Brief Definitive Report

The Presence of an Endogenous Murine Leukemia Virus Sequence Correlates with the Peripheral Expansion of γδ T Cells Bearing the BALB Invariant Delta (BID) T Cell Receptor δ

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Summary

γδ T cells participate in immune responses during viral, bacterial, and parasitic infections. However, it is not clear whether they recognize antigens produced by pathogens, or are actually reactive to self-ligands generated during the course of infection. In this paper, we report that the presence of the self-ligand that selectively expands a subset of γδ T cells correlates with the presence of an endogenous murine leukemia virus (MuLV) in inbred strains of mice. The implications of this observation for γδ T cell specificity and function is discussed.

γδ T cells constitute a small subset of T cells in the periphery lymphoid organs, yet they predominate in the epithelial linings of the skin, intestine, lung, and reproductive organs (1, 2). While it has been established that αβ T cells are selected for the recognition of peptide antigens in the context of self-MHC through positive and negative selection events in the thymus (3–5), the genetic factors that influence the selection and development of the γδ T cell repertoire are still not well understood. So far, there is no consensus on the molecules that present antigens to γδ T cells. Moreover, with few exceptions (6, 7), immunization with nominal antigens has failed to induce γδ T cell reactivity. In spite of this, there is ample documentation on the involvement of γδ T cells in infections and human diseases (8–13), although the modality by which they participate is unknown. To understand the role of γδ T cells in the immune system, it is important to identify the factors that govern γδ T cell selection and expansion, and to define the nature of the ligands that activate these cells. This issue can be addressed by identifying and characterizing polymorphic genetic elements involved in the selection of distinct γδ T cell repertoires in different inbred strains of mice (14, 15).

BALB invariant delta (BID) is a TCR-δ clonotype, defined by its specific VDJ junctional sequence (14). It is expanded in BALB/c but not C57BL/6 mice. In (C57BL/6 × BALB/c)F_1 mice, BID expression is dominant, indicating that the mechanism leading to the high frequency of BID is due to positive selection in BALB/c rather than negative selection in C57BL/6. Two types of evidence attest that this selection occurs extrathymically. First, BID is present at the same frequency in both the BALB/c and the C57BL/6 fetal thymus, but it is highly expanded only in the periphery of BALB/c mice (14, 16). Second, resident pulmonary γδ T cells isolated from athymic nude mice show a predominance of BID among the Vδ5 population in BALB/$c^{nu/nu}$ but not in C57BL/$6^{nu/nu}$ mice (14), attesting that the absence of the thymus has no influence on the BID phenotype. The extrathymic expansion of a γδ T cell subset (Vγ9Vδ2) has also been reported in human. However, no correlation with genetic background has been established (17). The differences in the level of BID expression between C57BL/6 (H-2^b) mice and both BALB/c (H-2^d) and BALB.B (H-2^b) mice bred in the same environment affirm that BID expression is regulated by genes that are polymorphic for mice of the BALB and the C57BL/6 background. Moreover, since BALB/c and BALB.B mice exhibit a similar BID phenotype, it appears that the polymorphic element(s) responsible for BID expansion map(s) outside of the classical H-2 region (14).

In this report, we present data indicating that the polymorphic genetic element that controls BID expression maps to an endogenous retroviral integration site, and could be the viral sequence itself. This observation has implications for γδ T cell function and reactivities.

Materials and Methods

Mice. The CXB series of recombinant inbred strains (18); CXBD, CXBE, CXBG, CXBH, CXBI, CXBJ, and CXBK mice as well as HRS/J and C57L/J mice were purchased from the Jackson Labs, Bar Harbor, ME. C3H/HeJ, A/J, and DBA2/J mice were purchased from IFFA, L'Arbresle, France.

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	Vδ 5	N1	Dδ 1	N2	Dδ 2	N3	Jδ 1	
GERMLINE	TGT GCC TCG GGG TAT.		GTGGCATATCA		ATCGGAGGGATACGAG		CT ACC GAC AAA	
CXB D:								
BID	TGT GCC TCG GGG TAT				ATCGGAGGGATACGAG		CT ACC GAC AAA	**x6**
others:								
D5	TGT GCC TCG GG	TTT			ATCGGAGGGA	C	CT ACC GAC AAA	
CXB E:								
BID	TGT GCC TCG GGG TAT				ATCGGAGGGATACGAG		CT ACC GAC AAA	**x1**
others:								
E1	TGT GCC TCG GGG	TC	TGGCAT	AAAT	ATCGGAGGGA	GAG	CT ACC GAC AAA	
E2	TGT GCC TCG GGG TAT	ATT			ATCGGAGGGATA	A	CT ACC GAC AAA	
E3	TGT GCC TCG GGG TAT	CG	GGCATAT	GGG	GGAGGGATACGAG	GCG	CT ACC GAC AAA	
E6	TGT GCC TCG GGG TAT				CGGAGGGATACG	GG	C GAC AAA	
E7	TGT GCC TCG GGG T	CCTCT			ATCGGAGGGATACGAG		CT ACC GAC AAA	
E9	TGT GCC TCG GGG		ATATC		GGAGGGATACG	GAGCCT	CT ACC GAC AAA	
CXB G:								
BID	TGT GCC TCG GGG TAT				ATCGGAGGGATACGAG		CT ACC GAC AAA	**x10**
others:								
G8	TGT GCC TCG GGG TAT	GC			CGGAGGGATACGAG		CT ACC GAC AAA	
G13	TGT GCC TCG G	CCCTT			ATCGGAGGGATACGAG		CT ACC GAC AAA	
CXB H:								
BID	TGT GCC TCG GGG TAT				ATCGGAGGGATACGAG		CT ACC GAC AAA	**x11**
others:								
H1	TGT GCC TCG GGG T	C			CGGAGGGATACGAG		CC GAC AAA	
H4	TGT GCC TCG GGG		ATC		ATCGGAGGGATACGAG		CT ACC GAC AAA	
H11	TGT GCC TCG GGG TAT	ATT	GGCATAT	TTGG	CGGAGGGATACGAG		CT ACC GAC AAA	
CXB I:								
BID	TGT GCC TCG GGG TAT				ATCGGAGGGATACGAG		CT ACC GAC AAA	**x2**
others:								
I1	TGT GCC TCG GGG TAT	GT	GGCA	CT	ATCGGAGGGATACGAG		CT ACC GAC AAA	
I2	TGT GCC TCG GGG TAT	TTTCC	TGGCATAT		ATCGGAGGGATACGAG	AG	CC GAC AAA	
I3	TGT GCC TCG GGG T	C			GAGGGATA	AGG	CT ACC GAC AAA	
I4	TGT GCC TCG GGG TAT	A	TGGCAT	CCCTC	ATCGGAGGGAT		T ACC GAC AAA	
I5	TGT GCC TCG GGG	CCG	TGGCAT		ATCGGAGGGATAC		CT ACC GAC AAA	
I10	TGT GCC TCG GG	T	CAT		ATCGGAGGGATACGAG		CT ACC GAC AAA	
I15	TGT GCC TCG GGG TAT		GTGGCA	CT	ATCGGAGGGATACGAG	CTACGG	ACC GAC AAA	
I16	TGT GCC TCG GGG TAT	ATC	TGGCATA	CGGT	ATCGGAGGGATA	TAGG	ACC GAC AAA	
I17	TGT GCC TCG GGG	CC	GTGGCAT		ATCGGAGGGATAC		CT ACC GAC AAA	
CXB J:								
BID	TGT GCC TCG GGG TAT				ATCGGAGGGATACGAG		CT ACC GAC AAA	**x2**
others:								
J2	TGT GCC TCG GGG TAT	AT			ATCGGAGGGATACGAG	TCCGG	T ACC GAC AAA	
J5	TGT GCC TCG GGG TA	C			CGGAGGG	CAG	CT ACC GAC AAA	
J6	TGT GCC TCG GGG TAT		ATA	GG	GGGATAC		ACC GAC AAA	
J8	TGT GCC T	TT	GGCAT	GCTC	ATCGGAGGGA	CAA	CT ACC GAC AAA	
J9	TGT GCC TCG GGG				GAGGGATACG		CT ACC GAC AAA	
J10	TGT GCC TCG GG	TCCAC	GTGGC		ATCGGAGGGATACGAG		CT ACC GAC AAA	
J14	TGT GCC TCG GGG T	G	GT	CG	ATCGGAGGGATACGAG		CT ACC GAC AAA	
J17	TGT GCC TCG GGG T		GGCAT		ATCGGAGGGATACGAG		CT ACC GAC AAA	
J18	TGT GCC TCG GGG TAT	GCCC			GAGGGATACGAG		CT ACC GAC AAA	
J19	TGT GCC TCG GGG TAT	ACTG			GGAGGG	GGG	CT ACC GAC AAA	
J20	TGT GCC TCG GGG TAT		TG		GGAGGGATACG	AGC	AC AAA	
J21	TGT GCC TCG GG	CCACC	GTGGCAT		ATCGGAGGGATACG	GGG	CT ACC GAC AAA	
CXB K:								
BID	TGT GCC TCG GGG TAT				ATCGGAGGGATACGAG		CT ACC GAC AAA	**x7**
others:								
K2	TGT GCC TCG GGG	G	AT	T	TCGGAGGGATACGAG		CT ACC GAC AAA	
K6	TGT GCC TCG GGG T		GGC		TCGGAGGGATACGAG	CTT	CC GAC AAA	
K13	TGT GCC TCG GGG TAT		GG		GGGATACGAG	CTTAG	C GAC AAA	

Figure 1. Differential expression of BID in CXB RI strains. The VDJ junctional sequences of Vδ5 cDNA clones isolated from pulmonary γδ T cells are shown. For each strain of mice, all clones that carry the BID rearrangement are grouped and the numbers of independently isolated clones are given in bold type at the end of the sequence.

RNA Preparation from γδ T Cells. Lungs were extensively perfused to remove circulating blood, dissected, and resident pulmonary lymphocytes purified from the dissected tissue according to our published protocol (19). Polyclonally activated cells were cultured for 72 h in a mixture of lymphokines essentially as described, except that PMA and ionomycin were included only in the first 24 h. The viable cells were separated from the dead cells by ficoll-hypaque centrifugation, and $\alpha\beta^+$ T cells were magnetically removed by treatment with biotin-conjugated anti-$\alpha\beta$-TCR monoclonal antibody H57-597 (20) followed by streptavidin coupled Dynal beads. Total cellular RNA was extracted by the acid phenol guanidinium chloroform (APGC) procedure (21). Trace amounts of contaminating DNA was further removed by treatment with RNAse free DNAse (Boehringer Mannheim Corp., Indianapolis, IN).

cDNA Cloning. This was performed essentially as previously described (14). Briefly, cDNA synthesis was performed in a 20-μl reaction volume, starting with RNA extracted from 10^6 cells, using 10 pmol of a Cδ-specific primer (5′-CGAATTCCACAA-TCTTCTTG-3′). After 1 h at 37°C, the reaction mixture was heated at 95°C for 2 min, and 2 μl was added directly to the PCR reaction for amplification. Both 5′ and 3′ primers were present at 0.5 mM. The Cδ primer for PCR is 5′-AACAGATGGTTTGGC-CGGAG-3′ and is internal to the Cδ primer used for cDNA synthesis. The Vδ5 primer is 5′-TCCACTGACCAGACAGTGGC-3′. Each PCR cycle consists of incubations at 94°C for 30 s, 55°C for 30 s, and 72°C for 3 min. Before the first cycle, the reaction mixture was denatured at 94°C for 1 min. After the last cycle, the incubation at 72°C was extended for another 6 min. 25 PCR cycles were performed for generating DNA for cloning. PCR products were gel purified, and the appropriate size fragments cloned into the SmaI site of pUC18.

DNA Sequencing. DNA sequencing was performed on double stranded plasmid DNA, by the dideoxy method using Sequenase (United States Biochemical Corp., Cleveland, OH) as described (14).

BID Typing. BID is a functional TCR rearrangement resulting from the joining of Vδ5 to Dδ2 and Jδ1 gene segments (14). The VDJ junction of BID is defined by the sequence TGTGCCTC-GGGGTATATCGGAGGGATACGAGCTACCGACAAA where the first 15 nucleotides are of Vδ5 origin, the next 16 of Dδ2 origin, and the last 11 are of Jδ1 origin. The VDJ joint is characterized by: (*a*) lack of deletion of any of the germline gene segment nucleotides, (*b*) no addition of any extra nucleotide at the VD or DJ junction, i.e., no N region added nucleotides.

Results and Discussion

To identify the locus regulating the differential selection of BID, we first analyzed BID expression in the CXB series of recombinant inbred (RI) strains of mice generated by D. W. Bailey (18), where the progenitor strains are BALB/c and C57BL/6. (C = BALB/cBy and B = C57BL/6By). γδ T cells from the lungs of each of the RI strains were isolated, and the Vδ5 population was typed as BID^+ or BID^- by cDNA cloning and sequencing analysis (14). A BID^+ population is one in which the VDJ junctional sequence characteristic of BID predominates in the population. The results are presented in Fig. 1. A summary of the mapping data is presented in Table 1, together with some of the linkage analysis generously performed by Dr. B. Taylor (Jackson Laboratories, Bar Harbor, ME).

Several observations emerge from this analysis. First, the discordance between CXBG and CXBK in BID and *H-2* expression (22) provides independent confirmation of our previous finding that the selection of BID is not governed by polymorphic determinants encoded in the classical *H-2* region. However, it does not rule out the involvement of a nonpolymorphic *H-2* encoded determinant. Second, the immunoglobulin heavy chain (*Igh*) locus does not appear to play any role in the selection of BID, since the expression of the BALB/c *Igh* allele (23) does not correlate with BID expression. This is noteworthy in light of the report of a B cell lymphoma that can stimulate γδ T cells (24). Third, it is not surprising that the TCR-α locus, within which is embedded the TCR-δ locus, has no influence on the BID phenotype (25). Data obtained in our lab have already shown that the BID-specific type of rearrangement is generated in the

Table 1. *Strain Distribution Pattern of BID in CXB Recombinant Inbred Mice*

Inbred CXB RI strains	No. of BID^+ sequences	No. of BID^- sequences	Percent BID^+ sequences	BID phenotype	*H-2* chr 17	*Igh-1* chr 12	TCR-α(δ) chr 14	*Xmmv-60* chr 1	*mpmv-30* chr 1
CXBD	6	1	85.7	C	C	B	C	C	C
CXBE	1	6	14.3	B	B	B	C	B	B
CXBG	10	2	83.3	C	B	C	B	C	C
CXBH	11	3	78.6	C	C	B	B	C	C
CXBI	2	9	18.1	B	B	B	C	B	B
CXBJ	2	12	14.2	B	B	C	C	B	B
CXBK	7	3	70.0	C	B	B	C	C	C
(BALB/c)	20	4	83.3	C	C	C	C	C	C
(C57BL/6)	1	37	2.6	B	B	B	B	B	B

The CXB RI strains are derived from BALB/cBY (*C*) and C57BL/6J (*B*) strains. C and B are used as generic symbols for alleles inherited from the C57BL/6 and BALB/c progenitor strains respectively. Strain distribution patterns of *H-2*, *Igh-1*, TCR-α, *Xmmv-60*, and *Mpmv-30* are referenced in the text.

Table 2. *Concordance of Mpmv-30 and the BID Phenotype in Inbred Mouse Strains*

	Xmmv-60	*Mpmv-30*	BID
BALB/cJ	+	+	+
C57BL/6J	–	–	–
C57L/J	–	–	–
C3H/HeJ	–	–	–
A/J	+	–	–
DBA/2J	–	–	–
HRS/J	+	+	+

The VDJ junctions of Vδ5 cDNA clones isolated from the resident pulmonary γδ T cells of each inbred strain of mice were determined as in Fig. 1. Six out of seven clones derived from HRS/J have the characteristic BID rearrangement (85.7%), while the level of BID expression in the nonexpanding strains range from 0 to 20%.

C57BL/6 fetal thymus, implying that no molecular impediment of DNA rearrangement accounts for the lack of BID expansion in these mice (15). Although there is a recent report linking the positive selection of γδ TCR to the TCR locus (26), data in Table 1 show that the selection of the BID δ-TCR is not linked to the TCR-δ locus. β2-microglobulin, a molecule that is noncovalently associated with all Class I- and Class I-like antigens such as TL, Qa, and CD1, is polymorphic between BALB/c and C57BL/6 and is known to cause different T cell responses (27). Nonetheless, it is also not a determining factor in BID expression.

The strain distribution pattern of BID among the CXB RI strains (CBCCBBC) coincides with that of two genetically linked endogenous murine leukemia virus–related sequences: *Xmmv-60* and *Mpmv-30* (Table 1; references 28, 29). These data suggest that the ligand/regulator of BID may map close to either of these retroviruses, both of which are integrated on chromosome 1. To obtain independent evidence of genetic linkage between BID expression and *Xmmv-60* or *Mpmv-30*, we took advantage of the fact that in a number of inbred strains of mice, the distribution patterns of the xenotropic (*Xmmv*), polytropic (*Pmv*) and modified polytropic (*Mpmv*) murine leukemia viruses are known (28–30). *Xmmv-60*, formerly known as XP-19, is defined by a 3.7-kb PvuII DNA fragment that hybridizes to the pXenv probe (28). This fragment is absent in most of the common mouse strains such as C57BL6/J, C57L/J, DBA/2, and C3H/HeJ, but is present in BALB/c, A/J, and the less common strain HRS/J. In the seven inbred strains mentioned above, *Mpmv-30* is carried only in the genomes of BALB/c and HRS/J (30). Accordingly, we analyzed the level of BID expression among the resident pulmonary γδ T cells in these mice for independent evidence of correlation between BID expression and the presence of these two endogenous retroviruses. The results are summarized in Table 2. Although all four *Xmmv-60*$^-$ strains are low in BID expression, only two of the three *Xmmv-60*$^+$ strains show high levels of BID expression. Since the presence of *Xmmv-60* in A/J does not result in the expansion of BID, it is unlikely that BID selection is governed by the presence of *Xmmv-60*, or an endogenous gene activated by the insertion of this retroviral sequence. On the other hand, among the seven inbred strains tested, there is a perfect concordance between the presence of *Mpmv-30* and BID expansion: all strains that carry *Mpmv-30* are BID$^+$, whereas all *Mpmv-30*$^-$ strains are BID$^-$ (Table 2). Thus, it appears that *Mpmv-30* is involved in the peripheral selection of γδ T cells carrying the BID-TCR chain.

By two independent criteria, recombinant inbred strain mapping and common inbred strain survey, it appears that the peripheral expansion of a γδ TCR is dependent on the presence of an endogenous retroviral sequence. In general, the number of recombinant inbred (RI) strains in a given set of RI mice is small, and a similar strain distribution pattern for two loci usually denotes close linkage rather than functional identity. Thus, data obtained from the CXB series of RI strains on the locus that regulates BID expansion can be taken to indicate probable genetic linkage between this locus and the two linked endogenous retroviruses, *Mpmv-30* and *Xmmv-60*. However, we should note the ease with which we subsequently dissociated functional linkage of the BID regulatory element from *Xmmv-60*, and established its concordance with *Mpmv-30*. Moreover, through similar linkage analysis, endogenous retroviruses of the MMTV family were identified as the genetic elements encoding the Mls antigens that stimulate specific Vβ subsets of T cells (31). It is likely that a functional identity exists between *Mpmv-30* and the BID regulatory locus itself.

It is clear that T cells respond to both exogenous and endogenous viral antigens. The profound relationship of retroviruses and the T cell repertoire is further exemplified in the case of the MAIDS virus (32), whereby infected B cells carrying the defective viral genome preferentially activate T cells bearing Vβ5, 11, and 12. It has long been known that endogenous type C viruses can be activated in vivo in mice by various means such as X irradiation, chemical carcinogens, and graft-versus-hosts reaction (33–35). Moreover, lymphocyte stimulation can also lead to the expression of endogenous retroviruses (36). In light of the present finding, we propose that various endogenous retroviral sequences may become activated in different cell types as a consequence of infection or of cellular injury. This can induce a transient expression of novel self-antigens responsible for γδ T cell activation. Moreover, γδ T cells activated by such self-antigens may not be autoaggressive.

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