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

By Gek-Kee Sim* and Andrei Augustin†

From the *Basel Institute for Immunology, Basel, CH-4005, Switzerland; the †Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado, 80206; and the ‡Department of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver, Colorado 80262

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.

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.
| Strain | VDJ Junctional Sequences |
|--------|--------------------------|
| CXB D  | BID TGT GCC TCG GGG TAT GTCGATATCA ATCGAGGAGGATACGAG CT ACC GAC AAA x6 |
|        | others: D5 TGT GCC TCG GG TATG ATCGGAGGA CC CT ACC GAC AAA |
| CXB E  | BID TGT GCC TCG GGG TAT GTCGATATCA ATCGAGGAGGATACGAG CT ACC GAC AAA x1 |
|        | others: E1 TGT GCC TCG GGG TC TGGCAT AAAT ATCGGAGGAGA CCC GAC AAA |
| CXB G  | BID TGT GCC TCG GGG TAT GTCGATATCA ATCGAGGAGGATACGAG CT ACC GAC AAA x10 |
|        | others: G8 TGT GCC TCG GGG TAT GC GTCGATATCA ATCGAGGAGGAGA CCC GAC AAA |
| CXB H  | BID TGT GCC TCG GGG TAT GTCGATATCA ATCGAGGAGGATACGAG CT ACC GAC AAA x11 |
|        | others: H1 TGT GCC TCG GGG TAT GC GTCGATATCA ATCGAGGAGGAGA CCC GAC AAA |
| CXB I  | BID TGT GCC TCG GGG TAT GTCGATATCA ATCGAGGAGGATACGAG CT ACC GAC AAA x2 |
|        | others: I1 TGT GCC TCG GGG TAT GC GTCGATATCA ATCGAGGAGGAGA CCC GAC AAA |
| CXB J  | BID TGT GCC TCG GGG TAT GTCGATATCA ATCGAGGAGGATACGAG CT ACC GAC AAA x2 |
|        | others: J2 TGT GCC TCG GGG TAT GC GTCGATATCA ATCGAGGAGGAGA CCC GAC AAA |
| CXB K  | BID TGT GCC TCG GGG TAT GTCGATATCA ATCGAGGAGGATACGAG CT ACC GAC AAA x7 |
|        | others: K1 TGT GCC TCG GGG TAT GC GTCGATATCA ATCGAGGAGGAGA CCC GAC AAA |

Figure 1. Differential expression of BID in CXB RI strains. The VDJ junctional sequences of V85 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 αβ⁺ T cells were magnetically removed by treatment with biotin-conjugated anti-αβ-TCR monoclonal antibody H57-597 (20) followed by streptavidin coupled Dynal beads. Total cellular RNA was extracted by the acid phenol guanidium chloroform (APGC) procedure (21). Trace amounts of contaminating DNA was further removed by treatment with RNase free DNase (Boehringer Mannheim Corp., Indianapolis, IN).

dDNA 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 10⁶ cells, using 10 pmol of a Cδ-specific primer (5'-CGAATTCCACAA-TCTTCTTGG-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'-AACAGATGGTTGTTGGC-CCGAG-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-GGGGTATATCGGACW~ATACGAGCTACCGACAAA 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 (Iγh) locus does not appear to play any role in the selection of BID, since the expression of the BALB/c Igδ 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 | Iγh-1 chr 12 | TCR-α(6) chr 14 | Xmmv-60 chr 1 | Mmmv-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/6By (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, Iγh-1, TCR-α, Xmmv-60, and Mmmv-30 are referenced in the text.
The VDJ junctions of V85 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 ranges 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 δ-TCR is not linked to the TCR-β 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 (CBCBCBBC) coincides with that of two genetically linked endogenous murine leukemia virus-related sequences: Xmmv-60 and Mpmv-30 (Table 1; references 28, 29).

| Xmmv-60 | Mpmv-30 | BID |
|---------|---------|-----|
| BALB/c | +       | +   |
| C57BL/6 | -       | -   |
| C57L/J | -       | -   |
| C3H/HeJ | -       | -   |
| A/J | +       | -   |
| DBA/2J | -       | -   |
| HRS/J | +       | +   |

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

The VDJ junctions of V85 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 ranges from 0 to 20%.

The VDJ junctions of V85 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 ranges 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 δ-TCR is not linked to the TCR-β 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.

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

The VDJ junctions of V85 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 ranges from 0 to 20%.

The strain distribution pattern of BID among the CXB RI strains (CBCBCBBC) 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 C57BL/6/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 endogenous 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 Vα5, 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-host 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.
We are grateful to A. Lanzavecchia and W. Hein for their critical reading of this manuscript. We thank L. Angman and M. McCarty for technical assistance.

This work was supported in part by National Institutes of Health grants to A. Augustin. The Basel Institute for Immunology was founded and is supported by F. Hoffmann-La Roche LTD, Basel.

Address correspondence to Gek-Kee Sim, Basel Institute for Immunology, Grenzacherstrasse 487, CH-4005, Basel, Switzerland.

Received for publication 21 June 1993.

References

1. Allison, J.P., and W.L. Havran. 1991. The immunobiology of T cells with invariant gamma delta antigen receptors. Annu. Rev. Immunol. 9:679.

2. Tonegawa, S., A. Berns, M. Bonneville, A. Farr, I. Ishida, K. Ito, S. Itohara, C.A. Janeway, Jr., O. Kanagawa, and M. Katsumi. 1989. Diversity, development, ligands, and probable functions of gamma delta T cells. Cold Spring Harbor Symp. Quant. Biol. 54:31.

3. Sha, W.C., C.A. Nelson, R.D. Newberry, D.M. Kranz, J.H. Spear, F.W. Fitch, and J.A. Bluestone. 1992. A murine CD4-, CD8- T cell receptor-gamma delta T lymphocyte done specific for herpes simplex virus glycoprotein I. J. Immunol. 148:2653.

4. Parker, C.M., V. Groh, H. Band, S.A. porcelli, C. Morita, M. Fabbi, D. Glass, J.L. Strominger, and M.B. Brenner. 1989. Evidence for extrathymic changes in the T cell receptor gamma delta repertoire. J. Exp. Med. 171:1597.

5. Kappler, J.W., N. Roehn, and P. Marrack. 1987. T cell tolerance by clonal elimination in the thymus. Cell. 49:273.

6. Janis, E.M., S.H.E. Kaufmann, R.H. Schwartz, and D.M. Pardoll. 1989. Activation of gamma delta T cells in the primary immune response to mycobacterium tuberculosis. Science (Wash. DC). 244:713.

7. Johnson, R.M., D.W. Lancki, A.I. Sperling, R.F. Dick, P.G. Spear, F.W. Fitch, and J.A. Bluestone. 1992. A murine CD4- , CD8- T cell receptor-gamma delta T lymphocyte clone specific for herpes simplex virus glycoprotein I. J. Immunol. 148:983.

8. Modlin, R.L., C. Firmez, F.M. Hofman, V. Terigian, K. Uyemura, T.H. Rea, B.R. Bloom, and M.B. Brenner. 1989. Lymphocytes bearing antigen specific gamma delta T cell receptors accumulate in human infectious disease lesions. Nature (Lond.) 339:544.

9. Holoshitz, J., F. Koning, J.E. Coligan, J. de Bruyn, and S. Strober. 1989. Isolation of CD4- CD8- mycobacterial reactive T lymphocyte clones from rheumatoid arthritis synovial fluid. Nature (Lond.) 339:226.

10. Hiromatsu, K., Y. Yoshikai, G. Matsuizaki, S. Ohga, K. Muramori, K. Katsumoto, J.A. Bluestone, and K. Nomoto. 1992. A protective role of gamma delta T cells in primary infection with Listeria monocytogenes in mice. J. Exp. Med. 175:49.

11. Rust, C., Y. Kooy, S. Pena, M.L. Mearin, P. Kluiin, and F. Koning. 1992. Phenotypical and functional characterizations of small intestinal TcR gamma delta + T cells in coeliac disease. Scand. J. Immunol. 35:459.

12. Wucherpfennig, K.W., J. Newcomb, H. Li, C. Keddy, M.L. Cuzner, and D.A. Hafler. 1992. gamma delta T cell receptor repertoire in acute multiple sclerosis lesions. Proc. Natl. Acad. Sci. USA. 89:4588.

13. Eichelberger, M., W. Allan, S.R. Carding, K. Bottomly, and P. Doherty. 1991. Activation status of the CD4-8- gamma delta T cells recovered from mice with influenza pneumonia. J. Immunol. 147:2069.

14. Sim, G.K., and A. Augustin. 1990. Dominantly inherited expression of BID, an invariant undiversified T cell receptor delta chain. Cell. 61:397.

15. Sim, G.K., and A. Augustin. 1991. Extrathymic positive selection of gamma delta T cells: Vgamma-jgamma rearrangements with Gammadelta junctions. J. Immunol. 146:2439.

16. Sim, G.K., and A. Augustin. 1991. Dominant expression of the T cell receptor BALB invariant delta (BID) chain is due to selection. Eur. J. Immunol. 21:859.

17. Potter, M., J.S. Finlayson, D.W. Bailey, E.B. Mushinski, B.L. Reamer, and J.L. Waiters. 1973. Major urinary protein and immunogenetics. Science (Wash. DC). 182:9.

18. Bailey, D.W. 1971. Recombinant inbred strains. Transplantation (Baltimore). 11:325.

19. Augustin, A., R.T. Kubo, and G.K. Sim. 1989. Resident pulmonary lymphocytes expressing the gamma delta T cell receptor. Nature (Lond.) 340:239.

20. Kubo, R.T., W. Born, J.W. Kappler, P. Marrack, and M. Pigeon. 1989. Characterization of a monoclonal antibody which detects all murine alpha beta T cell receptors. J. Immunol. 142:2736.

21. Chomczynski, P., and N. Sacchi. 1987. Single step method of RNA isolation by acid guanidinium thiocyanate phenol chloroform extraction. Anal. Biochem. 162:156.

22. Bailey, D.W. 1975. Genetics of histocompatibility in mice. Immunogenetics. 2:249.

23. Poter, M., J.S. Finlayson, D.W. Bailey, E.B. Mushinski, B.L. Reamer, and J.L. Wallers. 1973. Major urinary protein and immunoglobulin allotype of recombinant inbred mouse strains. Genet. Res. 22:325.

24. Sperling, A.L., and H.H. Wortis. 1989. CD4- CD8- gamma delta T cells from normal mice respond to a syngeneic B cell lymphoma and can induce its differentiation. Int. Immunol. 1:434.

25. Dembic, Z., B.A. Taylor, and M. Steinmetz. 1985. The gene encoding the T cell receptor alpha chain maps close to the Np-2 locus on mouse chromosome 14. Nature (Lond.). 314:271.

26. Sperling, A.L., R.Q. Cron, D.C. Decker, D.A. Stern, and J.A. Bluestone. 1992. Peripheral T cell receptor gamma delta variable gene repertoire maps to the T cell receptor loci and is influenced by positive selection. J. Immunol. 149:3200.

27. Perarnau, B., C.A. Siegrist, A. Gillet, C. Vincent, S. Kimura, and F. Lemonnier. 1990. 3-2 microglobulin restriction by antigen presentation. Nature (Lond.). 346:751.

28. Wejman, J.C., B.A. Taylor, N.A. Jenkins, and N.G. Copland. 1984. Endogenous xenotropic murine leukemia virus related
sequences map to chromosomal regions encoding mouse lymphocyte antigens. *J. Virol.* 50:237.

29. Frankel, W.N., J.D. Stoye, B.A. Taylor, and J.M. Coffin. 1990. A linkage map of endogenous murine leukemia proviruses. *Genetics.* 124:221.

30. Stoye, J.D., and J.M. Coffin. 1988. Polymorphism of endogenous proviruses revealed by using virus class specific oligonucleotide probes. *J. Virol.* 62:168.

31. Frankel, W.N., C. Rudy, J.M. Coffin, and B.T. Huber. 1991. Linkage of Mls genes to endogenous mammary tumour viruses of inbred mice. *Nature (Lond.)*. 349:526.

32. Hugin, A.W., M.S. Vacchio, and H.C. Morse. 1991. A virus-encoded “superantigen” in a retrovirus-induced immunodeficiency syndrome of mice. *Science (Wash. DC).* 252:424.

33. Kaplan, H. 1967. On the natural history of the murine leukemias. *Cancer Res.* 27:1325.

34. Igel, H.J., R.J. Huebner, H.C. Turner, P. Kotin, and H.L. Falk. 1969. Mouse leukemia virus activation by chemical carcinogens. *Science (Wash. DC).* 166:1624.

35. Hirsch, M.S., S.M. Philips, C. Solnik, P.H. Black, R.S. Schwartz, and C.B. Carpenter. 1972. Activation of leukemia viruses by graft-versus-host and mixed lymphocyte reactions in vitro. *Proc. Natl. Acad. Sci. USA.* 69:1069.

36. Stoye, J.P., and Moroni, C. 1983. Endogenous retrovirus expression in stimulated murine lymphocytes. 1983. *J. Exp. Med.* 157:1661.