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Murine Vβ3+ and Vβ7+ T Cell Subsets Are Specific Targets for the HERV-K18 Env Superantigen

Albert K. Tai, Miao Lin, Francesca Chang, Gang Chen, Francis Hsiao, Natalie Sutkowski, and Brigitte T. Huber

Superantigens are a class of proteins that are derived from microorganisms and have the unique characteristic of stimulating T cells in a TCR Vβ-specific manner, causing massive T cell proliferation and immune deregulation. For this reason, superantigens have been implicated in the development of multiple diseases. We have previously identified and cloned an EBV-associated superantigen, human endogenous retrovirus (HERV)-K18 envelope protein (Env). This superantigen is transactivated upon IFN-α treatment and EBV infection and stimulates human Vβ13+ T cells. Due to the limited scope of work that can be conducted with human samples and the complexity of HERVs in general, we set out to study the physiological effects of HERV-K18 Env in a murine model. In this report, we demonstrate the superantigen activity of HERV-K18 Env in mice and describe the generation of HERV-K18 transgenics, using a bacterial artificial chromosome as transgenes that allow the faithful reproduction of the expression pattern of this human provirus. From our in vitro and in vivo results we conclude that HERV-K18 Env stimulates Vβ3+ and Vβ7+ T cells in mice. The definition of the murine Vβ specificity and the establishment of a transgenic model will permit the investigation of the role of this superantigen in the life cycle of EBV and its implicated diseases.

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uperantigens are a class of proteins derived from microorganisms. One of the most prominent characteristics of a superantigen is its specific interaction with a particular family of Vβ TCRs. Furthermore, superantigens are presented in the context of MHC II molecules, but unlike conventional Ags, no Ag processing is required, and the T cell recognition is not MHC restricted. Because of this unique ability to interact with TCR and MHC II simultaneously, superantigens are capable of activating a large portion of T cells expressing a particular TCR Vβ family. This trimolecular interaction leads to a strong primary immune response and, hence, massive cytokine production; thus, exposure to superantigens can cause a toxic shock-like syndrome. In addition, the immense stimulation of superantigen-activated T cells results in lymphoproliferation. The superantigen-activated T cells are eventually eliminated via activation-induced cell death, leading to the deletion of whole T cell subsets of particular Vβ TCRs. The constitutive expression of endogenous superantigens results in partial or complete elimination of those T cells in a Vβ-specific manner, presumably due to sustained apoptosis of the reactive T cells and/or negative selection in the thymus.

An EBV-associated superantigen has been described by our laboratory. This superantigen is not encoded within the EBV genome, but by the envelope gene (env) of the human endogenous retrovirus (HERV)K18, which is normally transcriptionally silent. The EBV-associated superantigen is recognized by Vβ13+ and Vβ9+ and, to a lesser extent, Vβ7+ human T cells (1–3).

The significance of the HERV-K18 env transactivation by EBV remains unclear. We postulate that the superantigen plays a role in the life cycle of EBV and the pathogenesis of EBV-associated diseases, based on the findings with mouse mammary tumor virus (MMTV), a B type retrovirus that encodes a superantigen. This superantigen plays a crucial role in the completion of the MMTV life cycle; namely, the superantigen-activated T cells supply essential signals leading to the activation of MMTV-infected B cells, which, in turn, provide the critical environment for viral replication and relocation to appropriate tissues (4–6).

Because of this unique capability of activating T cells in a non-Ag specific manner, superantigens have been implicated in the development of autoimmune diseases, such as rheumatoid arthritis, Kawasaki disease, type I diabetes, and psoriasis (7); they are also associated with malignancies, such as cutaneous T cell lymphoma (8, 9). Interestingly, EBV has been linked to the etiology of similar types of disorders—autoimmune diseases, e.g., systemic lupus erythematosus (10–14), multiple sclerosis (15–18), and Sjogren’s syndrome (19); malignancies, e.g., Burkitt’s and Hodgkin’s lymphoma (20, 21), as well as, nasopharyngeal (22, 23) and gastric carcinoma (24–26); and lymphoproliferative diseases, e.g., infectious mononucleosis (27), X-linked lymphoproliferative disease (28), and posttransplant lymphoproliferative disorder (29). Due to the similarity of the types of diseases in which both superantigens and EBV have been implicated, we suspect that the HERV-K18 Env superantigen may contribute to the development of some of the EBV-associated diseases.

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4 Abbreviations used in this paper: HERV, human endogenous retrovirus; Env, envelope protein; BAC, bacterial artificial chromosome; env, envelope gene; LCL, lymphoblastoid cell line; MMTV, Murine mammary tumor virus.
Hence, it is of great interest to define the physiological effects of HERV-K18 Env. Currently, the characterization of this superantigen is limited to in vitro experimental systems using human samples; however, to understand the full impact of the HERV-K18 Env superantigen, an animal model is necessary. Here, we report the development of a HERV-K18 transgenic mouse. Furthermore, we have established that HERV-K18 Env functions as a superantigen for murine T cells both in vitro and in vivo, stimulating the Vβ3+ and Vβ7+ TCR subsets. This system will allow the analysis of the impact of HERV-K18 Env expression in vivo in the intact mouse.

Materials and Methods

Cell culture

All cell lines were grown in RPMI 1640, supplemented with 10% FCS, glutamine, HEPEs, sodium pyruvate, penicillin/streptomycin, and 2-MA (Invitrogen Life Technologies). A20/pCDLL, A20/K18.1, BL-41, and BL41/B95-8 have been described previously (2, 3). The EBV-transformed lymphoblastoid cell lines (LCL) (3), LCL-A and LCL-B, were derived from unrelated healthy adult donors, using previously described methods (3).

Antibodies

The FITC-labeled murine Vβ screening panel (catalog no. 557004), allophycocyanin-labeled anti-murine CD4 (catalog no. 553051), PE/Cy5-labeled anti-murine CD8 (catalog no. 553034), anti-murine CD3 (catalog no. 553238) were purchased from BD Biosciences. Anti-murine IgM F(ab′)2 (catalog no.115-006-006) was purchased from Jackson ImmunoResearch Laboratories.

Hybridoma assay

Murine T cell hybridomas of Vβ2 (EF2), Vβ3 (2B4; Leslie Berg, University of Massachusetts Medical School, Worcester, MA), KJ25 (John Kappler, National Jewish Hospital, Denver, CO), SKC (John Kappler), Vβ4 (42H11; Ref. 33), Vβ5 (EF31), Vβ6 (RG17, Ed Palmer, University of Basel), Vβ8 (MG4C2, MG1F4), Vβ9 (MG3A11), Vβ10 (MG4F5), and Vβ15 (EF13) TCR were used in the hybridoma assays as responders. EF2, EF31, EF13, MG4C2, MG1F4, and MG4F5 are newly derived murine T cell hybridomas. All T cell hybridomas were used as CD4+. The human LCL-A, LCL-B, BL-41, BL-41/B95-8, and murine A20/pCDLL, A20/K18.1 cell lines were used as stimulators/APCs. The hybridoma assays were setup, as previously described (30). In brief, a fixed amount of murine T cell hybridoma responders (2 x 10^5) was cocultured with the gamma-irradiated (5000 rad) stimulators/APCs at indicated ratios in a 96-well round-bottom plate. The cocultures were allowed to proceed for 24–48 h. Plate-bound anti-CD3-stimulated and unstimulated hybridomas were used as positive and negative controls, respectively. The concentration of IL-2 produced by the T cell hybridomas was then assayed by typical IL-2 ELISA, with recombinant IL-2 as standard.

In the antisense blocking experiments, the corresponding stimulators/APCs were pretreated with the anti-HERV-K18 Env antisense or preimmune serum for 1 h, followed by the hybridoma assays described above. The anti-HERV-K18 Env antisense has been described previously (2).

Transgenic mice

Bacterial artificial chromosomes (BAC) clones RP11-404F10 and RP11-575N16 (GenBank accession no. AL121985 and AC027082, respectively), carrying the complete HERV-K18 provirus and flanking genes, were used as transgenes. Transgenic mice were generated by pronuclear microinjection of the BAC into fertilized eggs of the FVB background. Offspring were screened by genomic DNA PCR, using primer pairs specific for the HERV-K18 provirus (forward: 5'-TCC GAA GAG ACA CGT ACA TGG A-3', reverse: 5'-GTA ATG ATC ATG CTG TCT AAG GT-3') and primers specific for sh2d1a as internal control (forward: 5'-AAG ATG CCA CAG GAA GCC TA-3', reverse: 5'-TGG CTA AGC CGT CTG TCT GT-3'). Three founder mice were selected that tested positive for the HERV-K18 transgene by PCR. They were then backcrossed for 6 to 10 generations with C57BL/6 transgenic mice that are on the 1A-/-, C57BL/6 background (31). All animals were bred at the Tufts University Division of Laboratory Animal Medicine. The Institutional Animal Care and Use Committee of Tufts University has approved all procedures.

IgM induction and HERV-K18 env real-time RT-PCR

The HERV-K18 transgenic mice were euthanized, and single cell suspensions of the spleen were obtained by meshing the spleen through a disposable 70-μm cell strainer. The B cells were then purified by negative selection with IMag Mouse B lymphocyte enrichment set—DM (BD Biosciences, catalog no. 557792), following manufacturer’s instructions. Purified B cells (95–97% purity) were cultured in complete medium in the presence or absence of anti-IgM (F(ab′)_2, 5 μg/ml) for 2 h. RNA was then isolated using RNeasy mini kit (Qiagen). The cDNA was generated from 1 to 2 μg of RNA using iScript reverse transcriptase (Bio-Rad). The cDNAs were subjected to duplex real-time PCR analysis on Sequence Detection System 7300. Primers and TaqMan MGB probes specific for the read-through transcript of HERV-K18 env (6-FAM conjugated, custom design; sequences for the forward and reverse primers and the TaqMan MGB probe are 5'-CCG CCT TTT GAG CAG AAG TAT AAG C-3P and 5'-TAA TGG CAA TGG CTA GTG CTG CAG ACA AAC TT-3P, respectively) and housekeeping gene 18S ribosomal RNA, in conjunction with TaqMan PCR master mix, were used to quantitate the mRNA level of HERV-K18 env in relation to 18S rRNA.

TCR Vβ repertoire analysis

Peripheral blood of the transgenic mice was obtained by tail bleeding. The RBC were lysed with 1× PhosphoLyse (BD Biosciences), following manufacturer’s instructions. The PBL were then stained with FITC-labeled anti-Vβ Abs, PE/Cy5-labeled anti-CD8, and allophycocyanin-labeled anti-CD4 at room temperature for 30 min. All data were acquired on a dual-laser 4-color FACSAlibur (BD Biosciences) and analyzed by FlowJo analytical software.

Results

HERV-K18 Env has specificity for murine Vβ3+ T cells in vitro

The most prominent characteristic of a superantigen is its capability to stimulate T cells expressing specific TCR Vβ chains. To investigate whether the HERV-K18 superantigen is also active on murine T cells, we screened a panel of murine T cell hybridomas expressing various Vβ chains (Fig. 1). We had previously reported that EBV-transformed LCLs express the HERV-K18 Env superantigen and are capable of stimulating T cell hybridomas of the human Vβ13 TCR family (2). Hence, we decided to use EBV+ LCLs as stimulators for this screening process.

Gamma-irradiated EBV-transformed LCL-A cells were used to stimulate a panel of T cell hybridomas, with plate-bound anti-CD3 serving as positive control. The read-out for these experiments is

**FIGURE 1.** Murine T cell hybridoma expressing Vβ3 TCR produces IL-2 in response to HERV-K18 Env. A panel of murine T cell hybridomas of indicated TCR Vβs was used as responders to test for the murine Vβ specificity of HERV-K18 Env. EBV-transformed LCL-A cells were used as APCs, and the amount of IL-2 produced by the respective T cell hybridomas was determined by ELISA measured in ng/ml. *p < 0.05, statistically significant difference between the experimental and control groups.
IL-2 production by the T cell hybridomas in response to stimulation. The results from these experiments, summarized in Fig. 1, demonstrate that only the murine Vβ3+ T cell hybridoma, 2B4, secretes significant amounts of IL-2 in response to LCL-A in a dose-dependent manner. These results indicate that murine Vβ3 is a potential target for the HERV-K18 Env superantigen. To a lesser extend, the Vβ6+ T cell hybridoma also produced IL-2 in response to LCL-A in a dose-dependent manner. Another characteristic of a superantigen is its dependency on MHC class II for presentation, although no MHC restriction of the response is seen. To test this property, three additional cell lines were used as stimulators in these experiments: 1) LCL-B, an EBV-transformed LCL with a different MHC class II haplotype from that of LCL-A; 2) BL-41/B95-8, an EBV-infected Burkitt’s lymphoma; and 3) A20/K18.1, a murine B cell lymphoma that is stably transfected with an expression vector encoding HERV-K18.1 env. The EBV-negative BL-41 and mock-transfected A20/pCDLI cell lines were used as negative controls, respectively. Both BL-41/B95-8, and A20/K18.1 have been shown to possess superantigen activity, stimulating human Vβ13+ T cell hybridomas (2).

Thus, the response of the murine Vβ3+ T cell hybridoma, 2B4, to these additional superantigen-expressing cell lines was tested under identical conditions as in the previous experiments. The results from these experiments are summarized in Fig. 2. As expected, 2B4 produced IL-2 in response to LCL-B in a dose-dependent manner (Fig. 2a). In addition, the EBV-infected Burkitt’s lymphoma, BL-41/B95-8, but not the EBV-negative control, BL-41, stimulated the Vβ3+ T cell hybridoma to produce IL-2 in a dose-dependent manner (Fig. 2b). We had previously demonstrated that HERV-K18 env is transactivated by EBV in the infected Burkitt’s lymphoma, resulting in the stimulation of human T cells (2). Furthermore, the murine B cell lymphoma, A20/K18.1, which is transfected with and stably expresses HERV-K18.1 env, also stimulated the Vβ3+ T cell hybridoma to produce significant amounts of IL-2 when compared with the control cell line (Fig. 2c; p < 0.05). These data suggest that the superantigen activity of HERV-K18 Env is maintained in the context of multiple human and murine MHC II haplotypes, as measured on murine T cells.

Up to this point, all experiments were performed with a single murine Vβ3+ T cell hybridoma, 2B4. Since a classical superantigen is capable of stimulating all T cells within a particular Vβ and murine MHC II haplotypes, as measured on murine T cells. Another characteristic of a superantigen is its dependency on MHC class II for presentation, although no MHC restriction of the response is seen. To test this property, three additional cell lines were used as stimulators in these experiments: 1) LCL-B, an EBV-transformed LCL with a different MHC class II haplotype from that of LCL-A; 2) BL-41/B95-8, an EBV-infected Burkitt’s lymphoma; and 3) A20/K18.1, a murine B cell lymphoma that is stably transfected with an expression vector encoding HERV-K18.1 env. The EBV-negative BL-41 and mock-transfected A20/pCDLI cell lines were used as negative controls, respectively. Both BL-41/B95-8, and A20/K18.1 have been shown to possess superantigen activity, stimulating human Vβ13+ T cell hybridomas (2).

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Up to this point, all experiments were performed with a single murine Vβ3+ T cell hybridoma, 2B4. Since a classical superantigen is capable of stimulating all T cells within a particular Vβ family, we reasoned that the HERV-K18 Env superantigen will be similarly capable of stimulating other T cell hybridomas expressing murine Vβ3, but differing in their Vα chains. Hence, additional Vβ3+ T cell hybridomas, JK25 and 5KC, were used as responders to LCL-B and A20/K18.1. Both JK25 and 5KC produced IL-2 in a dose-dependent manner in response to stimulation by LCL-B (Fig. 2, d and e). Similar results were obtained in experiments where the murine B cell lymphoma, A20/K18.1, was used as stimulator (Fig. 2, f and g). The results from these experiments confirm the notion that murine Vβ3 is a specific target for the HERV-K18 Env superantigen. It is important to note that A20 is of BALB/c origin, which expresses an endogenous MMTV provirus encoding a weak Vβ3 stimulatory superantigen. This accounts for the relatively high background IL-2 production observed with the control A20/pCDLI cells.

We had previously produced an antiserum against HERV-K18 Env that blocked superantigen stimulation of human T cells (2). Thus, we tested whether this antiserum would also block the stimulation of murine T cells. For this purpose, we preincubated A20/K18.1 cells with either anti-Env antiserum or preimmune serum as control. Untreated A20/pCDLI, A20/K18.1, and plate-bound anti-CD3 stimulation were included as additional controls. As predicted, the preimmune serum-treated A20/K18.1 cells showed stimulatory activity on the 2B4 T cell hybridoma. In contrast, A20/K18.1, pretreated with anti-Env antiserum, failed to stimulate this hybridoma. These data indicate that the anti-Env antiserum is capable of blocking HERV-K18 superantigen recognition by murine T cells (data not shown).

Taken together, these data indicate that murine T cells are able to recognize the HERV-K18 superantigen in a Vβ-specific and non-MHC II restricted manner. Furthermore, the antiserum blocking experiments provide direct evidence that the HERV-K18 Env protein is responsible for this stimulatory activity. Thus, we decided to use BAC for the generation of HERV-K18 transgenic mice, to study the primary T cell response to HERV-K18 Env in vivo.

HERV-K18 Env transgenic mice delete Vβ3+ and Vβ7+ T cells
To produce an animal model for studying the physiological effects of the HERV-K18 superantigen in vivo, we used BACs that contain the HERV-K18 provirus as transgenes. Since HERV-K18 maps to the first intron of cd48, an EBV-inducible gene, we hypothesized that these two genes may share regulatory elements. Hence, two different BACs, AL121985 and AC027082, each containing the complete coding sequence of cd48 and the HERV-K18 provirus, were chosen to generate the transgenic mice (Fig. 3a).

Pronuclear microinjection of the BACs into fertilized eggs of the FVB background were performed, and three founder mice were selected that screened positive for the transgene by PCR, two from the AL121985 BAC and the third from the AC027082 BAC injection. Each founder mouse was maintained as a separate line and was used in experiments only after having been backcrossed for 6 to 10 generations to C57BL/6 mice that were I-Ab+. In addition, the EBV-infected Burkitt’s lymphoma, BL-41/B95-8, but not the EBV-negative control, BL-41, stimulated the Vβ3+ T cell hybridoma to produce significant amounts of IL-2 when compared with the control cell line (Fig. 2c)

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blood of our transgenic lines, compared with DR4 only transgenic mice. Five to seven mice from each founder line were analyzed, and the mean frequency of the T cell subsets expressing each of the 13 Vβs is summarized in Fig. 4. Statistical significance of the differences in the frequencies of T cells of particular Vβ was determined by t test.

As predicted, the Vβ3+ T cell population measured in the HERV-K18 transgenic lines was significantly lower when compared with the control mice (p < 0.05). Furthermore, we observed that Vβ7+ T cells were also significantly reduced in all the HERV-K18 transgenic lines, when compared with the control mice (p < 0.05). In fact, the reduction we observed in the Vβ7 population is more prominent than that of the Vβ3 subset. In addition, T cells with Vβ13+ and Vβ9+ TCRs were slightly reduced in the HERV-K18 transgenic vs control mice; however, only two of three and one of three transgenic lines showed statistically significant (p < 0.05) reduction for Vβ13 and Vβ9, respectively. Because circular DNA of the BAC constructs was used for the production of the transgenic mice, each founder line differs in the amount of 5' and 3' regulatory elements. Thus, we plan to map the integrated constructs.

FIGURE 2. Vβ3+ T cell hybridomas respond to stimulation by multiple human and murine cell lines expressing HERV-K18 Env. Murine Vβ3+ T cell hybridoma 2B4 produced IL-2 in a dose-response manner when stimulated by (a) the EBV-transformed LCL-B cells, (b) the EBV-infected Burkitt’s lymphoma cell line, BL-41/B95–8, but not the EBV-negative parent line, BL-41, (c) the HERV-K18 env-transfected murine B cell lymphoma, A20/K18.1, but not control cell line, A20/pCDLI. Additional Vβ3+ T cell hybridomas 5KC (d and f) and KJ25 (e and g) produce IL-2 in a dose-dependent manner when stimulated by LCL-B (d and e) and significantly more IL-2 when stimulated by A20/K18.1, compared with A20/pCDLI control (f and g). * Statistically significant difference between the experimental and control groups.
of the various lines, because this may provide an explanation for the differential constitutive superantigen expression patterns observed.

It is interesting to point out that the T cells expressing Vβ5, Vβ11 and Vβ12 are clonally eliminated in both HERV-K18/DR4 double and DR4 single transgenic mice, demonstrating that the endogenous superantigens encoded by the Mtv-8, 9, and 17 proviruses of C57BL/6 mice are functional in the context of DR4, whereas these activities in the context of murine I-A<sup>+</sup> are weak, leading to peripheral T cell deletion over time only (32). Thus, we could not determine the extent to which HERV-K18 Env affects T cell activation/deletion of these Vβ families in this experiment. We are currently breeding our HERV BAC transgenic mice on a DR4/B6 background, where these particular Vβ subsets are not deleted.

Discussion
In this report, we have presented in vitro and in vivo data supporting the notion that HERV-K18 Env behaves as a superantigen in the murine system. Furthermore, we were able to define the murine...
Vβ specificity of the HERV-K18 superantigen. Results from the in vitro experiments strongly argue that the murine Vβ3 TCR is a specific target of this superantigen. The generation of HERV-K18 env transgenic mice was the natural choice for the in vivo characterization of this superantigen. From previously reported results and recent data accumulated in the laboratory, we learned to appreciate the complexity of the transcriptional regulation of HERV-K18 env, in which the expression is B cell restricted and can be up-regulated via several signal transduction pathways. Thus, it was essential to reproduce the expression of HERV-K18 Env in the transgenic model, if we want to study the physiological effects of this superantigen. Therefore, the choice of method to generate the transgenic mice was crucial for our study. Typically, the transgene of interest is expressed in the context of an exogenous promoter that regulates the expression. With this approach, the expression pattern of the transgene can be controlled to a certain extent by a well-characterized promoter. However, additional regulatory regions, located outside of the promoter region, control gene expression, such as enhancer and repressor elements that can be located at considerable distance from the transcriptional start site. Hence, a complete reproduction of the expression pattern of a gene using the above transgenic approach could be challenging. Furthermore, the regulatory elements of the HERV-K18 env have not been defined so far; thus, it is impossible to make a sensible choice of exogenous promoter that could reproduce the expression pattern of the HERV-K18 env in a murine transgenic system. For these reasons, we have chosen to take the less common approach, in which a BAC carrying the gene of interest is used as transgene. It has been reported that the use of BAC to generate transgenic mice enables the best reproduction of the endogenous expression pattern, as the large chromosome fragment contained within the BAC allows the inclusion of all transcriptional regulatory elements of a transgene. This is essential for the study of the physiological role of the HERV-K18 superantigen in a murine model system. HERV-K18 env maps to the first intron of cd48, and both HERV-K18 env and cd48 can be transactivated by EBV or induced by IgM/CD40 cross-linking on the B cell surface. This is an indication of shared regulatory elements. However, CD48 is also an activation marker for T cells, yet HERV-K18 env transcripts are not detected within this population. In addition, some signaling pathways are able to activate cd48 without affecting the transcription of HERV-K18 env. Furthermore, the 5′-long terminal repeat of the HERV-K18 provirus seems to retain a certain amount of regulatory activity, as demonstrated by in vitro reporter assays. Taken together, these data indicate that the use of BAC to generate HERV-K18 transgenic mice is currently the best available choice.

Upon generation of transgenic mice with the appropriate genetic background and HERV-K18 env transcriptional regulation, the T cell repertoires were assayed. As expected from the in vitro experiments with a panel of murine T cell hybridomas, a clonal reactivity of Vβ families was observed for HERV-K18 superantigen in our murine model, as T cells of these Vβ families are clonally deleted due to the endogenous Mtv-8, 9, and 17 superantigens that are recognized by these T cell subsets in the context of the transgenic DR4 molecule, whereas only partial deletion is seen in the context of the native I-Aβ (32). Thus, the BAC transgenic mice will be bred on a DR4−/B6 background, where no MMTV-mediated deletion of Vβ subsets is seen. Despite this limitation, the panel of anti-Vβ Abs that we used for our experiments, together with the collection of murine T cell hybridomas, is estimated to include the majority of Vβ families, accounting for >75% of the peripheral T cell repertoire in a normal mouse. Hence, our results represent a fairly comprehensive overview of the murine Vβ specificity of the HERV-K18 Env superantigen.

From the analyses of the superantigen activity of the various endogenous MMTV proviruses, we have learned to appreciate that clonal deletion/reduction in vivo is the most sensitive method for the detection of T cell reactivity, whereas T cell activation in an in vitro coculture system requires substantially higher retroviral superantigen activity. We have attempted to stimulate primary T cells from DR4 single transgenic mice with purified splenic B cells from the HERV-K18/DR4 double transgenic mice; however, we were not able to observe reproducible activation and/or proliferation of the primary murine T cells in vitro, even after cross-linking Ig on the B cell surface that led to increase in HERV-K18 env mRNA (data not shown). These negative results suggest that HERV-K18 Env is a weak superantigen in the context of our experimental system. Alternatively, either of the Vβ5, Vβ11, or Vβ12 CD4 T cell subsets may be the high responder to HERV-K18 Env.

Many factors contribute to the magnitude of the stimulatory capacity of a superantigen and the T cell reactivity to a superantigen, such as MHC II haplotypes, specific combinations of Vβ and Vα TCRs and the differential presentation capacity and reactivity among species. In our case, the chimeric DR4/I-Ek MHC II molecule may not be optimal for presentation of the HERV-K18 superantigen, despite its very efficient presentation of endogenous Mtv-8, 9, and 17 superantigens, leading to clonal deletion of Vβ5+, Vβ11+, and Vβ12+ T cell subsets. On the other hand, these endogenous superantigens are not efficiently presented in the context of I-Aβ, because no deletion of these subsets is seen in normal C57BL/6 mice. Further work will have to be conducted, testing additional MHC class II molecules for presentation of the HERV-K18 superantigen. As shown in Fig. 2, HERV-K18 Env can be efficiently presented by I-Eα, expressed by the HERV-K18.1 env-transfected A20 B lymphoma, which stimulates murine Vβ3+ T cell hybridomas. Alternatively, prestimulation of the HERV-K18-expressing B cells may be required. These approaches are currently being tested in our laboratory.

Two other groups have attempted to detect the superantigenic properties of HERV-K18 Env in the murine system by testing for murine Vβ specificity (33, 34), but have failed in this endeavor. In the first report (33), Azar and coworkers transfected the HERV-K18 env gene into human Raji cells and tested them for reactivity on a panel of murine T cell hybridomas, including KJ25, the identical Vβ3+ hybridoma that we used in this study. The authors did not observe significant IL-2 production by any of the hybridomas upon stimulation with the HERV-K18 env transfected Raji cells when compared with untransfected Raji cells. In our studies, we have observed that this particular Vβ3+ T cell hybridoma, KJ25, is a relatively weak responder to the HERV-K18 superantigen stimulation when compared with the other two Vβ3 hybridomas that we have tested, probably due to the contribution of the TCR α-chain. Furthermore, since Raji is an EBV-positive Burkitt’s lymphoma, it should have endogenous superantigen activity; i.e., the
background stimulation of untransfected Raji cells would overrule the effect of an additional ectopically expressed superantigen. Taken together, the weak responder and potential high background of the stimulator may have diminished any potential superantigen effect of the ectopically expressed HERV-K18 Env protein.

In the second report (34), Lapatschek and coworkers ectopically expressed truncated forms of the three alleles of HERV-K18 env in A20 cells and were able to demonstrate the expression of the HERV-K18 Env proteins, using tagged constructs. These cell lines were then used to stimulate a human Vβ7+ T cell hybridoma, T cell hybridomas with murine Vβ4, Vβ8, Vβ10, and Vβ14 TCRs, respectively, as well as primary murine T cells. The authors were unable to observe specific stimulation by the tagged HERV-K18 Env proteins in any of the experiments. Our experiments confirm that murine Vβ4, 8, 10, and 14 are not reactive with the HERV-K18 superantigen; hence, we would not expect to observe any specific stimulation by the superantigen with this panel of murine T cell hybridomas. This, in part, explains why these authors cannot detect the superantigen activity of the HERV-K18 Env.

With the establishment of HERV-K18 Env superantigen activity in the murine system and the generation of HERV-K18 transgenic mice that delete the appropriate TCR subsets in a Vβ-specific manner, we have created a new approach to study the physiological effects of this superantigen.

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Disclosures
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