mentioned inside the quadrants. (H) FACS stained cells numbers were plotted as bar graphs. Relative value in all activities are presented as the mean +/- SD of three independent experiments *, p < 0.05.

FIG. 4. FACS profile of T cells from lymph nodes expressing developmental markers. (A) Lymph nodes cells from either control littermate (LM) or transgenic (Tg) mice
were stained with either (A-B) CD4 and CD62-L. (D-E) CD4 CD45RB and (G-H) CD4 CD44. The cells were processed for FACS analysis and plotted as bar graphs. Plot showing the number of CD4 and CD62-L (C) cells, (F) CD4 CD45RB and (I) CD4 CD44 cells respectively. These results are representative of a single mouse from a set of three mice either from control or SMAR1-Tg mice.

**FIG. 5.** Flow cytometric analysis of Vβ expression on T cells from thymus and lymph nodes. (A) Whole thymocytes from SMAR1 transgenic mice or control mice were surface stained for anti-CD3ε-PE and anti-Vβ-FITC antibodies. Numbers on plots are the frequency of cells lying within the indicated regions. Transgenic mice expressing SMAR1 shows diminished frequencies of T cells corresponding to Vβ5.1/5.2, Vβ8.1/8.1 Vβ8.3 or Vβ13. (B) T cells from lymph nodes of SMAR1 transgenic mice or littermate control mice were surface stained for anti-CD3ε-PE and anti-Vβ-FITC antibodies. Numbers on plots are the frequency of cells lying within the indicated regions. The frequency of indicated Vβs is significantly reduced in lymph node of SMAR1 transgenic mice. These results are representative of analysis from single mouse from a set of three mice either from control littermate or Transgenic.

**FIG. 6.** Defective V(D)J gene rearrangement in SMAR1 transgenic mice. (A) Schematic diagram showing TCRβ region containing Vβ, Dβ, Jβ and Cβ gene segments. The arrows showing the regions of primers used. Forward and Reverse primers used for PCR analysis is shown by the directions. The PCR products were run on an agarose gel as described in the Methods. The DNA fragments were transferred onto nitrocellulose membrane and hybridized using Vβ, Dβ1 or Jβ1 probes. (B) Southern blots of PCR products obtained by using genomic DNA of thymi from non-
transgenic (LN) and transgenic (Tg) mice. Jβ1 primer along with primers for either 
Dβ1 or various Vβs such as Vβ6, 5.1, 5.2, 8.1, 8.2 and 8.3 were used. One microgram 
of template was used for both normal and transgenic mice. Thy1 was used as a loading 
control using the same amount of genomic DNA as a template. Unlike other Vβs, there 
was no significant defect in the rearrangement pattern of Vβ6 in the transgenic mice 
compared to the control mice. (C) Southern blots of PCR products obtained by using 
genomic DNA of thymi from control and transgenic mice. Jβ2 primer along with 
primers for either Dβ2 or various Vβs such as Vβ5.1, 5.2, 8.1 and 8.3 were used. Thy1 
was used as a loading control using the same amount of genomic DNA as a template. 
Like Vβ-Dβ1-Jβ1 gene rearrangement, Vβ-Dβ2-Jβ2 gene rearrangement was also 
defective in the transgenic mice. (D) Semi-quantitative PCR for Thy-1 using serially 
diluted genomic DNA from thymus of both normal littermate (LM) and SMAR1 
transgenic mice (Tg).

FIG. 7. Expression of RNA for specific Vβ’s, Cβ1 and Cβ2. Total RNA was isolated 
from thymocytes of LM and Tg mice by Trizol method (Qiagen). Twenty 
microgram RNA was loaded on 1% formaldehyde gel and processed for Northern blot 
analysis using either Vβ4, Vβ5.1, Vβ8.2, Vβ 8.3 or Cβ probe. β-actin probe was used 
to hybridize same filter shown in the bottom panels. The β-actin expression showing 
equal gel loading. These results are representative of three independent mice either 
from control littermate (LM) or SMAR1-Tg mice. The results shown in the bar graph 
represents the mean +/- SD of three independent experiments *, p < 0.05.
**FIG. 8.** (A) and (B) Schematic diagram of plasmids corresponding to either Eβ or HS1/MARβ under the control of Vβ13 or Vβ14 promoters. (C) 5 x 10^5 cells (4980) were transfected with reporter constructs pGL2 or pGL2-Eβ or pGL2-MARβ-Eβ in absence or in presence of increasing amounts of SMAR1. The lysates were made as described in Methods and Luciferase assays were performed. For MARβ or Eβ constructs, either 5 or 10 μg of SMAR1 expressing plasmid DNA. (D) For cotransfection with Vβ13-MARβ-Eβ, 1-10 μg of SMAR1 plasmid was used. Lanes, a and b show relative luciferase activity in the absence of SMAR1 whereas lanes d-f exhibit transcription in the presence of increasing amounts of SMAR1. (E) Similarly, 5x10^6 P4980 cells were cotransfected with either Vβ14-Eβ or Vβ14-MARβ-Eβ alone or along with increasing amounts of SMAR1 (5-10μg). Lanes, b and e show relative luciferase activity in the absence of SMAR1 whereas lanes c-d and f-g exhibit transcription in the presence of increasing amounts of SMAR1. The SD value was calculated from minimum of 3 independent results done in triplicate. The mean +/- SD used, were generated from three independent experiments *, π < 0.001.

**FIG. 9.** Schematic diagram of TCRβ locus depicting a model that can explain the mechanism and control of V(D)J recombination by SMAR1-Cux complex. The thick bend line shows the blockage of Eβ enhancer by SMAR1-Cux repressor complex. The straight arrow shows the possible cross-talk between SMAR1-Cux complex and the MAR sequences flanking the respective Vβ regions. The arrangement of the Vβ 8.3-5.3P segments within the 6.0 kb fragment is shown. Vβ segments are shown by hollow rectangles and MARs next to the specific Vβ segments are shown by black triangles.
Open circles and numbers show the various DNaseI hypersensitive sites. The solid circle represents position of Eβ enhancer.
FIGURES

Fig. 1A-E; Kaul-Ghanekar et al.
Fig. 2A-H; Kaul-Ghanekar et al.
Fig. 3A–H; Kaul-Ghanekar et al.
Fig. 4A-I; Kaul-Ghanekar et al.
Fig. 5A-H; Kaul-Ghanekar et al.
Fig. 6A-D; Kaul-Ghanekar et al.
Fig. 7; Kaul-Ghanekar et al.

Fig. 8A-E; Kaul-Ghanekar et al.
Fig. 9; Kaul-Ghanekar et al.
Abnormal V(D) J recombination of T cell receptor β locus in SMAR1-transgenic mice
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