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Decrease in Pool of T Lymphocytes with Surface Phenotypes of Effector and Central Memory Cells under Influence of TCR Transgenic β-Chain Expression

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Abstract—Peripheral T lymphocytes can be subdivided into naive and antigen-experienced T cells. The latter, in turn, are represented by effector and central memory cells that are identified by different profiles of activation markers expression, such as CD44 and CD62L in mice. These markers determine different traffic of T lymphocytes in the organism, but hardly reproduce real antigenic experience of a T lymphocyte. Mechanisms of homeostasis maintenance of T lymphocytes with different activation phenotypes remain largely unknown. To investigate impact of T cell receptor (TCR) transgenic chains on formation of T lymphocytes, their peripheral survival and activation surface phenotypes, we have generated the transgenic mouse strain expressing transgenic β-chain of TCR 1D1 (belonging to the Vβ6 family) on the genetic background B10.D2(R101). Intrathymic development of T cells in these transgenic mice is not impaired. The repertoire of peripheral T lymphocytes in these mice contains 70-80% of T cells expressing transgenic β-chain and 20-30% of T cells expressing endogenous β-chains. The ratio of peripheral $CD4^+CD8^-$ and $CD4^-CD8^+$ T lymphocytes remained unchanged in the transgenic animals, but the percent of T lymphocytes with the "naive" phenotype $CD44^-CD62L^+$ was significantly increased, whereas the levels of effector memory $CD44^+CD62L^-$ and central memory $CD44^+CD62L^+$ T lymphocytes were markedly decreased in both subpopulations. On the contrary, T lymphocytes expressing endogenous β-chains had surface phenotype of activated T cells $CD44^+$. Thus, for the first time we have shown that the pool of T lymphocytes with different activation phenotypes depends on the structure of T cell receptors.

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Key words: T cell receptor, transgenic animals, lymphocyte, effector, memory cell

Among peripheral T lymphocytes, several subpopulations with different functional characteristics can be identified: naive T lymphocytes, still not activated by a specific antigen, effector T lymphocytes activated after engagement with a specific antigen, whose functions are aimed at elimination of this antigen, and memory cells that represent a small pool of T cells remaining after contraction of the immune response and that are capable of accelerated response to the same antigen. Actually, these subpopulations are identified by the presence of activation markers on their surface, for example, CD44 and CD62L in mice, that determine capability for extravasation into non-lymphoid tissues and specialized high endothelium of lymph nodes, respectively [1, 2]. During an immune response, naive T lymphocytes $CD44^-CD62L^+$ really acquire the surface phenotype of effector T cells $CD44^+CD62L^-$ and, then, central memory cells $CD44^+CD62L^+$. Nevertheless, it is unclear whether expression of these markers manifests real status of a T lymphocyte functioning as an effector or memory cell. Particularly, acquiring of the memory phenotype can occur without engagement of a specific antigen as well as during lymphopenia [3-6], but the data demonstrating functional activity of cells with such phenotype are extremely controversial [7, 8]. Therefore, the question about mechanisms of acquiring various surface phenotypes remains open.

It is well known that homeostasis of naive T lymphocytes is maintained by tonic signals provided by interactions with self MHC–peptide complexes during recircu-

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lation via lymphoid tissues [9]. We have proposed that similarly the profile of T lymphocyte activation markers can be determined by interactions of their T cell receptor (TCR) with self MHC molecules. This hypothesis can be suggested by the observation of higher affinities of memory TCRs with a specific ligand as compared with affinities of naive T cells specific to the same antigen [10]. It is also well known that lymphopenia, resulting in acquisition of the activated phenotype by T lymphocytes, frequently provokes development of autoimmune diseases [11, 12]. It would be possible to verify this hypothesis experimentally on animals possessing a changed repertoire of TCRs, in particular, on TCR transgenic animals. Existing models with transgenic TCRα/β have grave shortcomings, which do not allow using them in studies of T cell homeostasis. Among these shortcomings are: abnormal intrathymic differentiation of T lymphocytes and unphysiologically high frequency of T lymphocytes with the same specificity, compelling investigators to apply invasive methods and approaches, e.g. adoptive transfers of transgenic T cells into lymphopenic wild type animals. This prompted us to develop our own transgenic model in which the transgene encodes only one chain of memory TCR, allowing normal rearrangement of another chain and formation of a diverse repertoire of T lymphocytes.

Earlier, we have shown a valuable means to induce selective response of memory T cells, specific to the allogeneic class I MHC molecule, without concomitant activation and involvement of clones of naive T lymphocytes [13]. To do this, we used the mixed lymphocyte reaction (MLR) of lymphocytes from animals immunized by allogeneic tumor cells as responder, and allogeneic splenocytes, killed by severe heat shock (45°C, 1 h) as stimulator cells. We have shown that this experimental system allows selective detection of proliferation of memory CD8 T cells specific to the immunizing MHC class I molecule and capable of recognizing its mutant forms [14, 15]. This simple approach enabled us to obtain clones and T hybridomas of memory cells that in turn opened the way to molecular identification and cloning of their TCRs [16]. Our next step was to generate animals with transgenic expression of individual TCR chains [17]. Due to full or partial (functional) allelic exclusion of TCR β- and α-chains, the repertoire of T cells in these animals is altered in varying degrees, which allows us to investigate the impact of individual chains of TCR on a great number of immunological phenomena, such as intrathymic selection of T lymphocytes, their survival in the periphery, allogeneic recognition, autoimmunity, and formation of memory T cells. In this work we describe a mouse strain expressing an individual β-chain of transgenic memory TCR and show that expression of the transgenic β-chain of this TCR did not impair intrathymic development of T lymphocytes, but significantly influenced abundance of the pools with surface phenotypes of naive and earlier activated T cells.

MATERIALS AND METHODS

Animals. Mice of the C57BL/6 ($K^bI\text{-}A^bD^b$), C57BL/10 ($K^bI\text{-}A^bD^b$), B10.D2(R101) ($K^dI\text{-}A^dI\text{-}E^dD^b$) strains were obtained from the breeding facility of the Blokhin Cancer Research Center. The F_1 hybrid (CBA × C57BL/6) was from Stolbovaya nursery. Transgenic mice, expressing the TCR β-chains of the memory hybridoma 1D1 were generated in the Laboratory of Transgenesis, Institute of Gene Biology, Russian Academy of Sciences, and bred in the Laboratory of Regulatory Mechanisms in Immunity, Blokhin Cancer Research Center, Russian Academy of Medical Sciences.

Cloning cDNA encoding the β-chain of memory TCR. The full-length cDNA encoding the β-chain of TCR was cloned into the hCD2 cassette vector, allowing specific expression of the gene in T lymphocytes (a kind gift from Prof. Dimitris Kioussis, MRC – National Institute for Medical Research, London, Great Britain) [18]. We used primers to the variable TCRBV6S1BamHI (5′-caga**ggatcc**agaaagtccctccaaact-3′) and the constant CB2BamHIBglII (5′-gaa**ggatcc**agatctcataaaagtttgtc-3′) regions, containing the restriction site for *BamH*I (shown as bold), to clone the full-length cDNA encoding the β-chain of the memory TCR. The products amplified from hybridoma 1D1 cDNA were cloned into the TA vector using a TA Cloning Kit (Invitrogen, USA) and sequenced (Genome Center, Engelhardt Institute of Molecular Biology). The sequence results were analyzed using programs Chromas v.1.45, DNAssist v.1.0, and BLAST (online). Then, the full-length cDNA cloned into the TA vector was digested with *BamH*I (Fermentas, Lithuania), purified from agarose gel with a QIAEX II Gel Extraction Kit (Qiagen, USA), and recloned into the hCD2 cassette vector linearized with *BamH*I. Prior to injection of this genetic construct, ampicillin resistance gene was excised with *Sal*I and *Not*I (Fermentas). For microinjections, DNA solution (5 ng/μl) in TE buffer for microinjections was used [19].

Generation of transgenic animals. To obtain primary transgenic animals, the genetic construct described above (5 ng/μl in TE buffer) was injected into the male pronuclei of fertilized eggs of F_1(CBA × C57BL/6) mice, and surviving after microinjection zygotes were grafted to pseudopregnant recipients [19].

Eggs were obtained by induction of superovulation. To do this, young F_1(CBA × C57BL/6) female mice (12-13 g) were injected intraperitoneally 8 units of pregnant mare serum gonadotropin (PMSG; Mosagrogen, Russia) and, after 46 h, 8 units of human chorionic gonadotropin (HCG; Moscow Endocrine Company, Russia). The females were placed with F_1(CBA × C57BL/6) males. The fact of mating was verified next morning by the presence of a copulative plug. Females with copulative plugs were sacrificed via cervical dislocation and their oviducts were isolated. Eggs were washed from the oviducts with the medi-

um HEPES-KSOM supplemented with hyaluronidase (Sigma, USA) under a Zeiss Stemi DV4 binocular microscope (magnification ×32), using glass capillaries with an inner diameter of approximately 100 μm made on a Narishige PC10 puller and a Narishige MF900 microforge (Japan). Eggs were cultured in a HEPES-KSOM or KSOM drop [20] covered with mineral oil (Sigma) for 2 h (37°C, 5% CO_2) and then placed into a microinjection cell. Microinjections were performed in HEPES-KSOM under a Zeiss Axiovert 200M microscope (magnification ×400-×600) using Narishige micromanipulators. Microinjection needles were made using a Sutter Instrument Co P-97 puller (USA), and a holder pipette was made on a Narishige PC10 puller and a Narishige MF900 microforge. After microinjection, surviving cells were transferred into a drop of KSOM or HEPES-KSOM covered with mineral oil and cultured for 1 h to identify viable cells. Media were prepared using earlier published protocols [17].

To obtain recipients, adult F_1(CBA × C57BL/6) females weighing not less than 24 g were placed with vasectomized males of the same strain. Pseudopregnant recipients were selected the next morning by the presence of copulative plugs.

After microinjection, viable zygotes were transplanted into the left oviduct of a pseudopregnant female. Depending on the number of cells surviving after microinjection, 10-20 zygotes were transplanted to each female. The females were immobilized during surgery with Avertin (a 2.5% aqueous solution of the preparation containing 1 g of 2,2,2-tribromoethanol in 1 ml of 2-methyl-2-butanol) (Sigma). Avertin was injected intraperitoneally at 15 μl per gram body weight.

Progeny and its testing for the transgene. If delivery did not occur 21 days after grafting of microinjected eggs, the recipient was sacrificed via cervical dislocation, cesarean section was performed, and the pups were fed by foster-mothers. Tail tips (2-3 mm) of the mice, born from microinjected zygotes were cut off 14-21 days after birth, and DNAs were isolated and tested for the transgene by PCR.

Genotyping of mice by PCR. DNA isolation followed a standard protocol (Molecular Cloning, USA). Amplification with Taq DNA polymerase (Evrogen, Russia) was performed in the buffer (containing 2.5 mM $MgCl_2$) supplied with the enzyme in the presence of 2 mM dNTP and 10 pmol of each primer and included denaturation at 94°C for 3 min; four cycles of 94°C for 20 s, 62°C for 30 s, and 72°C for 35 s; eight cycles of 94°C for 20 s, 60°C for 30 s, and 72°C for 35 s; 25 cycles of 94°C for 20 s, 58°C for 30 s, and 72°C for 35 s; and the last synthesis at 72°C for 10 min. The primers for amplification of the transgenic β-chain were shown above. For amplification of the CD4 gene, the following primers were used: CD4w-S (5′-gaggttcgccttcgcagtttgat-3′) and CD4w-AS (5′-tggacatggagagaactttgga-3′). Amplification products (10 μl) were analyzed by electrophoresis on 1.5% agarose gels in TAE-buffer containing ethidium bromide according to the Molecular Cloning protocol. Gels were photographed using a transilluminator with CD-camera.

Isolation of mononuclear cells from peripheral blood. Blood samples were obtained from the retro-orbital venous sinus with a Pasteur pipette. Heparin (5000 U/ml) was immediately added to the blood samples (30 μl of heparin solution per 100 μl of blood). Then erythrocytes were lysed with the lytic buffer (0.826 g NH_4Cl, 0.1 g $NaHCO_3$, 0.0037 g Na_2EDTA per 100 ml H_2O), and the mononuclear cells were twice washed by centrifugation in PBS (200*g*, 5 min).

Estimation of quantity of lymphocytes expressing transgene. To estimate the quantity of T lymphocytes expressing the transgenic β-chain of the memory TCR on the surface, mononuclear cells of peripheral blood, obtained as described above, were stained with fluorochrome-conjugated antibodies to mouse CD3 and Vβ6, and then the percent of $CD3^+$ T cells carrying transgenic TCR was determined on a BD FACSCanto II flow cytometer (Becton Dickinson, USA).

Cell suspension. Lymphocytes were gently squeezed from the stroma of mouse spleen and thymus in a Potter homogenizer with a conic pestle. Erythrocytes were lysed by addition of lytic buffer to a cellular pellet, and thymocytes and splenocytes were settled by centrifugation (200*g*, 5 min) and resuspended in PBS. Viable cells were counted after staining with trypan blue–eosin in a Goryaev chamber.

Antibodies. To analyze cells on the BD FACSCanto II flow cytometer, the following monoclonal antibodies were used: FITC-conjugated anti-CD8α (clone 53-6.7; BD Pharmingen, USA), PE-conjugated anti-mouse TCR Vβ6 (clone RR4-7; BD Pharmingen), APC/Cy7-conjugated anti-mouse CD62L (clone MEL-14; BioLegend, USA), APC-conjugated anti-mouse CD44 (clone IM7; BioLegend), Alexa Fluor®647 conjugated anti-mouse CD3 (clone 17A2; eBioscience, USA), eFluor®450-conjugated anti-mouse CD3 (clone 17A2; eBioscience), APC-conjugated anti-mouse CD4 (clone L3T4; BD Pharmingen). To exclude dead cells, propidium iodide (PI; Sigma) was used at final concentration $7.5 \cdot 10^{-5}$ M.

Staining and analysis on flow cytometer. Staining with antibodies was performed at 4°C, 35 min. Analysis was performed on the BD FACSCanto II flow cytometer using the BD FACSDiva 6.0 program. Dead cells were excluded from the analysis based on scatter signals and staining with PI. No less than $1 \cdot 10^5$ events were collected to characterize peripheral populations of T lymphocytes. Further processing of results was performed using Flow Jo 7.6.

RESULTS

Obtaining of primary transgenic animals. Primary transgenics 1D1b were obtained after microinjection of DNA into pronuclei of fertilized eggs. The presence of transgene in the genome of mice born after injection of

the 1D1b genetic construct was determined by PCR. Figure 1a demonstrates results of the PCR analysis. Two primary transgenics (Nos. 1 and 2, respectively) were generated. The presence of the transgene in the genome does not guarantee its efficient expression [21]. Thus, additional analysis by flow cytometry was performed to detect T lymphocytes expressing the transgene (Fig. 1b). A variable segment of the β-chain of TCR 1D1 is referred to the Vβ6 family that allow detection of transgene-positive cells by monoclonal antibodies to Vβ6. In wild type mice, the percent of T cells expressing certain Vβ-segment usually does not exceed 10%, whereas in transgenic animals it may be highly elevated. In the transgenic No. 1, about 70% of peripheral blood T lymphocytes carry the transgenic TCR β-chain, and about 26% of T cells with the transgenic β-chain were detected in the blood of the primary transgenic No. 2 (Fig. 1b, bottom panel). The absolute number of T lymphocytes in peripheral blood of both primary transgenics was not decreased as compared with wild type animals (Fig. 1b, top panel), which indirectly shows the lack of impairments in their ontogeny.

Obtaining transgenic mouse strain on genetic background of B10.D2(R101). The initial T cell receptor containing the studied β-chain was primarily formed and selected in the intrathymic environment of MHC molecules of recombinant haplotype H-2^{g1} of B10.D2(R101) mice ($K^dI^dD^b$). For a number of reasons, primary transgenics were obtained on the genetic background of F_1 (CBA × C57BL/6) mice, whose progeny has mixed genetic background and thus is not suitable for immunological experimentation. To transfer the transgene to the genetic background of the B10.D2(R101) strain, primary transgenics were back-crossed to R101 mice for several generations. Both primary transgenics were founders of transgenic mouse strains: the sub-strain derived from the primary transgenic No. 1 was called 1D1bFM, and from the transgenic No. 2 – 1D1bFF. At present, surface phenotype of thymocytes and mature T lymphocytes of the substrain 1D1bFM has been characterized in more detail. Up to the generation F_2, the quantity of $CD3^+V\beta6^+$ cells in peripheral blood of transgenic animals highly varied (data not shown); however, in the generation F_2 expression of the transgene was stabilized and further not changed in a number of generations (Fig. 2b). We have never detected transgenic animals without expression of the transgene (data not shown). The expression of the transgene did not result in any changes of absolute number of $CD3^+$ cells in the peripheral blood (Fig. 2a). We have not observed any changes in cellularity of lymphatic organs of transgenic animals as compared with control animals. Lifetime of transgenics also did not change as compared with wild type control animals (data not shown).

Thereby, we have obtained a mouse strain transgenic on the β-chain of the memory TCR on the genetic back-

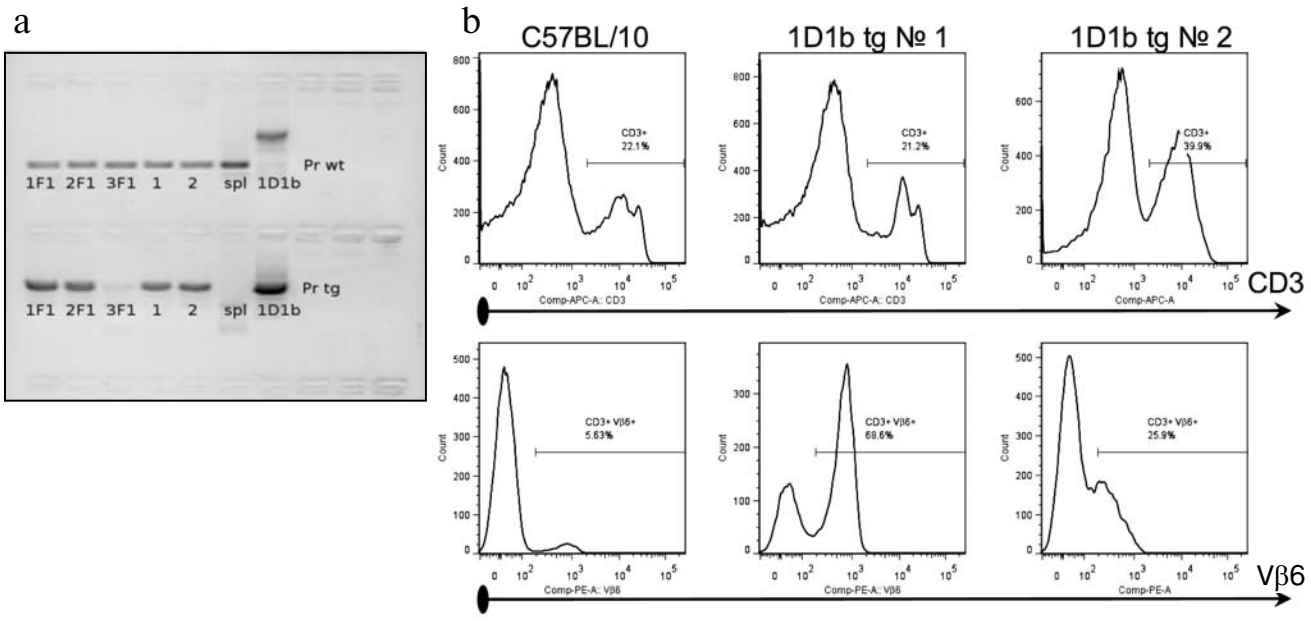

Fig. 1. a) PCR-based analysis of genomic DNA of founder mice transgenic for the hCD2 containing the TCR β-chain of the memory hybridoma 1D1 and F1 progeny of the transgenic strain 1D1bFM. Pr wt, PCR with primers directed to CD4 (control); Pr tg, PCR with primers directed to the 1D1 TCR transgenic β-chain; 1F1, 2F1, and 3F1, genomic DNA of the mouse strain 1D1bFM F_1 progeny, mouse No. 1, 2, and 3, respectively; *1*, *2*) genomic DNAs of the primary transgenics 1D1b; spl, genomic DNA of wild type C57BL/6 mice (negative control); 1D1b, cDNA coding the TCR transgenic β-chain cloned into the expression vector hCD2 (positive control). b) Flow cytometry analysis of $CD3^+$ peripheral blood mononuclear cells (PBMC, top panel) and expression of the transgenic β-chain on the surface of peripheral blood T lymphocytes (bottom panel) of primary transgenics 1D1b. C57BL/10, PBMC of wild type C57BL/10 mice; 1D1b tg No. 1, PBMC of the primary transgenic No. 1; 1D1b tg No. 2, PBMC of the primary transgenic No. 2.

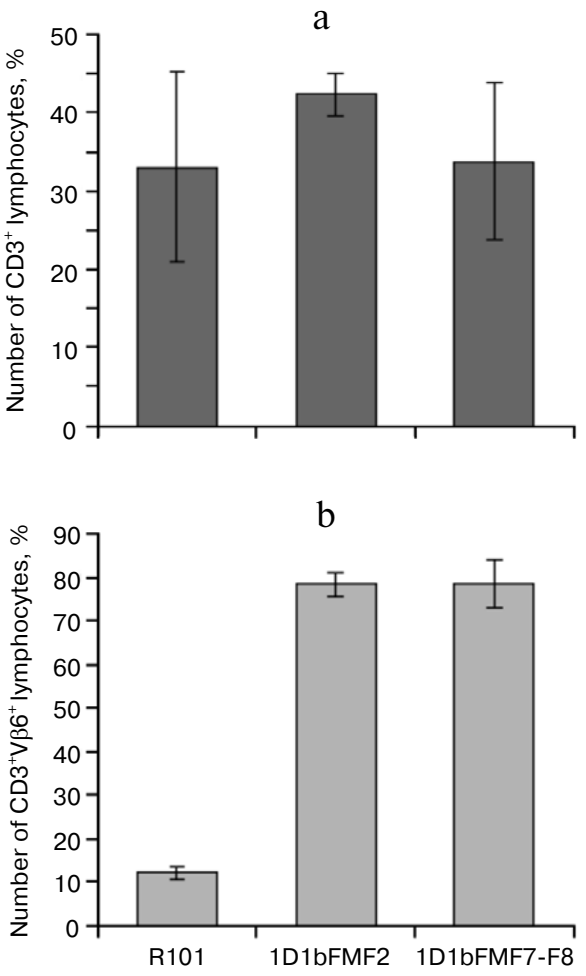

Fig. 2. Relative quantity of $CD3^+$ lymphocytes (a) and expression of the 1D1 TCR transgenic β-chain (b) in PBMC of generations F_2, F_7, and F_8 of 1D1bFM mice. R101, PBMC of B10.D2(R101) wild type mice (n = 7); 1D1bFM F2, PBMC of transgenic 1D1bFM F_2 (n = 6); 1D1bFM F7-F8, PBMC of transgenic 1D1bFM mice, generations F_7 and F_8 (n = 25).

ground of B10.D2(R101) mouse strain. This mouse strain is characterized by stable tissue-specific expression of the transgene, because only $CD3^+$ T lymphocytes were stained with anti-Vβ6.

Surface phenotype of mouse transgenic strain 1D1bFM thymocytes. It is known that expression of transgenic TCR α- and β-chains may influence intrathymic development of T lymphocytes. In part, this may be due to inhibitory effects of TCR transgenic chain expression on rearrangement of endogenous chains of TCR [22, 23]. On the other hand, expression of TCR transgenes may affect interactions of T lymphocytes with MHC molecules in the thymus and thereby influence the processes of their positive and negative selection [24]. So, we analyzed intrathymic subpopulations of lymphocytes in transgenic 1D1bFM and wild type B10.D2(R101) mice. The density plot presented in Fig. 3a shows that the

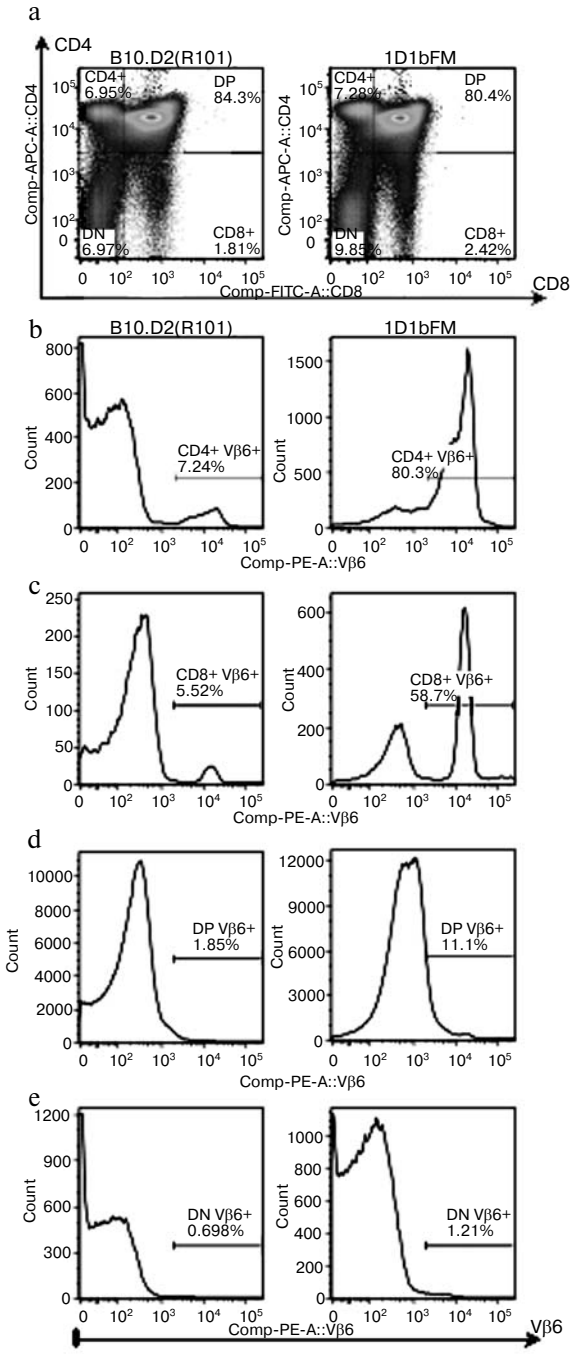

Fig. 3. a) Flow cytometric analysis of CD4 and CD8 expression on the surface of thymocytes isolated from 1D1bFM mice on genetic background B10.D2(R101). b-e) Flow cytometric analysis of 1D1 TCR transgenic β-chain expression on the surface of different subpopulations of thymocytes isolated from 1D1bFM mice on genetic background B10.D2(R101): b) SP $CD4^+$; c) SP $CD8^+$; d) DP $CD4^+CD8^+$; e) DN $CD4^-CD8^-$. B10.D2(R101), thymocytes of wild type B10.D2(R101) mice; 1D1bFM, thymocytes of transgenic 1D1bFM mice, generation F_7. Only singlets were gated to be analyzed. Six control and six transgenic animals were analyzed. Representative data of one of three experiments are shown.

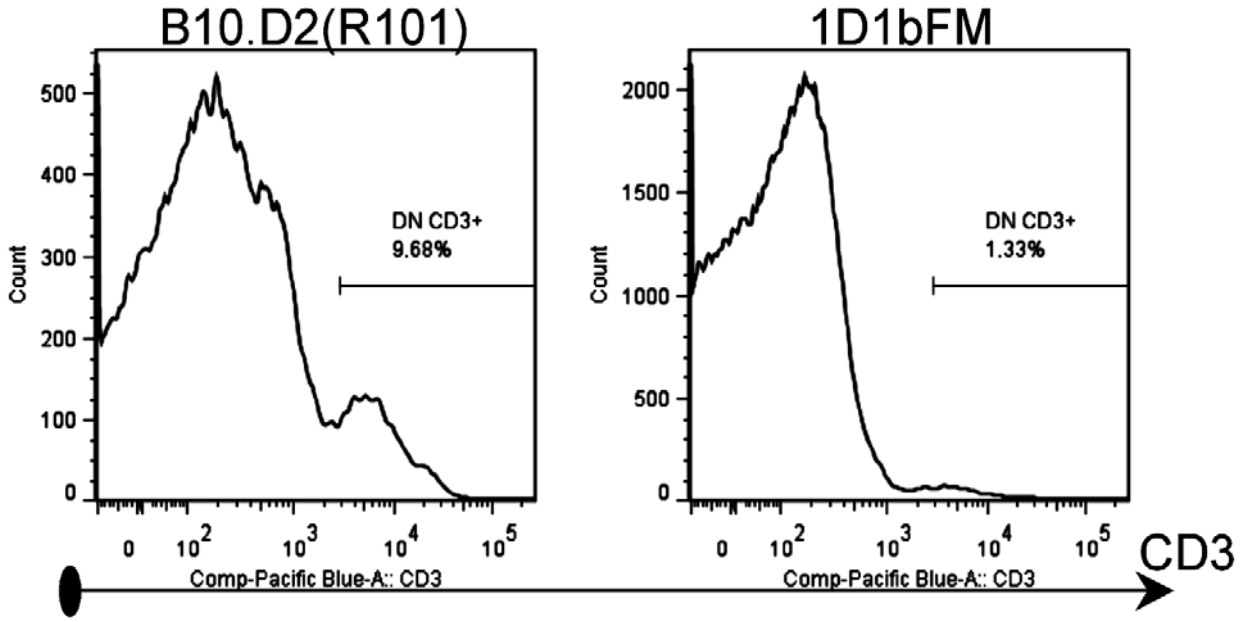

Fig. 4. Flow cytometric analysis of CD3 expression on the surface of DN thymocytes ($CD4^-CD8^-$) isolated from wild type B10.D2(R101) and 1D1bFM transgenic mice on genetic background B10.D2(R101). Only singlets were gated to be analyzed. B10.D2(R101), thymocytes of wild type B10.D2(R101) mice; 1D1bFM, thymocytes of 1D1bFM transgenic mouse strain, generation F_7. Four control and four transgenic animals were analyzed. Representative data of one of three experiments are shown.

percentage of double-negative (DN) $CD4^-CD8^-$ thymocytes is slightly increased in the thymus of the transgenic mice, whereas ratios of another subpopulations (double-positive (DP) $CD4^+CD8^+$ and single-positive (SP) $CD4^+CD8^-$ and $CD4^-CD8^+$) remain virtually unchanged. Probably, expression of transgene increases the percent of DN thymocytes due to better survival of T cells that normally die "by neglect" as result of non-productive rearrangements of endogenous β-chains and inability to form functional pre-TCR necessary for further differentiation of T cells.

The emergence of transgenic β-chains on the surface of thymocytes evidently correlates with their developmental stages. In the transgenic mice, the β-chain of TCR is expressed under control of the hCD2 promoter, i.e. expression of the transgenic β-chain begins simultaneously with the β-selection of endogenous TCR β-chains in the stage DN3. Perhaps that is why about 20% of T cells have successfully rearranged endogenous β-chains and do not express the transgene.

Among DN thymocytes, only a small number of cells are stained with anti-Vβ6 antibody (Fig. 3e). The percentage of T cells carrying the transgenic β-chain is markedly increased on the DP stage (Fig. 3d), when successful rearrangements of α-chains initiate emergence of mature TCR/CD3 complexes on the surface of T lymphocytes [25]. The large majority of SP $CD4^+$ and $CD8^+$ thymocytes express the β-chain transgene and its product on the surface. The ratio of SP $CD4^+$ and $CD8^+$ thymocytes, expressing transgene, remains unchanged, which indicates the absence of prevalence for lineage commitment of transgenic T cells to differentiate into $CD4^+$ or $CD8^+$ T lymphocytes.

The emergence of CD3 on the surface of thymocytes of transgenic animals correlates with maturation stages and expression of transgene (data not shown). The percentage of $CD3^+$ DN thymocytes is significantly decreased as compared with wild type animals, indicating possible suppression of γ-chain rearrangements under the influence of functional transgenic β-chain expression (Fig. 4).

Surface phenotype of peripheral 1D1bFM $CD3^+$ transgenic mouse lymphocytes. After passing the processes of intrathymic selection, transgenic $CD4^+$ and $CD8^+$ T cells go on to seed the periphery. In our study, it was important to evaluate their capacity for recirculation, how the normal ratio between $CD4^+$ and $CD8^+$ subpopulations is maintained, and what percentage of T cells in these subpopulations expresses transgene. In spleen, as well as in thymus of transgenic animals, the ratio of $CD4^+$ and $CD8^+$ T lymphocytes is unchanged as compared with wild type animals (Fig. 5a). Both subpopulations of spleen T cells express transgene (Fig. 5, b and c), but the percentage of $V\beta6^+$ lymphocytes in $CD8^+$ subpopulation (Fig. 5c) is slightly higher than in $CD4^+$ subpopulation (Fig. 5b). Thereby, T cells expressing the 1D1 TCR transgenic β-chain successfully pass intrathymic selection, seed peripheral lymphatic organs, and recirculate.

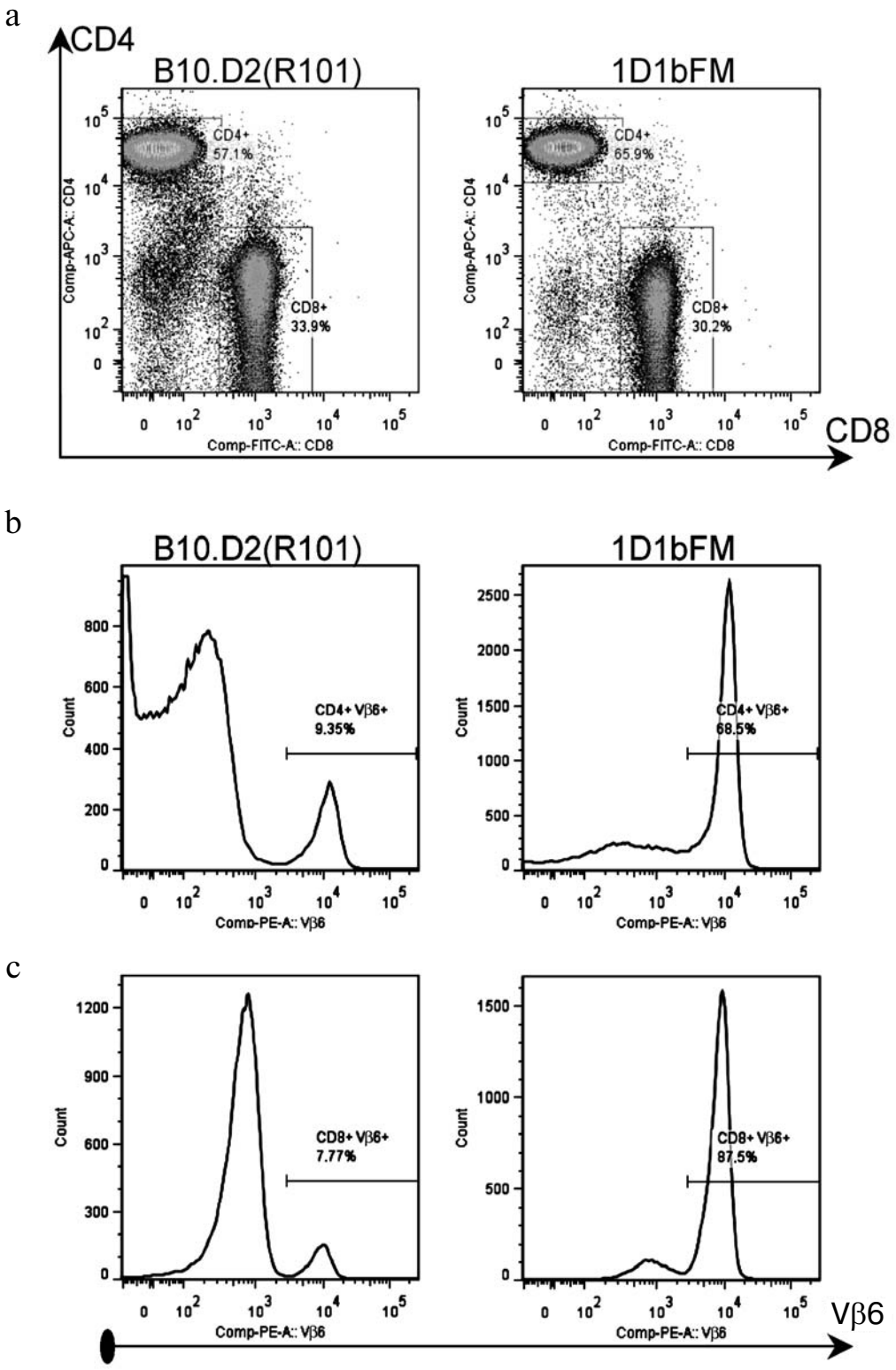

Fig. 5. Flow cytometric analysis of splenocytes isolated from wild type B10.D2(R101) and 1D1bFM transgenic mice on genetic background B10.D2(R101): a) CD4 and CD8 expression on the surface of $CD3^+$ splenocytes. Only singlets were gated to be analyzed. b, c) Vβ6 expression on different subpopulations of $CD3^+$ splenocytes—$CD4^+$ (b) and $CD8^+$ (c). B10.D2(R101), splenocytes of wild type B10.D2(R101) mice; 1D1bFM, splenocytes of TCR 1D1bFM transgenic mice, generation F_7. Six control and eight transgenic animals were analyzed. Representative data of one of three experiments are shown.

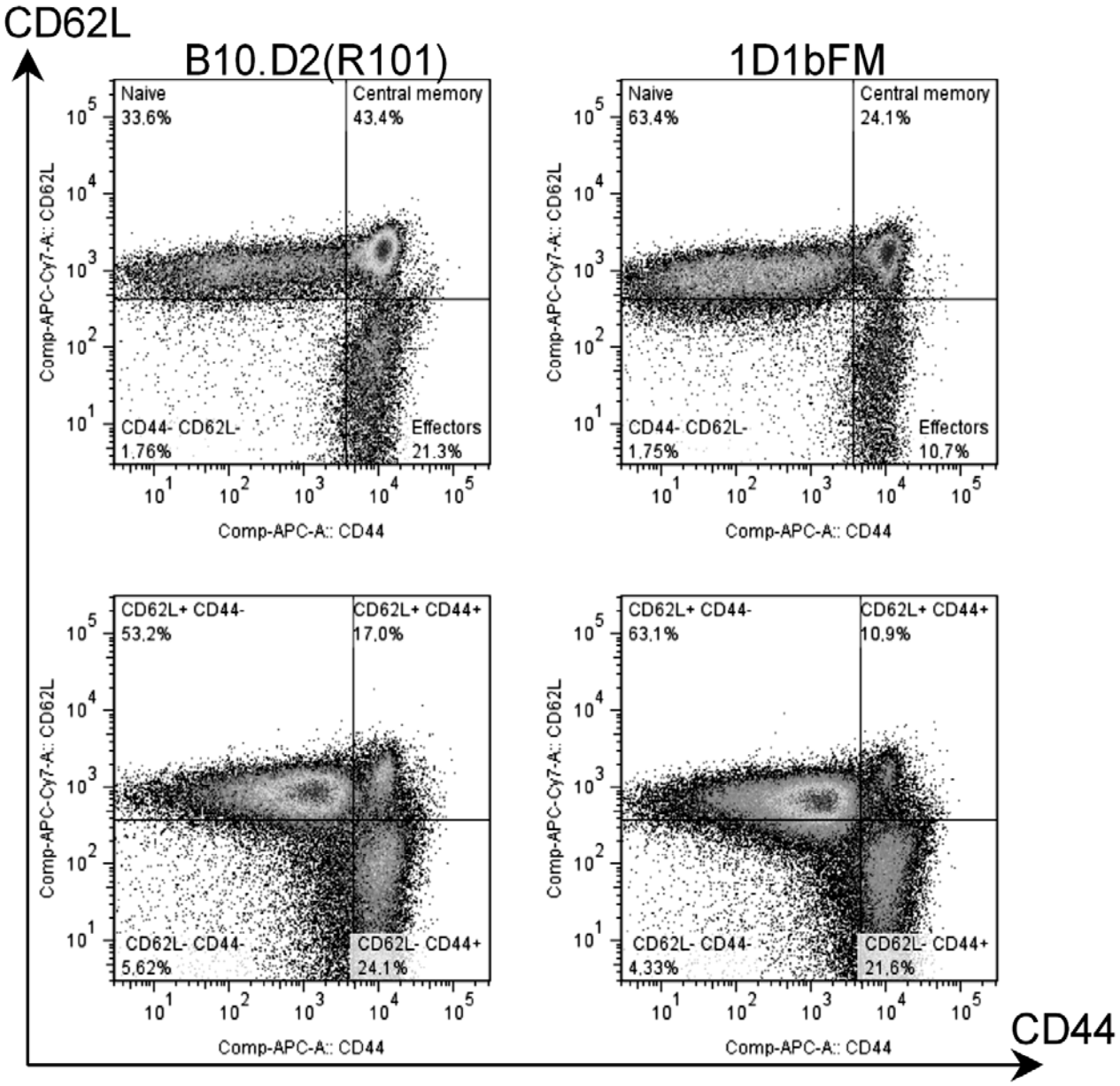

Fig. 6. Flow cytometric analysis of coexpression of CD62L and CD44 molecules on the surface of different subpopulation of T cells in mouse spleen. Top panel, $CD8^+$ splenocytes; bottom panel, $CD4^+$ splenocytes. Only singlets were gated to be analyzed. B10.D2(R101), splenocytes isolated from B10.D2(R101) wild type mice; 1D1bFM, splenocytes isolated from 1D1bFM transgenic mice, generation F_7. Relative number of $CD4^+$ and $CD8^+$ T lymphocytes with the surface phenotypes $CD44^-CD62L^+$ and $CD44^+CD62L^+$ in transgenic mice significantly differed from wild type animals ($P < 0.05$). Six control and eight transgenics were analyzed. The data shown were obtained in three independent experiments.

As opposed to wild type animals, 70-80% of T lymphocytes in the transgenic animals have TCRs with the transgenic β-chain, whereas 20-30% carry TCRs formed as result of normal rearrangements of gene segments encoding α- and β-chains. Herewith, we have a possibility to evaluate effects of the TCR transgenic β-chain expression on formation of T lymphocytes with surface phenotypes of naive and activated T cells, as well as to estimate their balance between T lymphocytes expressing endogenous and transgenic chains of the TCR.

We have analyzed expression of activation markers CD62L and CD44 in $CD4^+$ and $CD8^+$ subpopulations of spleen T lymphocytes (Fig. 6). The number of T lymphocytes with naive phenotype ($CD44^-CD62L^+$) was significantly increased in transgenic animals as compared with the wild type control both in $CD8^+$ (Fig. 6, top panel) and $CD4^+$ (Fig. 6, bottom panel) subpopulations of T lymphocytes. On the contrary, the number of cells with phenotypes of effector ($CD44^+CD62L^-$) and central memory ($CD44^+CD62L^+$) T cells was decreased in transgenic

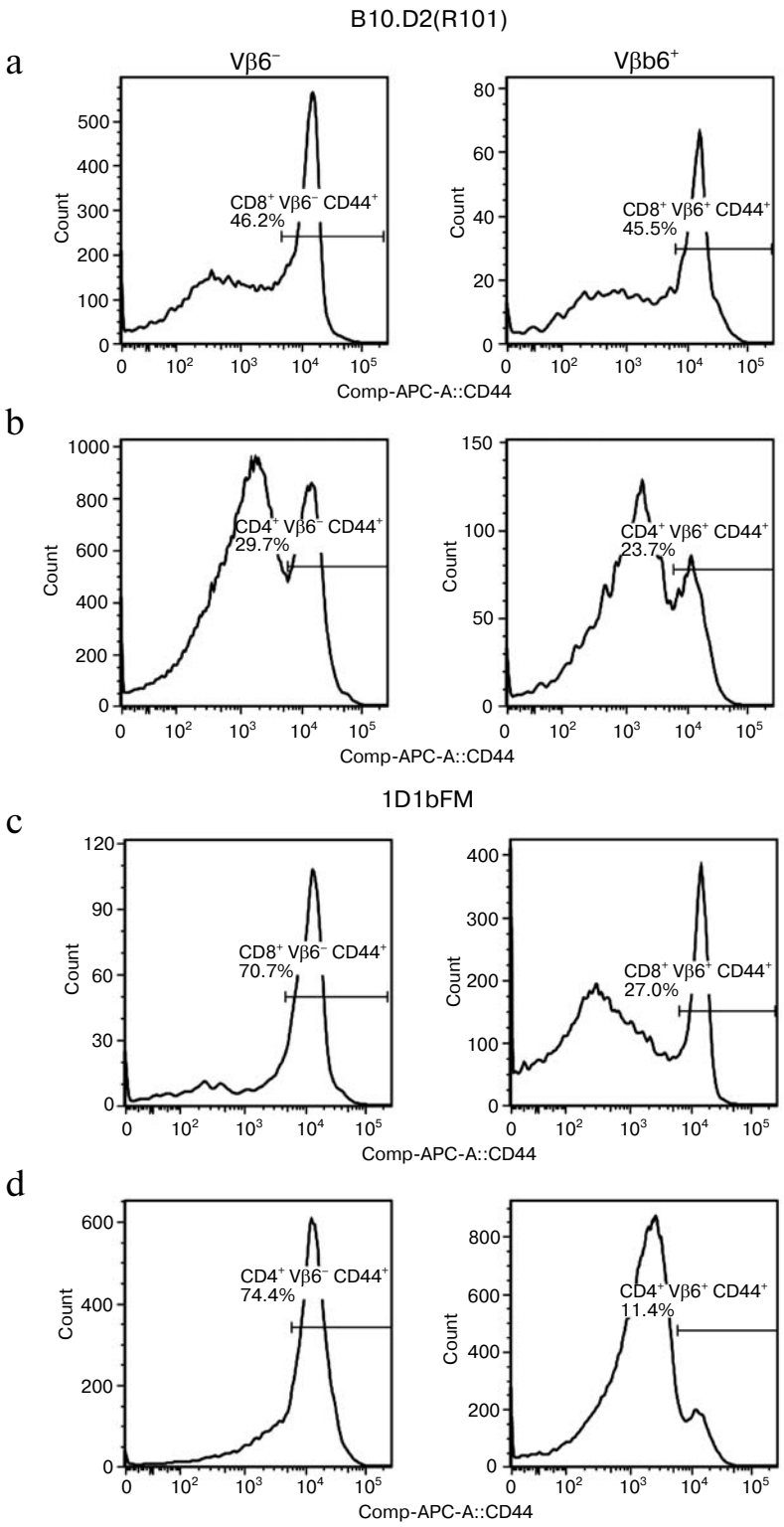

Fig. 7. Percentage of T lymphocytes expressing the activation phenotype ($CD44^+$) in different subpopulations of splenocytes isolated from wild type B10.D2(R101) and 1D1bFM transgenic mice on genetic background B10.D2(R101): $CD8^+$ (a, c), $CD4^+$ (b, d), $V\beta6^-$ (left), and $V\beta6^+$ (right). Six control and eight transgenic mice were studied. Only singlets were gated to be analyzed. The data shown were obtained in three independent experiments.

animals. At the same time, T lymphocytes expressing the transgenic β-chain had phenotype of naive T cells $CD44^-$, whereas T cells expressing endogenous β-chains were $CD44^+$, characteristic for activated T cells (Fig. 7). Thereby, the balance between T lymphocytes with phenotypes of naive and activated T cells in subpopulations $V\beta6^+$ and $V\beta6^-$ was significantly impaired in transgenic mice as compared with wild type animals.

DISCUSSION

In this work we studied outcome of expression of the transgenic TCR β-chain we cloned earlier from the memory T hybridoma [26]. Under examination of thymus of transgenic animals, we have not revealed any impairment of T cell development. After passing the processes of intrathymic selection, $CD4^+$ and $CD8^+$ T cells expressing the transgene go on to seed the periphery, successfully recirculate, and their ratio remains unchanged.

The main goal of this study was to investigate the impact of TCR structure on phenotypic features of T lymphocytes, particularly whether expression of the memory TCR transgenic β-chain influences the surface phenotype of T cells. Obviously, identification of mouse naive, effector, and central memory T cells by flow cytometry is based on expression of CD44 and CD62L markers. The phenotype $CD44^-CD62L^+$ is characteristic of naive T cells, $CD44^+CD62L^+$ of central memory, and $CD44^+CD62L^-$ of effector memory T cells [27]. Analysis by flow cytometry of expression of these markers on the surface of splenocytes in transgenic mice revealed substantial decrease in the percentage of T cells with the activation phenotype, and increase in the percentage of T lymphocytes with the phenotype of naive T cells. Therefore, we have ascertained that the balance of subpopulations of naive and activated T cells was influenced by TCR transgenic β-chain expression. The most interesting finding of this work is that T lymphocytes expressing endogenous chains of TCR have the phenotype of activated T cells; this observation obviously happens in the course of lymphopenia induced by irradiation or cytostatic therapy.

Earlier published works mostly investigated effects of transgenic TCRs containing α- and β-chains together that resulted in hard impairment of a T cell repertoire and unphysiologically high frequency of T lymphocytes with the same specificity. This made significant problems for their adequate intrathymic selection and further survival in the periphery. In this respect, the model developed by us has benefits, because it allows formation of a diverse repertoire of TCRs due to rearrangements of endogenous α-chains. There are few publications devoted to effects of individual chains of TCR on the functional capabilities of a T cell repertoire. It was demonstrated that expression of TCR DO-11.10 (Vβ8.2) transgenic β-chain resulted in decreased responses of T lymphocytes to different antigens, including allogeneic MHC molecules as well as the burden of autoimmune disease developed in MRL-lpr mice. At the same time, accumulation of $CD4^+CD44^+$ $V\beta8.2^-$ T cells takes place in transgenic MRL-lpr mice, and this is responsible for the progress of disease in transgenic animals [28, 29].

The principal distinction of our work is that in intact animals we have seen similar T lymphocytes with the activation phenotype and carrying endogenous TCRs without antigenic stimulation and manifestations of autoimmune diseases.

In our study it was surprising that T cells expressing the memory TCR transgenic β-chain acquired the surface phenotype of naive, but not memory T cells, whereas T cells expressing endogenous β-chains became $CD44^+$ similar to T cells under conditions of lymphopenia. Mechanisms of this phenomenon remain unclear. We propose that our results may be explained by competition of T lymphocytes for the endogenous MHC–peptide complexes in the lymphatic tissue. Anyway, it is evident that the results demonstrate close association of the structural features of TCR with the surface activation phenotype of individual T cells.

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