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Murine V\(\text{\textbeta}^3^+\) and V\(\text{\textbeta}^7^+\) 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 V\(\text{\textbeta}\)-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-\(\alpha\) treatment and EBV infection and stimulates human V\(\text{\textbeta}^3^+\) 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 V\(\text{\textbeta}^3^+\) and V\(\text{\textbeta}^7^+\) T cells in mice. The definition of the murine V\(\text{\textbeta}\) 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. The Journal of Immunology, 2006, 177: 3178–3184.
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-ME (Invitrogen Life Technologies). A20/CDLL, 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 Fab(ab')2, and allophycocyanin-labeled anti-CD4 at room temperature for 30 min. All data were acquired on a dual-laser 4-color FACS Calibur (BD Biosciences) and analyzed by FlowJo analytical software.

Hybridoma assay
Murine T cell hybridomas of Vβ2 (EF2), Vβ3 (2B8; Leslie Berg, University of Massachusetts Medical School, Worcester, MA), KJ25 (John Kappler, National Jewish Hospital, Denver, CO), 5KC (John Kappler), Vβ4 (42H11; Ref. 33), Vβ5 (EF31), Vβ6 (RG17, Ed Palmer, University of Basel), Vβ8 (MG14C2, MG1F4), Vβ9 (MG3A11), Vβ10 (MG4F5), and Vβ15 (EF13) TCR were used in the hybridoma assays as responders. EF2, EF31, EF13, MG14C2, MG1F4, and MG4F5 are newly derived murine T cell hybridomas. All T cell hybridomas used were CD4+ . The human LCL-A, LCL-B, BL-41, BL-41/B95-8, and murine A20/CDLL, A20/K18.1 cell lines were used as stimulators/APCs. The hybridoma assays were set up, as previously described (30). In brief, a fixed amount of murine T cell hybridoma responders (2 × 10^4) 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 antisera blocking experiments, the corresponding stimulators/ APCs were pretreated with the anti-HERV-K18 Env antisera or preimmune serum for 1 h, followed by the hybridoma assays described above. The anti-HERV-K18 Env antisera 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'-GGG AGA CTG CGT ATT TCA-3' , reverse: 5'-GTA ATG GCA ATG CTG GAT TAA GT-3'), and primers specific for sh2d1a as internal control (forward: 5'-AAG ATG GCA CAG GAA GAG ACA ATG ACA TCG A-3', reverse: 5'-CTC GTA ATG GCA ATG CTG GAT TAA 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 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 (Fab')2 (5 µg/ml) for 2 h. RNA was then isolated using RNasey 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 CAG ATT AAG A-3', 5'-CAG TAA TGG CAA TGC TGG CTA TG-3' and 5'-TAA GTC GCA CAG ACA AAC TT-3', respectively) and housekeeping gene 18S ribosomal RNA, in conjunction with TaqMan PCR master mix, were used to quantitate the RNA 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× PharmLyse (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 FASCAlibur (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](http://www.jimmunol.org/)
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 extent, 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 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 can be 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 antisera against HERV-K18 Env that blocked superantigen stimulation of human T cells (2). Thus, we tested whether this antisera would also block the stimulation of murine T cells. For this purpose, we preincubated A20/ K18.1 cells with either anti-Env antisera 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 antisera, failed to stimulate this hybridoma. These data indicate that the anti-Env antisera 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 antisera 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−/−/DR4−/−. The DR4 transgenes of these mice consist of the variable regions from the DRB1*0401 and DRA1 alleles and the constant regions from the murine I-Eα and β alleles, encoding a chimeric class II molecule that can be recognized in the context of murine CD4, as has been shown in several experimental systems. Thus, proper development of CD4 T cells occurs in these mice, which is crucial for an in vivo model system.

Anti-IgM cross-linking on primary human B cells induces HERV-K18 Env mRNA and the expression of the corresponding superantigen. Thus, we tested whether IgM cross-linking would also lead to HERV-K18 Env expression in our three BAC transgenic lines. As can be seen in Fig. 3b, this is indeed the case, confirming the proper control of expression of the HERV-K18 Env in these transgenic mice; namely, anti-IgM treatment of transgenic B cells enhanced the expression of HERV-K18 Env transcripts in all three transgenic lines. It is important to point out, though, that HERV-K18 Env expression at a basal level is seen in the resting B cells of all transgenic lines. Similarly, we had observed a basal level of HERV-K18 Env transcripts in human resting peripheral B cells, although these cells showed no superantigen stimulatory activity when tested in in vitro stimulation assays (Fig. 3).

It has been well documented that endogenous MMTV superantigen expression in mice results in reduction or deletion of the T cell subset expressing the TCR Vβ that matches the specificity of the particular superantigen. We, therefore, reasoned that the HERV-K18 superantigen might behave similarly in our transgenic mice, leading to partial or complete deletion of Vβ3+ T cells, in agreement with our in vitro results. Because our panel of T cell hybridomas did not include all murine Vβ families, deletion of T cell subsets other than Vβ3 could also be expected. To test this prediction, we looked for evidence of clonal deletion of T cell subsets by flow cytometry. For this purpose, the frequency of T cells expressing 13 different Vβs was determined in the peripheral
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). 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
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 pro-
viruses of C57BL/6 mice are functional in the context of DR4,
whereas these activities in the context of murine I-Ab 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/
H11002 background, where these particular Vβ subsets are not deleted.

Discussion
In this report, we have presented in vitro and in vivo data support-
ning the notion that HERV-K18 Env behaves as a superantigen in
the murine system. Furthermore, we were able to define the murine

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FIGURE 3. Generation of HERV-K18 transgenic mice. a, Schematic diagram of the SLAM family locus on chromosome 1q23-24. The name and transcriptional orientation of each gene are indicated. The exons of the genes are shown in black. The HERV-K18 provirus (light gray) is located within the first intron of cd48. The HERV-K18 env gene is shaded in dark gray. The transcriptional direction of HERV-K18 is in reverse with respect to cd48. The regions included in the BAC clones RP11-404F10 and RP11-575N16 (accession nos. AL121985 and AC027082, respectively), used to generate the transgenic mice, are shown. The Roman letter represents the number of exons of each gene that are included in the BACs. b, Representative results from screening of the transgene by PCR. PCR products from the transgene and the endogenous control are expected to be 1.15 kb and 0.35 kb, respectively. c, All three lines of the transgenic mice (two from RP11-404F10, lines 14b and 4; one from RP11-575N16, line 5) express basal level of HERV-K18 env in purified splenic B cells, as measured by quantitative real-time PCR. The HERV-K18 env is up-regulated at least 2-fold upon IgM cross-linking in all three transgenic lines.

FIGURE 4. CD4 T cells of multiple TCR Vβ families are partially deleted in HERV-K18 env transgenic mice. The proportion of the indicated TCR Vβ families within CD4⁺ T cells in the peripheral blood was measured by flow cytometry. Five to seven mice were analyzed from each of the HERV-K18/DR4/E-E-E double transgenic lines (lines 5, 14b, and 48) and from the control line (DR4/DR4 single transgenic mice). The mean proportion ± SE of each Vβ subset is shown. Statistical analyses were performed by comparing data from individual transgenic and control lines, as well as by comparing data from all transgenic lines combined to the control. *p < 0.05, statistically significant lower proportion, when compared with the control.
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 reduction of peripheral Vβ3+ T cells was detected in these HERV-K18 transgenic mice. Detailed analyses provide evidence for additional Vβ families as targets for the HERV-K18 superantigen, namely Vβ7, and possibly Vβ13 and Vβ9. It remains to be tested whether the non-deleted T cells of these families are anergic. Although the Vβ6+ T cell hybridoma responded weakly to LCL-A in vitro, a clonal reduction or Vβ6+ T cells was not observed in vivo. This discrepancy is likely due to the differences of the experimental systems and various factors that affect the presentation of superantigens, which will be discussed below. Due to limitations of the experimental system and the availability of reagents, it is currently impossible to test all the murine Vβ families for reactivity to the HERV-K18 superantigen. For instance, we were unable to assess the reactivity of Vβ5, Vβ11, and Vβ12 to this 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-Ak (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 provirus, 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-Eα 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

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background stimulation of untransfected Raji cells would overrule the effect of an additional ectopically expressed supernatant. Taken together, the weak responder and potential high background of the stimulator may have diminished any potential supernatant 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 supernatant; hence, we would not expect to observe any specific stimulation by the supernatant with this panel of murine T cell hybridomas. This, in part, explains why these authors cannot detect the supernatant activity of the HERV-K18 Env.

With the establishment of HERV-K18 Env supernatant 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 supernatant.

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Disclosures

The authors have no financial conflict of interest.

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