Diverse Repertoire of the MHC Class II-Peptide Complexes Is Required for Presentation of Viral Superantigens[1]

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Among other features, peptides affect MHC class II molecules, causing changes in the binding of bacterial superantigens (b-Sag). Whether peptides can alter binding of viral superantigens (v-Sag) to MHC class II was not known. Here we addressed the question of whether mutations limiting the diversity of peptides bound by the MHC class II molecules influenced the presentation of v-Sag and, subsequently, the life cycle of the mouse mammary tumor virus (MMTV). T cells reactive to v-Sag were found in mice lacking DM molecules as well as in A^bEp-transgenic mice in which MHC class II binding grooves were predominantly occupied by an invariant chain fragment or $E\alpha^{52-68}$ peptide, respectively. APCs from the mutant mice failed to present v-Sag, as determined by the lack of Sag-specific T cell activation, Sag-induced T cell deletion, and by the aborted MMTV infection. In contrast, mice that express I-A^b with a variety of bound peptides presented v-Sag and were susceptible to MMTV infection. Comparison of v-Sag and b-Sag presentation by the same mutant cells suggested that presentation of v-Sag had requirements similar to that for presentation of toxic shock syndrome toxin-1. Thus, MHC class II peptide repertoire is critical for recognition of v-Sag by the T cells and affects the outcome of infection with a retrovirus. ***The Journal of Immunology,*** **2001, 166: 2244–2250.**

Peptides constitute an integral part of the MHC proteins without which these molecules aggregate and get degraded. The tertiary complex between peptide and α- and β-chains of the MHC is seen by the TCR as a single entity. The nature of a peptide modifies the complexes in such a way that the overall conformation of the MHC molecule is changed. The changes in conformation could be estimated by determination of SDS stability and hydrophobicity (1–5) and by mAb binding (6–9).

MHC class II molecules are also critical for presentation of superantigens (Sag),[4] which are protein products of bacterial (b-Sag) or viral (v-Sag) origin. Sag are able to activate large numbers of T cells bearing TCR β-chains that interact with Sag-MHC class II complexes. For exogenous mouse mammary tumor virus (MMTV) the Sag presentation is an absolutely critical step in its life cycle (10, 11). Cells of the immune system are the first targets of this virus (12). The infected B cells present v-Sag in the context of MHC class II molecules to T cells, leading to stimulation and consequent proliferation of specific Vβ-bearing T cells (13). These events result in viral amplification and transport to the mammary glands. Without recognition of v-Sag by cognate T cells, there is no T cell activation, no infection of mammary epithelium, and no viral transmission to the progeny via milk (10, 11). Sags that are present in the germline cause deletion of the Sag-reactive T cell subsets during formation of the immune repertoire (14). The MHC class II isotype and allotype matters for v-Sag presentation: I-E molecules are more efficient than I-A (14) and among I-A allotypes there is a hierarchy of v-Sag presentation (14, 15). As a result, mice lacking Sag-cognate T cells (10, 11) or animals with inappropriate MHC alleles (16) are either completely protected from, or are relatively resistant to, MMTV infection.

Whether v-Sag presentation by a given MHC molecule depends on the nature of a bound peptide was unknown. Targeted disruption of genes involved in MHC class II Ag presentation (DM, Refs. 17–19 and invariant chain (Ii), Ref. 20), as well as generation of mice expressing single MHC-peptide complexes (21), allowed the studies of the importance of the peptide repertoire limitation for T cell selection. Here, taking advantage of the array of animals with altered peptide presentation we address a specific question whether qualitative differences between MHC class II complexes affect presentation of v-Sag.

Materials and Methods

Mice and approved symbols

CBA/J, CBA/CaJ, C57BL6/J (B6), 129/J, (B6 × 129/J) F_1, BALB/cJ, LP/J, D1.LP/J (D1.LP-$H2^b$ H2-$T18^{b?}$/Sn), and DM knockout (KO) (B6, 129S-*H2-Ma*^{tm1Luc}; Ref. 18) mice were obtained from The Jackson Laboratory; Ii KO mice (20) were backcrossed to B6 mice for more than six generations at The Jackson Laboratory. To generate *Mtv7*-positive mutant mice, D1.LP/J (H-2^b, *Mtv7*$^+$) females were crossed to *Mtv7*-negative DM KO (H-2^b, *Mtv7*$^{-/-}$) males, and resulting F_1 females were backcrossed to DM KO males. N_2 generation animals were screened by *Mtv7*-specific PCR (22) and by FACS analysis to identify *Mtv7*$^{+/-}$, *Mtv7*$^{-/-}$, DM$^{+/-}$, and DM$^{-/-}$ offspring. *Mtv7*$^{+/+}$ Ii KO mice expressing single $E\alpha^{52-68}$ peptide-MHC class II complexes (A^bEp) (21) were provided by Dr. Philippa Marrack (National Jewish Center, Denver, CO). To generate *Mtv7*$^{-/-}$ A^bEp-transgenic mice, original *Mtv7*$^{+/+}$ A^bEp-transgenic females were crossed to MHC class II KO, Ii KO, *Mtv7*$^{-/-}$ males, and resulting F_1 females (*Mtv7*$^{+/-}$) were backcrossed to MHC class II KO, Ii KO, *Mtv7*$^{-/-}$ males. N_2 generation animals were screened by PCR to identify *Mtv7*$^{+/-}$ and

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[4] Abbreviations used in this paper: Sag, superantigen(s); b-Sag, bacterial superantigen; v-Sag, viral superantigen; MMTV, mouse mammary tumor virus; Ii, invariant chain; KO, knockout; A^bEp, covalent complex of A^b with a peptide derived from Eα molecule; SE, staphylococcal enterotoxin; TSST-1, toxic shock syndrome toxin-1; SI, stimulation index.

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Mtv7$^{-/-}$ mice, and by FACS analysis to identify A^{b}Ep-transgenic and nontransgenic mice.

Superantigens

b-Sag were purchased from Toxin Technology (Sarasota, FL). A mixture of three exogenous MMTVs (BALB2, BALBLA, and BALB14; Ref. 23) was a gift from Dr. Isabele Piazzon (Instituto de Investigaciones Hematologicas, Buenos Aires, Argentina). These viruses were passed on BALB/cJ mice (called BALB/cLA throughout).

RNase T_1 protection assays were performed as previously described (24), using BALBLA/(*Mtv7*)-specific probe (22). RNA was isolated from milk of mice after the second pregnancy by the method of Chirgwin et al. (25), and 5 μg was used per assay. The same probe was used to test expression of *Mtv7* in spleens. Forty micrograms of total RNA isolated from spleens was used for this analysis. X-ray film exposure was standard (12 h) in all experiments.

T cell activation

CD4$^+$ T cells were purified from lymph nodes by treatment with mAbs against MHC class II (Y3JP) (26) and 25-9-17 (27), and anti-CD8 (53-6.72) (28) for 45 min at 4°C (10^7 cells per ml of culture supernatant) followed by negative selection with the mixture of magnetic beads (anti-mouse IgG, anti-mouse IgM, anti-rat IgG) from PerSeptive Biosystems (Framingham, MA) according to manufacturer's protocol. Sag-presenting cells were 2000 rad irradiated, T cell-depleted spleen cells (for b-Sag), or nonfractionated splenocytes (for v-Sag). For T cell depletion a mixture of anti-CD8 and anti-CD4 (GK1.5) (29) mAbs was used, followed by treatment with anti-rat Ig magnetic beads.

Proliferation of CD4$^+$ T cells in response to b-Sag was measured by [^{3}H]thymidine incorporation after 3 days of cocultivation of 2×10^5 purified B6 CD4$^+$ cells with 3–4 $\times 10^5$ irradiated (2000 rad), T cell-depleted splenocytes and variable amounts of b-Sag in 96-well plates (Becton Dickinson, Lincoln Park, NJ) in the total volume of 150 μl. Culture medium was Click's Eagle's Hank's Amino Acids Medium (Irvine Scientific, Santa Ana, CA) supplemented with 5% FCS (Sigma, St. Louis, MO), 20 mM L-glutamine (Life Technologies, Grand Island, NY), 5×10^{-5} M 2-ME (Bio-Rad, Richmond, CA), and 100 U/ml penicillin/streptomycin mixture (Life Technologies). Stimulation index (SI) was calculated as follows: $SI = (a - b)/(c - d)$, where a = counts in response to Sag in an experiment, c = counts in response to Sag presented by MHC class II KO APCs, and b and d = counts in the same respective cultures without Sag.

Proliferation of T cells bearing specific TCR β-chains in response to v-Sag was estimated by FACS analysis of cells obtained from 3-day coculture of 2–3 $\times 10^6$ purified CD4$^+$ T cells from B6 or mutant mice with 5×10^6 irradiated stimulator splenocytes in 24-well plates (Becton Dickinson).

Virus isolation

Milk collected from MMTV-free or MMTV-infected BALB/c mice was diluted 1:10 with PBS and centrifuged at 600 $\times$ *g* for 15 min. Skim fraction of milk was centrifuged at 129,000 $\times$ *g* for 1 h, and pellets containing viral particles were resuspended in PBS. Fifty microliters of the original milk volume was injected into footpads as previously described (22, 30). After 4 days, cells from popliteal lymph nodes were stained with anti-CD4 and anti-Vβ6 mAb and analyzed by FACS.

mAbs and FACS analysis

Anti-TCR Vβ mAbs coupled with FITC were obtained from PharMingen (San Diego, CA). Anti-CD4 mAbs coupled to PE were purchased from Sigma. FACS analysis was performed using a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) and CellQuest software.

Results and Discussion

Lack of v-Sag presentation by DM KO cells

In DM KO mice most of class II molecules are occupied by class II binding Ii peptide. This complex alters recognition of A^b by allogeneic T cells (17, 19) and affects T cell selection (31, 32). In the following experiments we tested whether APCs of DM KO are capable of presenting the endogenous and exogenous v-Sag. To do that we first crossed *Mtv7*$^{-/-}$ DM KO mice to *Mtv7*$^{+/+}$ D1.LP/J (H-2^b) mice and then backcrossed hybrid F_1 females (*Mtv7*$^{+/-}$) to DM KO males. TCR Vβ profile was analyzed in the N_2 generation of mice with or without *Mtv7* and DM molecules. Deletion of T cells expressing cognate β-chains (Vβ6) was used as a read-out. Should v-Sag be presented normally, the cognate T cells should be deleted. As expected all DM-sufficient *Mtv7*$^+$ N_2 mice have deleted Vβ6$^+$ T cells (Fig. 1*A*). In contrast, none of DM-negative *Mtv7*$^+$ mice showed deletion of Sag cognate T cells, even though expression of this provirus could be easily detected in the spleens of these mice by *Mtv7*-specific RNase T_1 protection assay (Fig. 1*A*).

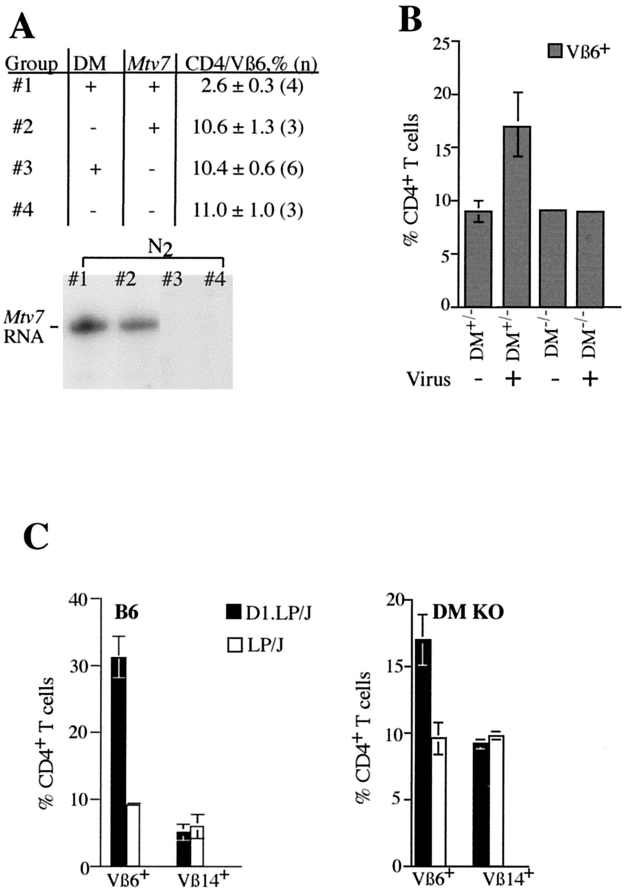

FIGURE 1. DM KO mice fail to present endogenous (*A*) and exogenous (*B*) v-Sag but have functional v-Sag-reactive T cells (*C*). *A*, *Mtv7*$^+$ DM KO mice do not delete v-Sag-reactive Vβ6$^+$ T cells. Four groups (1–4) of animals differing in *Mtv7* and DM expression were analyzed for the presence of Vβ6$^+$ cells among peripheral CD4$^+$ T cells. Data shown as mean percentage ± SE. *n*, number of mice used per group. *Bottom*, Expression of endogenous *Mtv7* by splenocytes from the four groups of analyzed mice detected by RNase T_1 protection assay with the *Mtv7* Sag-specific probe. *B*, No response to v-Sag could be elicited in DM KO mice upon MMTV injection. Four days after footpad injection of MMTV-containing or MMTV-free milk, cells derived from popliteal lymph nodes were double-stained with anti-CD4 PE and anti-Vβ6 FITC-labeled mAbs and analyzed by FACS. Results are expressed as mean percentages of Vβ6$^+$ T cells within the CD4$^+$ subset ± SE. $n = 6$. *C*, Purified CD4$^+$ T cells from the wild-type and DM KO mice responded to *Mtv7* v-Sag in vitro by selective amplification of T cells bearing *Mtv7*-reactive TCR (Vβ6$^+$), but not of Vβ14$^+$ cells. Expression of TCR β-chains was compared after stimulation with *Mtv7*$^{+/+}$ D1. LP/J and *Mtv7*$^{-/-}$ LP/J splenocytes. B6 CD4$^+$ T cells were used as positive control. The increase in frequency of CD4$^+$Vβ6$^+$ cells in response to D1. LP/J vs LP/J cells among DM-negative CD4$^+$ T cells was significant ($p < 0.001$). DM KO mice strongly respond to syngeneic I-A^b due to improper negative selection. Three B6 and six DM KO mice were used in two independent experiments.

We also tested the anti-v-Sag-specific response of DM KO mice to a mixture of exogenous MMTVs. Mice were injected with partially purified virus derived from BALB/cLA milk. BALB/cLA mice produce a complex of three viruses with Vβ2-, Vβ14- and Vβ6-specific Sag, respectively (22, 23). BALBLA virus encodes a Sag with the same Vβ specificity as *Mtv7*, capable of interacting with both I-E and I-A MHC class II molecules (22, 30). A significant increase in the percentage of $CD4^+V\beta6^+$ T cells was detected in the regional lymph nodes of DM-sufficient mice injected into foot pads with virus-containing milk compared with control mice injected with MMTV-free milk (Fig. 1*B*). In contrast, no T cell-specific stimulation was observed in DM KO mice injected with virus-containing milk (Fig. 1*B*). These results indicated that APCs from DM KO mice failed to present exogenous and endogenous v-Sag.

T cells from DM KO mice are fully capable of recognizing a v-Sag

The lack of T cell deletion (Fig. 1*A*) or proliferation (Fig. 1*B*) in response to v-Sag could be explained by a lack of the proper thymic positive selection in DM KO mice. Thus, we tested the ability of T cells from DM KO mice to react to v-Sag presented by DM-sufficient APC. To determine whether $CD4^+$ T cells from DM KO mice were able to recognize v-Sag, we tested their ability to increase the proportion of cells with appropriate TCR Vβ-chains in response to an endogenous v-Sag in vitro. Purified $CD4^+$ T cells from DM KO and control B6 mice were cultured with irradiated splenocytes from *Mtv7*-positive D1.LP/J ($H\text{-}2^b$) mice. T cells expressing several Vβs were expected to expand in reaction to *Mtv7*, including $V\beta6^+$ T cells. After 3 days in culture the cells were stained with anti-Vβ mAbs. In two independent experiments, responding T cells have shown an increase in the percentage of $V\beta6^+$ T cells after stimulation with *Mtv7*-expressing cells (Fig. 1*C*). At the same time the numbers of noncognate $V\beta14^+$ cells were not increased. In contrast, activation of the same cells with *Mtv7*-negative splenocytes from LP/J mice did not cause any special increase in $V\beta6^+$ T cell subsets. The relatively small increase in $CD4^+$ $V\beta6^+$ T cells from DM KO mice is most likely due to vigorous proliferation of T cells in response to MHC class II A^b molecules. This is because A^b-reactive cells are not deleted in DM KO mice (18, 31). Thus, DM KO mice have T cells that can respond to the endogenous v-Sag.

Lack of v-Sag presentation by A^bE-transgenic APC

A^bEp-transgenic mice express a single A^b-peptide complex (21). This complex is SDS stable and is recognized by A^bEp-specific Ab YAe (33, 34). Furthermore, it is not recognized by an anti-A^b mAb 25-9-17 (8), suggesting a special conformation of MHC class II complexes imposed by the peptide. We sought to determine whether APC from A^bEp-transgenic mice are capable of presenting endogenous v-Sag. The Ii KO mice were used as controls for these experiments because A^bEp-transgenic mice lacked Ii molecule (21). Both A^bEp-transgenic mice and Ii KO mice have reduced MHC class II expression (20); however, Ii KO mice express a diverse peptide repertoire (34). A^bEp-transgenic mice were found to inherit and express *Mtv7* (data not shown) contributed by one of the embryonic stem cell donors during generation of these mice (21). To determine whether A^bEp-transgenic mice delete $CD4^+V\beta6^+$ T cells when *Mtv7* is present we generated A^bEp-transgenic mice without *Mtv7* (see *Materials and Methods*). None of the A^bEp-transgenic $Mtv7^{+/-}$ mice have deleted their $CD4^+V\beta6^+$ T cells even though expression of the provirus could be easily detected in the spleens (Fig. 2*A*) and in lymph nodes (data not shown) of these mice. Thus, like DM KO mice with limited MHC peptide repertoire, the A^bEp-transgenic animals expressing the single MHC class II-peptide complex were unable to present endogenous v-Sag to the T cells.

T cells from A^bEp mice are fully capable of recognizing a v-Sag

Next we sought to determine whether A^bEp-transgenic mice were able to generate $CD4^+$ T cells capable of v-Sag recognition. Purified $CD4^+$ from A^bEp-transgenic and control Ii KO mice were stimulated with $Mtv7^{+/+}$ cells from D1.LP/J mice (Fig. 2*B*). $CD4^+$ T cells from both A^bEp-transgenic and Ii KO mice recognized v-Sag as determined by their ability to increase the percent of $V\beta6^+$ T cells after stimulation in vitro (Fig. 2*B*). In contrast, activation with $Mtv7^{-/-}$ LP/J cells did not show any increase in $V\beta6^+$ subsets (Fig. 2*B*). Although this increase was consistent in several experiments ($p = 0.0015$), it was very small. That relatively small increase was explained by a strong reactivity to A^b molecules with various peptides (21), which was obviously involving TCRs with diverse Vβ-chain repertoire. To circumvent this problem we used a pair of allogeneic $H\text{-}2^k$ strains CBA/J and CBA/CaJ, which are positive and negative for *Mtv7*, respectively. We expected that the frequency of cells responding to MHC class II $H\text{-}2^k$ molecules would be lower and that we would be able to detect the anti-v-Sag response. In fact, in this case $CD4^+$ T cells isolated from A^bEp-transgenic mice responded to *Mtv7* v-Sag by selective proliferation of the $V\beta6^+$ cells, whereas proliferation to $H\text{-}2^k$ molecules was nonselective in terms of Vβ usage (Fig. 2*C*). It is unlikely that the *Mtv7* v-Sag in A^bEp mice elicits stronger response when complexed with the MHC class $H\text{-}2^k$ compared with MHC class II $H\text{-}2^b$ molecules because the response of control B6 T cells to the *Mtv7* v-Sag presented by $H\text{-}2^k$ and $H\text{-}2^b$ MHC class II molecules did not differ (Figs. 1*C* and 2*C*). It could have been a problem for another v-Sag, but it is very unlikely for such a strong activator as *Mtv7* v-Sag. Thus, $CD4^+$ cells in A^bEp-transgenic mice are capable of reacting to v-Sag that is processed and presented by normal APC with a diverse MHC-peptide repertoire.

Exogenous MMTV infection depends on diversity of peptides presented by MHC class II

MMTV life cycle is dependent on the v-Sag T cell activation, and viruses that lack Sag sequences cannot propagate in vivo (35). Having established that peptides bound to MHC class II are important for presentation of endogenous MMTVs, we sought to determine whether exogenous MMTVs could infect MHC class II presentation mutants. Thus, we allowed DM KO and $(B6 \times 129/J)F_1$ control females to ingest infected milk from BALB/cLA females and later analyzed their own milk for the presence of MMTVs. All three viruses could be readily detected in all milk samples of $(B6 \times 129)F_1$ females but not in DM KO mice (Fig. 3*A*, data for the most abundant LA virus is shown).

We then applied the same strategy for infection of Ii KO and A^bEp-transgenic mice. Ii KO and A^bEp animals were infected with LA viruses by foster-nursing on BALB/cLA females. The control Ii KO mice became infected and produced virus into the milk (Fig. 3*B*). In contrast, there was no detectable virus in the milk of A^bEp-transgenic mice exposed to MMTV (Fig. 3*B*). Thus, even though T cells from mutant mice respond to v-Sag in vitro (Figs. 1*C* and 2, *B* and *C*) they fail to do so in vivo due to a failure of Sag presentation leading to an aborted MMTV infection.

The failure to present v-Sag by mutant APC could not be attributed to the differences in the amount of MHC class II expressed on the cell surface. The total class II expression in the wild-type and DM KO mice is very similar (17–19). The level of MHC class II in Ii KO and A^bEp-transgenic mice is below the levels of expression of MHC class II in the wild-type mice, but similar to each

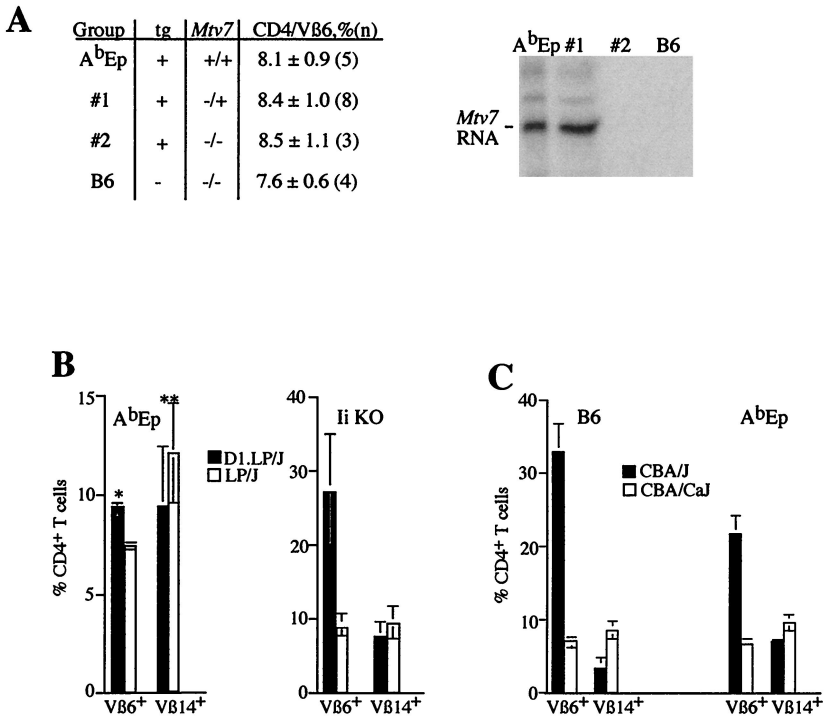

FIGURE 2. A^bEp-transgenic mice fail to present v-Sag (*A*) although they harbor T cells capable of recognizing it (*B* and *C*). *A*, $Mtv7^{+/+}$, A^bEp-transgenic mice do not delete v-Sag-reactive $V\beta6^+$ T cells. $Mtv7^{+/+}$ A^bEp-transgenic females were crossed to $Mtv7^{-/-}$ MHC class II KO and Ii KO males to generate two groups (1 and 2) differing in *Mtv7* provirus inheritance. *Right*, Expression of endogenous *Mtv7* by splenocytes from the analyzed groups of mice. The presence of $V\beta6^+$ cells among peripheral T cells was determined by FACS analysis. Data are expressed as mean percentage ± SE. n = number of mice used. *B*, T cells from A^bEp-transgenic mice respond to *Mtv7* Sag presented by MHC class II A^b molecules. Purified $CD4^+$ T cells from two A^bEp and control Ii KO mice responded to *Mtv7* v-Sag in vitro by selective amplification of *Mtv7*-reactive $V\beta6^+$ T cells after stimulation with D1.LP/J ($Mtv7^{+/+}$) splenocytes. Due to a high frequency of A^b-reactive $CD4^+$ T cells in A^bEp-transgenic mice, the overexpansion of the $V\beta6^+$ T cells was not impressive compared with Ii KO control mice. However, the difference in $Vb6^+$ T cell response to D1.LP/J cells was statistically significant (*, $p = 0.0015$), whereas the difference in activation of $V\beta14^+$ T cells was not (**, $p = 0.3$). Three A^bEp-transgenic and three Ii KO mice were used. *C*, Reactivity of Sag-cognate T cells from A^bEp-transgenic mice was better revealed when *Mtv7* Sag was presented by MHC class II molecules of the $H\text{-}2^k$ haplotype. Purified $CD4^+$ T cells from both wild-type (B6) and A^bEp mice responded to *Mtv7* Sag in vitro by a selective amplification of the T cells bearing *Mtv7*-reactive $V\beta6^+$ TCR. $V\beta6$ frequencies were compared after stimulation with $Mtv7^{+/+}$ CBA/J and $Mtv7^{-/-}$ CBA/CaJ splenocytes (both $H\text{-}2^k$). $V\beta14^+$ $CD4^+$ T cells were used as negative control. Summarized data from four independent experiments.

other (21). In addition, the low total number of $CD4^+$ T cells in those mutants could not be at fault, because Ii KO mice have similar $CD4^+$ T cell numbers as DM KO mice (31). However, the viral life cycle is complete in Ii KO mice but not in DM KO mice (Fig. 3). Furthermore, CD4-negative mice that have very few functional non-$CD8^+$ T cells are susceptible to MMTV infection (36). Moreover, mice that had over 90% of v-Sag-responding T cells deleted due to transgenic expression of a v-Sag (10) were producing detectable amounts of MMTV after two or more pregnancies (37). Thus, even practically invisible populations of v-Sag-reactive T cells may promote MMTV infection. In our experiments, all MMTV-infected mice were analyzed after the second pregnancy, and none of the mutant mice became MMTV infected. Thus, qualitative differences between MHC class II complexes must be responsible for the observed differences in the v-Sag presentation.

Variable presentation of b-Sag by MHC class II-positive cells from mutant mice

The mode of v-Sag presentation by MHC class II molecules could be better understood if compared with the presentation of b-Sag by MHC class II-positive cells from wild-type and mutant mice. b-Sag vary in the manner they bind MHC class II molecules as demonstrated by analyses of Sag mutants and crystal structures of Sag-MHC complexes. For instance, staphylococcal enterotoxin (SE)A binds MHC class II at two sites (one on α- and one on β-chain) (38, 39), and binding to His81β involves a Zn^{2+} ion. Another b-Sag, SED also cross-links MHC class II molecules using Zn^{2+} (40). In contrast, SEB has a single MHC binding site and does not use Zn^{2+} (41–44). Toxic shock syndrome toxin-1 (TSST-1) can bind Zn^{2+} but does not require it to elicit T cell responses and uses a single site for binding MHC (45, 46).

Such differences in binding properties of b-Sag would likely be detected in MHC class II presentation mutants. Therefore, we compared b-Sag-dependent activation of normal $CD4^+$ T cells from B6 mice by T cell-depleted normal and mutant splenocytes. We readily observed the differences in proliferation of responding $CD4^+$ T cells (Fig. 4). Some of our observations were in line with the previously published studies of b-Sag binding to MHC class II presentation mutants (31, 47). However, to evaluate quantitatively the ability of mutants to present Sag, all of the mutants had to be compared in the same experiment. Although the range of protein concentrations that elicit maximal T cell activation varies for different b-Sag (nanogram range for TSST-1 and SEA, and 10 μg range for SEB), the hierarchy of presentation of different b-Sag by the mutants can be found. Results of the experiments shown in Fig. 4 revealed the rank of presentation of the three tested b-Sag by wild-type and mutant APCs. It became clear that each Sag has its own pattern (Fig. 5). For example, presentation of TSST-1 is weaker by Ii KO cells, but is much weaker by DM KO cells when compared with the wild-type cells. In contrast, SEA is presented

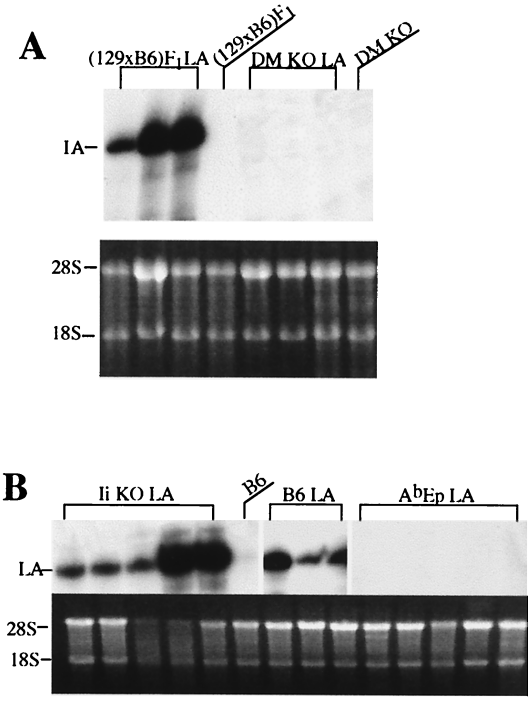

FIGURE 3. DM KO (*A*) and A^bEp-trangenic (*B*) mice are resistant to exogenous MMTV infection. *A*, (129 × B6)F_1 DM-sufficient females, but not DM-negative females became infected with BALBLA virus when foster-nursed by infected BALB/cLA females (labeled as (129 × B6)F_1 LA and DM KO LA, respectively). RNA isolated from milk of three individual mice per group was subjected to RNase T_1 protection analysis. The full-length protected band corresponds to exogenous MMTV RNA expression. ((129 × B6)F_1 and DM KO), RNA from the milk of uninfected mice. *Bottom*, Integrity of the RNA samples used for the assay. *B*, Ii KO, A^bEp-transgenic mice but not the control Ii KO mice are resistant to the exogenous MMTV infection. The same RNase T_1 protection assay was used to detect the presence of MMTV in the milk of mutant mice fed with BALB/cLA milk. (Ii KO LA, B6 LA, and A^bEp LA), RNA from the milk of mice foster-nursed on BALB/cLA milk. B6, Milk RNA sample from uninfected B6 mice. *Bottom*, Integrity of the RNA samples used for the assay.

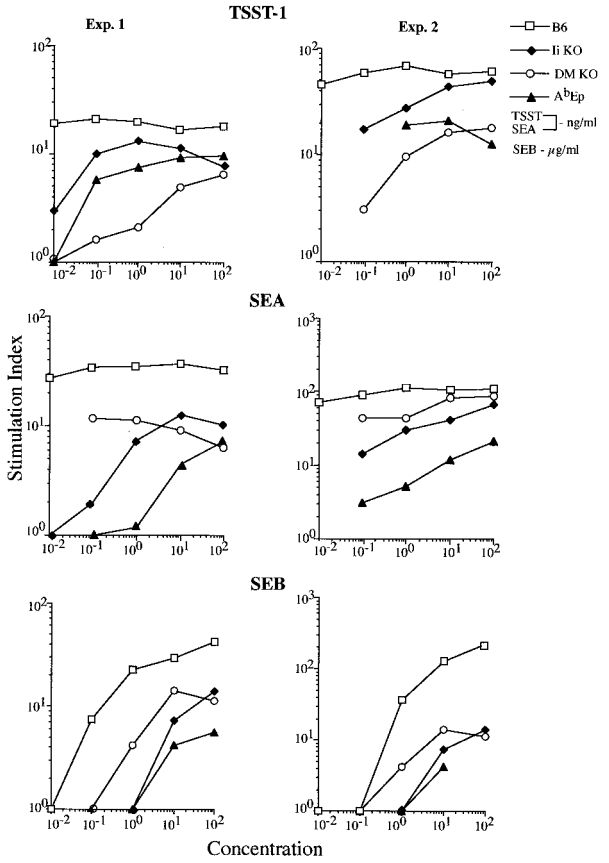

FIGURE 4. b-Sag are presented differently by MHC class II Ag presentation mutants. Purified $CD4^+$ T cells from B6 mice were stimulated in vitro by b-Sag presented by T cell-depleted splenocytes from B6 and mutant mice. [^{3}H]Thymidine incorporation was measured 72 h after activation. Vertical axis: SI, calculated as follows: $SI = (a - b)/(c - d)$, where a = counts in response to Sag, c = counts in response to Sag presented by MHC class II-negative splenocytes, and b and d = counts in the same respective cultures but without Sag. Horizontal axis: b-Sag concentration (TSST-1 and SEA, ng/ml; SEB, μg/ml).

relatively well by DM KO cells, but poorly by A^bEp-transgenic cells. Our data indicates that v-Sag presentation resembles (but is not identical to) the mode of presentation of TSST-1 than of other b-Sag tested, suggesting that these two Sag may favor similar MHC class II-peptide complexes for binding. Both SEA and TSST-1 have been previously shown to block the *Mtv7* Sag-peptides binding to MHC class II in vitro. However, the concentrations of blocking b-Sag were 10^3-10^4 times higher than required for T cell activation (48). The similar mode of presentation such as of TSST-1 and v-Sag does not necessarily imply that the two types of Sag use exactly the same interaction sites on the MHC class II molecules (49).

How do MHC class II variants affect Sag presentation? The most probable mechanism accounting for the Sag-presenting properties is the change in affinity of interaction between MHC class II molecule and Sag. Such differences have been shown to influence T cell activation quite dramatically; even a slight increase in MHC-Sag interaction could compensate for a huge loss of TCR-Sag interaction (50).

b-Sag bind MHC on the cell surface and do not need any additional processing. Presentation of v-Sag has been more enigmatic since their discovery. MMTV Sag is believed to be a type II protein processed from a longer precursor (51, 52). Recent compelling biochemical evidence suggests that v-Sag expression on the plasma membrane is independent of class II expression and that the Sag-MHC complexes are formed on the cell surface (53, 54) even in solution without any accessory proteins (55). That is important, because different MHC-peptide complexes may have their own peculiarities of intracellular trafficking, which theoretically could affect the rate of v-Sag binding and presentation.

That MHC class II-bound peptides can influence the b-Sag presentation has been well documented (56–58), and MHC-binding residues within a peptide as well as the C terminus of the peptide have the most influence on b-Sag binding (58). Two of MHC class

SAg	WT	Ii KO	DM KO	A^bEp
TSST-1	++++	+++	+	++
SEA	++++	++	+++	+
SEB	++++	+	++	+
vSAg	++++	++	-*	-*

FIGURE 5. Patterns of v-Sag and b-Sag presentation by MHC class II Ag presentation mutants. The responses to individual b-Sag and v-Sag were ranked relative to maximal responses to the same stimuli in the wild-type (WT, B6) animals. The presentation of v-Sag mostly (but not completely) resembles the mode of presentation of TSST-1. *, No detectable T cell deletion and MMTV infection.

II presentation mutants used in our study have peptide repertoire limited to either a single peptide (A^bEp) or to almost completely a single peptide (class II binding Ii peptide in DM KO mice). It is likely that peptides influence v-Sag binding indirectly by inducing MHC class II conformations that either do or do not favor v-Sag binding. However, the direct involvement of peptide residues in v-Sag binding cannot be excluded. Either way, the presentation of v-Sag (as well as the outcome of the retroviral infection) appears to be sensitive to the nature of the MHC-peptide complexes.

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