Expression and Presentation of Endogenous Mouse Mammary Tumor Virus Superantigens by Thymic and Splenic Dendritic Cells and B Cells[1]

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Tolerance against superantigens (SAgs) encoded by endogenous mouse mammary tumor virus (*Mtv*) loci involves the intrathymic deletion of SAg-reactive T cells expressing a particular TCR Vβ-chain, presumably upon presentation of the SAg by specialized APC. However, although the role of dendritic cells (DC) in the induction of tolerance against conventional Ags has been demonstrated, little is known about the role played by DC in tolerance induction against *Mtv* SAgs. Moreover, there is conflicting evidence concerning the capacity of DC to express and present *Mtv* SAgs. In this report we have analyzed the expression of *Mtv* SAgs in highly purified thymic and splenic DC and B cells by reverse transcriptase-PCR, using primers amplifying *Mtv* SAg-specific spliced mRNAs. DC express *Mtv* SAgs at levels comparable to B cells, but display a differential expression pattern of the various *Mtv* loci compared with B cells. Furthermore, our results show that DC are able to induce the deletion of SAg-reactive thymocytes in an in vitro assay, indicating that *Mtv* SAgs are functionally expressed on the DC surface. Collectively, our data are consistent with the hypothesis that DC play a role in the induction of intrathymic tolerance to *Mtv* SAgs. *The Journal of Immunology,* 1996, 157: 2789–2794.

Superantigens (SAgs)[4] encoded by an open reading frame (ORF) in the 3′ long terminal repeat (LTR)[4] of both endogenous and infectious mouse mammary tumor viruses (1, 2) were first described as minor lymphocyte stimulatory (Mls) Ags (3). Mls tolerance is induced in mice expressing the relevant endogenous *Mtv* SAg by intrathymic clonal deletion of T cells bearing SAg-reactive TCR Vβ elements (4, 5); however, the identity of the cells responsible for Mls tolerance induction within the thymus remains to be defined. In this sense, the ability of peripheral B cells to stimulate Mls-reactive T cells has been firmly demonstrated (6), but little is known about the participation of their thymic counterparts in the induction of tolerance against SAgs, although recently, Inaba et al. (7) reported that neonatal intrathymic injection of purified thymic B cells expressing the *Mtv*-7 SAg (Mls-1^a) induced clonal deletion of Vβ6$^+$ T cells. In addition, there is conflicting evidence on whether DC, which are highly efficient as APCs for conventional Ags, can express *Mtv* SAgs and thereby contribute to Mls tolerance. Whereas Webb et al. (8) found that splenic DC were poor stimulators of Mls-1^a-reactive T cells, the potential of DC to elicit anti-Mls responses in MLR assays has been described by Sunshine et al. (9) and by Inaba et al. (7). Moreover, Mazda et al. (10) reported that both splenic B cells and DC are required to induce clonal deletion of Vβ6$^+$ T cells in fetal thymic organ cultures, and Bhardwaj et al. (11, 12) have shown that DC are 10- to 50-fold more efficient than B cells as APCs for microbial SAgs.

Recently, two different groups have analyzed the expression of *Mtv* SAgs by DC at the mRNA level (13, 14). The results obtained are again controversial; whereas Jarvis et al. (13) did not detect the 1.7-kb spliced mRNA product predicted to encode *Mtv* SAgs in purified DC, Moore et al. (14) observed a differential expression of *Mtv* loci in DC derived from 7-day-old thymic organ cultures. In the latter study, *Mtv* mRNA expression was examined by reverse transcriptase-PCR using LTR-specific primers, allowing amplification of all *Mtv* transcripts including a 8.5-kb full-length genomic transcript and a 3.5-kb transcript encoding the envelope gene. However, the *Mtv* SAg is most likely translated from a 1.7-kb subgenomic mRNA initiated within the 5′ LTR and spliced to a site immediately upstream of the 3′ LTR (15, 16). Importantly, this 1.7-kb spliced transcript is expressed at much lower levels than the full-length genomic RNA transcript (17).

Therefore, in the present report we have analyzed the expression of *Mtv* SAg mRNA in highly purified thymic and splenic DC and B cells by reverse transcriptase-PCR using primers specifically amplifying the spliced 1.7-kb message. The SAg expression from several endogenous *Mtv* loci has been assessed simultaneously by taking advantage of size heterogeneities in the SAg COOH-terminal sequences (allowing the identification of particular *Mtv* loci on a sequencing gel after digestion with the restriction enzyme *RsaI*) as recently described by Waanders et al. (18). Finally, the capacity of DC to present *Mtv* SAg in a functional form has been assessed by testing their ability to induce the deletion of SAg-reactive thymocytes in an in vitro assay.

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[4] Abbreviations used in this paper: SAg, superantigen; ORF, open reading frame; LTR, long terminal repeat; Mls, minor lymphocyte stimulatory antigen; *Mtv*, endogenous mouse mammary tumor virus; DC, dendritic cell; DP, double positive.

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Materials and Methods

Mice

DBA/2 and BALB/c mice were purchased from HO Harlan OLAC Ltd. (Bicester, U.K.). *Mtv*-7$^+$ (Mls-1^a) congenic BALB.D2 mice were bred from breeding pairs originally obtained from Dr. H. Festenstein (London Hospital Medical College). P14 TCR-$\alpha\beta$ transgenic mice (Vα2-JαTA31/Vβ8.1DJβ2.4) expressing a TCR with double specificities for lymphocytic choriomeningitis virus and the *Mtv*-7 SAg (19) were obtained from Dr. H. Pircher (Institute of Experimental Pathology, University Hospital, Zurich, Switzerland) and crossed on a BALB/c background. In all experiments, 5- to 6-wk-old female mice were used.

Antibodies

The following mAbs were used: KT3–1.1, anti-CD3; GK1.5 and CT-CD4, anti-CD4; CT-CD8, anti-CD8; III-5 and AT83, anti-Thy-1; PC61.5, anti-IL-2Rα; C1.A3–1, anti-macrophage Ag F4/80; RB6.8C5, anti-granulocyte Ag Gr-1; TER-119, anti-erythrocyte Ag TER-119, FD11–54.3, anti-MHC class II; RA3–6B2, anti-B220; and NLDC-145, anti-DC marker NLDC-145.

Isolation of DC and B cells

Thymic and splenic DC were purified as previously described (20). Thymuses and spleens were cut into small fragments and then digested with collagenase (0.5 mg/ml)) and DNase I (1 mg/ml) (Boehringer Mannheim, Mannheim, Germany) in DMEM (adjusted to mouse osmolarity and supplemented with HEPES buffer, pH 7.2) for 10 min at 37°C with continuous agitation. Digested fragments were filtered through a stainless steel screen, and the cell suspension was washed in DMEM at 37°C and recovered by centrifugation at 500 × *g* for 7 min. The cells were then resuspended in cold isosmotic metrizamide solution (Nyegaard Diagnostics, Oslo, Norway), pH 7.2 (density, 1.068 g/cm^3), containing 5 mM EDTA to dissociate DC-thymocyte complexes, and a low density cell fraction, accounting for 1 to 2% of the starting cell population, obtained by centrifugation at 1700 × *g* for 10 min, and washed in PBS containing 5 mM EDTA and 5% FCS (PBS-EDTA-FCS) supplemented with HEPES buffer, pH 7.2. T lineage cells, macrophages, granulocytes, and erythrocytes were depleted by treating the recovered low density cells for 30 min at 4°C with a mAb mixture including anti-CD3, anti-CD4, anti-Thy-1.2, anti-IL-2Rα, anti-macrophage Ag F4/80, anti-granulocyte Ag Gr-1, and anti-erythrocyte Ag TER-119, then magnetically removing the unwanted cells after incubation for 30 min at 4°C with anti-rat Ig-coated magnetic beads (Dynabeads, Dynal, Oslo, Norway), using a 10:1 bead to cell ratio.

The DC-enriched preparation obtained after immunomagnetic bead depletion was double stained with FITC-conjugated anti-B220 mAb and biotinylated anti-MHC class II mAb plus streptavidin-phycoerythrin (Caltag, San Francisco, CA). All staining steps were performed at 0 to 4°C in PBS-EDTA-FCS. Propidium iodide (Calbiochem, La Jolla, CA) was included at 10 μg/ml in the final wash to selectively stain dead cells.

Thymic and splenic DC were then sorted (Fig. 1) as MHC class II^{high}B220$^-$ cells on a FACStar flow cytometer (Becton Dickinson Co., Mountain View, CA). Thymic and splenic B cells were sorted from the same DC-enriched preparation as MHC II^{high}B220$^+$ cells (Fig. 1). Alternatively, splenic B cells were purified by depletion of T cells by complement-mediated cytotoxicity using the anti-Thy-1 mAb AT83 and sorting for B220$^+$ cells after staining with FITC-conjugated anti-B220 mAb. Analysis of MHC class II, B220, and NLDC-145 DC Ag expression confirmed that the sorted cell preparations had a purity >96% for DC (Fig. 1) and >98% for B cells (data not shown).

PCR analysis

Cellular RNA was isolated from 5 × 10^5 thymic DC or 10^6 splenic DC or thymic or splenic B cells, (supplemented with 10^6 COS monkey kidney cells as carrier cells), by denaturation with guanidinium isothiocyanate, separation of the RNA by ultracentrifugation on a cesium chloride gradient, and acid-phenol extraction (21). COS carrier cells were not used for isolating RNA from B cells purified by complement-mediated cytotoxicity. Purified RNA was resuspended at 1 μg/μl, and the reverse transcriptase reaction was performed with 1 μl RNA solution as described previously (22), omitting reverse transcriptase in the control samples. In this study two different sets of primers were used to compare the different patterns of expression obtained when using primers amplifying all *Mtv* transcripts or specifically the 1.7-kb spliced subgenomic mRNA encoding the SAg. Oligonucleotides to amplify all transcripts were chosen on the basis of a high degree of sequence conservation between the 3′ LTR of various *Mtv* loci. In particular, the 5′ oligo ORF-100 (CTCAGGAAGAAAAAGACGAC AT) was used in combination with the 3′ oligo VJ71 (CCCAAACCAAG TCAGGAAACCACTTG), yielding a 250- to 290-bp PCR product. To specifically amplify the 1.7-kb spliced mRNA the 5′ oligo *orf* mRNA (CAGGGAACTGCAGTCTCGCCTA) corresponding to the 5′ noncoding region at the 5′ splicing site was used in combination with VJ71, yielding a 1.1-kb PCR product. Longer cDNA molecules are not amplified under these conditions. For PCR, cDNA was boiled for 3 min in the presence of the oligonucleotides; the conditions for PCR were 1 min at 60°C, 1 min at 72°C, and 1 min at 93°C for 40 cycles and 7 min at 72°C in 1× PCR buffer containing 20 mM Tris-HCl (pH 8.4), 3 mM $MgCl_2$, 100 mM KCl, 0.02% gelatin, and 0.3 mM dNTP. [α-^{32}P]ATP (6 μCi) and 5 U of Taq polymerase (AmpliTaq, Perkin-Elmer Corp., Pomona, CA) were added per PCR reaction. The PCR products were digested for 45 min at 37°C with 1 U *Rsa*I/reaction (Boehringer Mannheim). The ^{32}P-labeled *Rsa*I-digested PCR products were then size fractionated on a 6% denaturing polyacrylamide sequencing gel for 4 h using standard techniques. After fixation, the dried gels were autoradiographed on Kodak X-OMAT films (Eastman Kodak, Rochester, NY) and used to expose phosphor screens to perform a signal quantitation using a PhosphorImager SF (Molecular Dynamics, Inc., Sunnyvale, CA). The relative expressions of the different endogenous DBA/2 *Mtv*s (*Mtv*-1, -6, -7, -8, -11, -13, -14, and -17) were assessed by taking advantage of length polymorphisms in the SAg COOH-terminal sequences. The size of the *Rsa*I-digested PCR products obtained from SAg mRNAs is 214 nucleotides for *Mtv*-1, *Mtv*-6, and *Mtv*-13 (*Mtv*-1, -6, -13 locus group), 239 nucleotides for *Mtv*-8 and *Mtv*-11 (*Mtv*-8, -11 locus group), and 254 nucleotides for *Mtv*-7. Finally, the *Mtv*-14 SAg is not expressed in thymocytes and splenocytes (23) and *Mtv*-17 SAg is not transcribed due to a defect in its promoter (24).

In vitro deletion experiments

Single cell suspensions of thymocytes were prepared by squeezing the whole thymuses through a wire screen. Thymocytes (2 × 10^5/well) from *Mtv*-7-reactive P14 TCR-$\alpha\beta$ transgenic mice were cultured with purified thymic DC or splenic B cells (10^5/well), from either BALB/c (H-2^d, *Mtv*-7$^-$) or BALB/D2 (H-2^d, *Mtv*-7$^+$) mice, in RPMI medium supplemented with 10% FCS in 96-well plates for 24 h. Deletion of double positive (DP) thymocytes was determined after double staining with phycoerythrin-conjugated anti-CD4 mAb and FITC-conjugated anti-CD8 mAb (Caltag) and analyzed on a FACScan flow cytometer (Becton Dickinson). Propidium iodide (Calbiochem) was included at 10 μg/ml in the final wash to selectively stain dead cells. APCs were excluded in the analysis of thymocytes on the basis of their CD4 vs CD8 profile. The percent deletion of DP thymocytes was calculated using the formula 100 × (1 − % DP with APCs/% DP without APCs).

Results and Discussion

Endogenous Mtv loci in DBA/2 mice

The endogenous Mtv loci present in DBA/2 mouse genomic DNA were analyzed by PCR using the ORF-100/VJ71 set of primers by taking advantage of size heterogeneities in the SAg COOH-terminal sequences, as previously described by Held et al. (25). When separated on a sequencing gel, the *Rsa*I-digested PCR products obtained from the different *Mtv* loci gave the electrophoretic pattern shown in Figure 2: a 258-nucleotide band corresponding to *Mtv*-14; a 254-nucleotide band corresponding to *Mtv*-7; a 239-nucleotide band corresponding to *Mtv*-8, *Mtv*-11, and *Mtv*-17; and a 214-nucleotide band corresponding to *Mtv*-1, *Mtv*-6, and *Mtv*-13. The precise size of the *Rsa*I-digested PCR product obtained with the indicated primers for *Mtv*-14 was calculated after sequencing the *Mtv*-14 SAg (M. Braun and H. Acha-Orbea, unpublished observations). The relative positions of the *Mtv*-14 and *Mtv*-7 bands were confirmed by comparing the band pattern obtained with DBA/2 and BALB.D2 genomic DNA, the latter containing only *Mtv*-6, *Mtv*-7, *Mtv*-8, and *Mtv*-9 (Fig. 2).

Expression of Mtv SAgs by splenic B cells

The low levels of expression of the 1.7-kb spliced transcript encoding the *Mtv* SAg (17) make its detection difficult by Northern blot. Furthermore, Northern blot analysis does not allow discrimination among the multiple endogenous *Mtv* loci expressed by a given inbred mouse strain. To circumvent these problems, we took

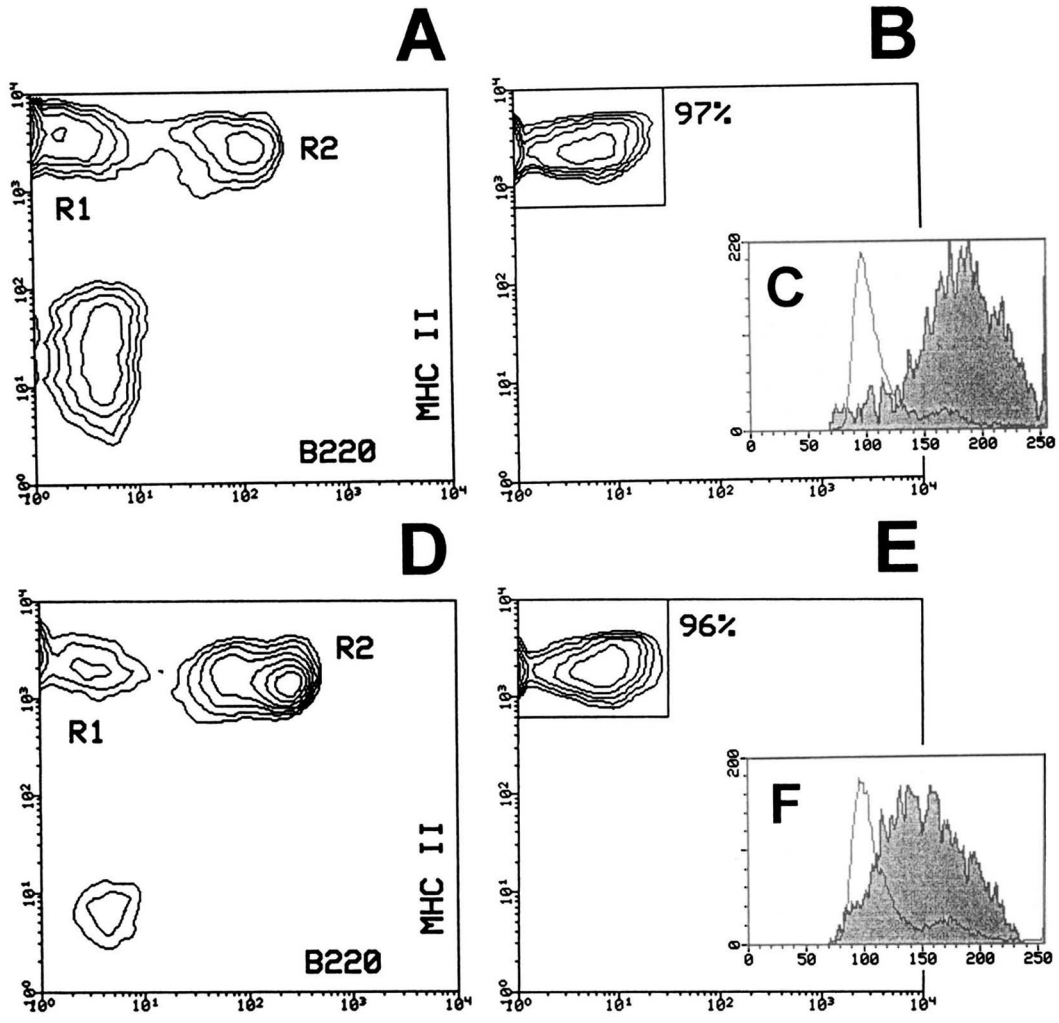

FIGURE 1. Isolation of thymic and splenic DC and B cells. A DC-enriched preparation, obtained after collagenase digestion, centrifugation in a discontinuous metrizamide gradient, and immunomagnetic bead depletion, was double stained with FITC-conjugated anti-B220 mAb and biotinylated anti-MHC class II mAb plus streptavidin-phycoerythrin. DC were then sorted as MHC class $II^{high}B220^{-}$ cells (R1), whereas B cells were sorted as MHC $II^{high}B220^{+}$ cells (R2). In the experiment shown, R1 and R2 represented 29 and 23%, respectively, of the thymic DC-enriched preparation (*A*) and 21 and 57%, respectively, of the splenic DC-enriched preparation (*D*). Reanalysis of the thymic and splenic DC preparations (*B* and *E*) showed that the purities obtained after sorting were 97 and 96%, respectively. The forward scatter profile of thymic and splenic DC and B cells is shown in *C* and *F*, in which the shaded profiles correspond to the DC and the open profiles correspond to the B cells.

advantage of a recently developed highly sensitive reverse transcriptase-PCR-based technique (18) that distinguishes endogenous *Mtv* locus transcripts on the basis of length heterogeneity in the SAg COOH-terminal sequences.

Figure 3 shows the differential expression of DBA/2 *Mtv* SAgs by splenic B cells and illustrates the different band patterns obtained when using the ORF-100/VJ71 set of primers (amplifying all *Mtv* mRNAs) or the *orf* mRNA/VJ71 primers (specifically amplifying *Mtv* SAgs from the 1.7-kb spliced mRNA). PhosphorImager analysis of the bands obtained with ORF-100/VJ71 (Fig. 4) indicated that the predominant *Mtv* mRNA species originates from *Mtv*-7 (71%), followed by *Mtv*-8 and -11 (24%) and *Mtv*-1, -6, and -13 (4%). However, when the expression of *Mtv* SAgs in splenic B cells is determined from the SAg-specific spliced mRNA using the primer set *orf* mRNA/VJ71, the pattern of expression changed dramatically (Figs. 3 and 4); the highest level of expression actually corresponded to *Mtv*-8 and -11 (68%), *Mtv*-1, -6, and -13 expression represented 28% of the signal, and the *Mtv*-7 SAg-specific

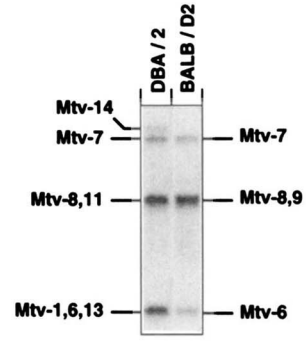

FIGURE 2. Endogenous *Mtv* loci present in DBA/2 mice. Genomic DNA from DBA/2 (and control BALB.D2) thymic DC was subjected to PCR analysis with the *Mtv* LTR-specific primer set ORF-100/VJ71. Separation of the ^{32}P-labeled PCR products on a sequencing gel allows the identification of the different *Mtv* loci based on length polymorphisms in the SAg COOH-terminal sequences.

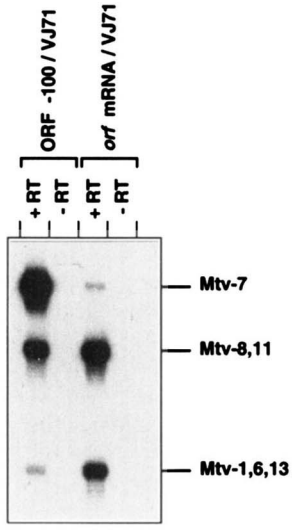

FIGURE 3. PCR analysis of *Mtv* SAgs expressed by splenic B cells in DBA/2 mice. RNA isolated from splenic B cells was reverse transcribed and subjected to PCR using the ORF-100/VJ71 set of primers (amplifying *Mtv* SAgs from all *Mtv* mRNAs) or the *orf* mRNA/VJ71 primers (specifically amplifying *Mtv* SAgs from the 1.7-kb spliced mRNA). The ^{32}P-labeled PCR products were separated on a sequencing gel and visualized autoradiographically. In control samples, reverse transcriptase (RT) was omitted.

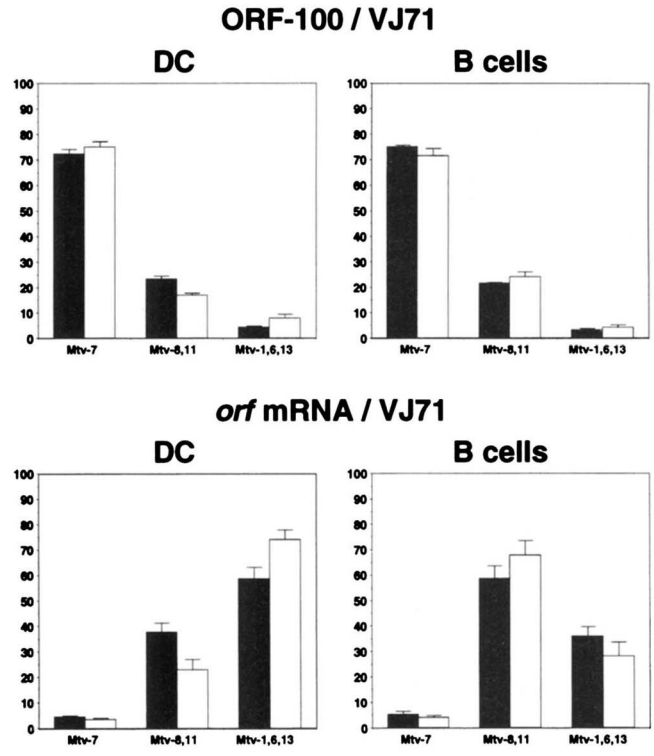

FIGURE 4. PhosphorImager analysis of *Mtv* SAg expression by thymic and splenic DC and B cells. Quantitation of ^{32}P-labeled PCR products separated on a sequencing gel (e.g., Figs. 3 and 5) was performed by PhosphorImager analysis as described in *Materials and Methods*. For both the ORF-100/VJ71 and the *orf* mRNA/VJ71 sets of primers, the results are indicated as a percentage of the expression of the different *Mtv* locus groups for cells of thymic (closed bars) or splenic (open bars) origin. Data are expressed as the mean ± SD of three or four experiments.

mRNA showed the lowest expression level (4%). These results emphasize the importance of using primers amplifying specifically the 1.7-kb spliced mRNA in studies seeking to determine the relative expression of *Mtv* SAg transcripts.

Expression of Mtv SAgs by thymic and splenic DC and B cells

The *orf* mRNA/VJ71 primer set was then used to compare the expression of *Mtv* SAgs by thymic B cells and that by thymic and splenic DC (although the results obtained with the ORF-100/VJ71 set are also considered to discuss the data existing in the literature). Analysis of *Mtv* SAg expression by thymic B cells using both sets of primers showed an almost identical differential expression of the *Mtv* loci compared with splenic B cells (Figs. 4 and 5). Similarly, when highly purified DC were analyzed, their patterns of *Mtv* SAg expression were similar regardless of their splenic or thymic origin (Figs. 4 and 5). However, interestingly, the relative expressions of the various DBA/2 *Mtv* loci were different when DC were compared with B cells using the *Mtv* SAg mRNA-specific set of primers *orf* mRNA/VJ71 (Fig. 4). For both thymic and splenic DC, the *Mtv* SAg locus group with the highest expression was *Mtv*-1, -6, and -13 (65%), followed by *Mtv*-8 and -11 (32%) and *Mtv*-7 (4%). In the DC samples, but not in the splenic or thymic B cell samples, an extra band located below the *Mtv*-7 band appeared (Fig. 5). This band does not correspond in size to any of the predicted *Rsa*I-digested PCR products obtained for the different DBA/2 *Mtv* SAgs, and its origin is currently unknown.

In summary, our results demonstrate that DC globally express *Mtv* SAg mRNA at levels comparable to those in B cells in DBA/2 mice; however, DC and B cells show a differential pattern of expression, as DC display higher *Mtv*-1, -6, and -13 SAg expression levels but lower *Mtv*-8 and -11 SAg expression levels than B cells. The reason for this apparent lineage-specific differential expression of *Mtv* SAg loci remains to be elucidated. Since total *Mtv* transcripts showed similar expression patterns in DC and B cells, it

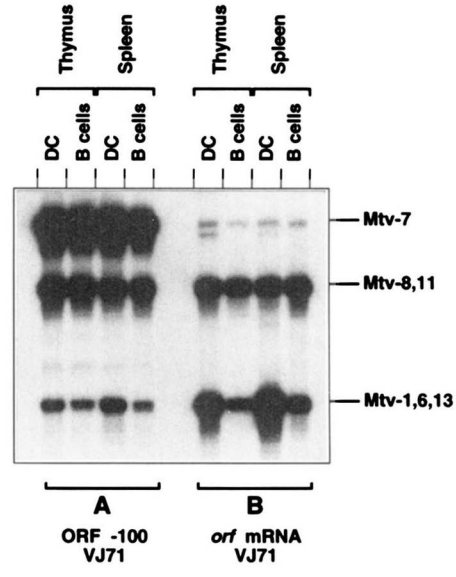

FIGURE 5. Expression of *Mtv* SAgs by thymic and splenic DC and B cells. RNA isolated from highly purified thymic and splenic DC and B cells was reverse transcribed and subjected to PCR analysis using the ORF-100/VJ71 (*A*) or the *orf* mRNA/VJ71 (*B*) set of primers. The ^{32}P-labeled PCR products were separated on a sequencing gel and visualized autoradiographically.

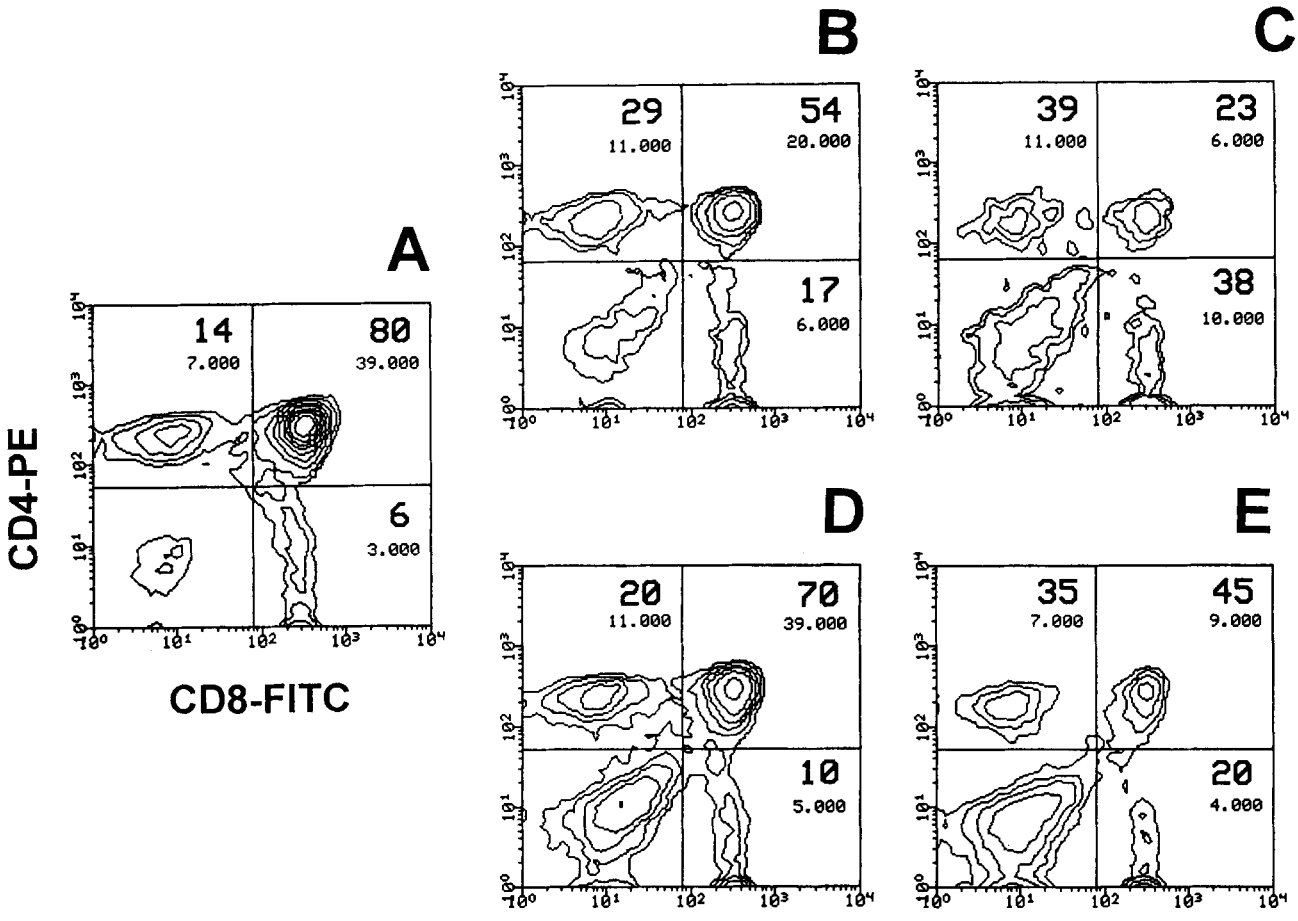

FIGURE 6. In vitro deletion of DP TCR transgenic thymocytes by DC and B cells expressing *Mtv*-7 SAg. Thymocytes from P14 $\alpha\beta$ TCR transgenic mice (Vβ8.1, Vα2) were cultured for 24 h alone (*A*) or with APC from BALB/c (*B–D*) or BALB/D2 mice (*C–E*) and double stained with phycoerythrin-conjugated anti-CD4 mAb and FITC-conjugated anti-CD8 mAb. *B–C* and *D–E* correspond, respectively, to the cultures in which thymic DC and splenic B cells were used as APCs. Each panel shows the percentage (large figures) and the absolute viable cell number per well (small figures) for the different thymocyte subsets after 24 h. The percentages of single positive (SP) $CD4^+$, SP $CD8^+$, and DP thymocytes shown have been calculated considering that SP + DP = 100%, to exclude double negative thymocytes and APCs from the analysis. The absolute viable cell number per well has been estimated by applying these percentages to the total number of viable cells (negative for propidium iodide staining) recovered per well. The percent deletion based on the recovery of DP thymocytes was 44% in *B*, 68% in *C*, 12% in *D*, and 48% in *E*. This experiment was performed four times with similar results.

appears unlikely that enhanced lineage-specific transcription in the vicinity of the *Mtv* integration site(s) can account for this phenomenon. Rather, it could be speculated that variations in the efficiency of splicing of certain *Mtv* SAg transcripts may occur in DC and B cells. Indeed, lineage-specific differential splicing has been well documented for other genes expressed in hemopoietic cells, such as CD45 (26); however, the molecular mechanism controlling this phenomenon remains obscure. Alternatively, it is possible that the activity of a SAg-specific promoter (27) may be differentially regulated in DC and B cells. Whatever the mechanism, differential transcription of *Mtv* SAg loci in DC and B cells provides compelling evidence that the two populations are not cross-contaminated to a significant degree.

Two previous reports have considered *Mtv* SAg expression by DC at the mRNA level with apparently contradictory results. Jarvis et al. (13), using PCR primers detecting spliced *Mtv* SAg mRNAs, detected SAg transcripts in splenic B cells and Con A-activated peripheral $CD8^+$ T cells, but not in DC of C3H mice (expressing *Mtv*-1, -6, -8, -11, and -14). Discrepancies between the results of Jarvis and our findings may be due to differences in the isolation procedure of DC, although neither the origin (peripheral vs thymic) nor the method of DC purification was described by these authors. On the other hand, Moore et al. (14) found detectable levels of expression of *Mtv* transcripts in DC from BALB/c mice (expressing *Mtv*-6, -8, and -9) isolated from 7-day-old fetal thymic organ cultures and maintained for 14 to 21 days with granulocyte-macrophage CSF. However, these researchers used PCR primers amplifying all *Mtv* mRNAs (equivalent to the ORF-100/VJ71 set) and, hence, did not directly address the issue of *Mtv* SAg expression by DC.

In vitro deletion of SAg-reactive thymocytes by DC

To determine whether DC not only expressed *Mtv* SAg at the mRNA level, but also in a functional form on the cell surface, we investigated the capacity of thymic DC expressing the potent *Mtv*-7 SAg to delete SAg-reactive Vβ8.1 thymocytes in vitro, and we compared them to splenic B cells, which are well characterized as *Mtv*-7 SAg-presenting cells (6). For this purpose, P14 TCR-$\alpha\beta$ transgenic thymocytes were cultured with purified thymic DC or splenic B cells from *Mtv*-7^- (BALB/c) or *Mtv*-7^+ (BALB/D2) mice. Figure 6 shows that after 24 h, BALB/D2 thymic DC induced a 68% deletion of DP thymocytes, whereas splenic B cells caused a 48% deletion. The increased efficiency of deletion of DP thymocytes by thymic DC in this system was highly reproducible

(68 ± 3% deletion by thymic DC vs 49 ± 4% deletion by splenic B cells after 24 h; mean ± SD; $n = 3$). These data are in agreement with previous reports showing that in vivo intrathymic deletion of SAg-reactive T cells occurs at the DP stage (28–30) and support the results obtained by Bhardwaj et al. (11, 12) demonstrating that DC are more efficient than B cells as microbial SAg-presenting cells. Moreover, our data on the deletion of P14 TCR-$\alpha\beta$ transgenic DP thymocytes induced with APCs expressing the *Mtv*-7 SAg are comparable to the results described by Pircher et al. (31), using the same transgenic thymocytes, with APCs in the presence of the antigenic lymphocytic choriomeningitis virus peptide. In this system, in which clonal deletion was peptide dose dependent, these researchers obtained a 50% deletion of DP thymocytes with thymic DC at an optimal peptide concentration of 10^{-5} M.

Therefore, the in vitro approach used in this study mimicked the negative selection process taking place in the intact thymus. However, a partial deletion (44%) of DP thymocytes occurred when DC from *Mtv*-7$^-$ BALB/c mice were cultured with P14 TCR-$\alpha\beta$ transgenic thymocytes. A much lower, but significant, deletion (12%) was also observed with BALB/c splenic B cells. Interestingly, previous reports have shown partial deletion of DP cells in the case of the in vitro deletion of H-Y-specific TCR transgenic thymocytes when APCs not expressing the male Ag or the correct MHC molecules were used (32, 33). Although this point was not discussed by these authors, a partial stimulation of DP thymocytes by syngeneic DC could be an explanation for this phenomenon. In this context, a syngeneic stimulation of peripheral T cells by DC has been reported in MLR experiments dealing with both *Mtv* SAgs (7) and conventional Ags (34).

In conclusion, our collective data clearly indicate that DC express functional *Mtv* SAgs and, as suggested by previous results (7, 10), may be responsible for the in vivo clonal deletion of SAg-reactive T cells. In this regard, the recent finding that deletion of *Mtv* SAg-reactive T cells occurs normally in B cell-deficient mice (35) is also consistent with a role for thymic DC in this process.

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