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The *xid* mutation plays an important role in delayed development of murine acquired immunodeficiency syndrome

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Abstract

Infection of C57BL/6 mice with LP-BM5 murine leukemia virus (MuLV) leads to the development of murine acquired immunodeficiency syndrome (MAIDS) characterized by abnormal lymphoproliferation, hypergammaglobulinemia and severe immunodeficiency. Progression of MAIDS is delayed in X chromosome-linked immunodeficient (XID) mice, which have an abnormality of Bruton's tyrosine kinase (Btk) and lack functionally mature B cells including $CD5^+$ B cells. In this study, we report the following four major findings. (i) Susceptibility to disease induction is not reconstituted by transfer of $CD5^+$ B cells to XID mice. (ii) Spleen cells from asymptomatic XID mice are able to transmit MAIDS to wild-type mice. (iii) MAIDS can be transmitted to XID mice with the transfer of B cells, but not T cells, from C57BL/6 mice with MAIDS. (iv) Cells which undergo massive lymphoproliferation in XID mice with MAIDS by cell transfer are of host origin, but are not from the donor. We suggest from these results that a B cell subpopulation that is impaired in XID mice plays an important role in the initiation of MAIDS.

Introduction

Murine acquired immunodeficiency syndrome (MAIDS) is a disease characterized by abnormal lymphoproliferation, polyclonal T and B cell activation, enhanced production of cytokines, hypergammaglobulinemia, including a vast elevation of IgG2a and severe immunodeficiency, with death occurring at 15–20 weeks after infection (1–3). The development of MAIDS is caused by infection of susceptible strains of mice, such as C57BL/6 (B6) mice, with a mixture of LP-BM5 murine leukemia virus (MuLV). The virus mixture contains non-pathogenic replication-competent MuLV and a disease-inducing defective virus (4–6). The progression of disease is a consequence of complex host–virus interactions that are only partially understood. The presence of both $CD4^+$ T cells and B cells is critical for the development of this disease (7,8).

It has been reported that the majority of cells infected with LP-BM5 MuLV belong to B-lineage cells (6,9–12), although peritoneal macrophages (13) and T cells (14,15) can serve as targets for infection. Using mice with a B cell deficiency due to a targeted mutation of the membrane exon of the IgM heavy chain gene, it has been shown that B cells are the primary target for infection and expression of defective virus, and that there is inefficient infection of T cells and macrophages in the absence of B cells (10). In a previous study, we and others demonstrated that X chromosome-linked immunodeficient (XID) mice, which have a single amino acid substitution in the pleckstrin homology domain of Bruton's tyrosine kinase (Btk) (16) and lack functionally mature B cells (17–22), show a delayed progression of MAIDS (12,23). However, the cellular mechanisms of this delayed progression of MAIDS in XID mice are still unclear.

In this study, we examined the susceptibility of XID mice to MAIDS by transferring B cells from wild-type mice that have developed MAIDS. We have found that XID mice become fully susceptible to MAIDS when B cells from wild-type mice with MAIDS are transplanted and that cells from asymptomatic XID mice are also effective in the transmission of the disease to B6 mice. Finally, we discuss the role of a B cell subpopulation lacking in XID mice that triggers the progression of MAIDS.

Methods

Mice

C57BL/6 (B6) female mice were purchased from Japan SLC (Hamamatsu, Japan). C57BL/6.*xid* (XID) mice were

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maintained in the animal facility at the Institute of Medical Science, University of Tokyo, under specific pathogen-free conditions. BALB.B and C57BL/6-Ly5.1-Pep^{3b} (B6-Ly5.1) mice were kindly provided by Dr J. Hayakawa (Kanazawa University, Kanazawa, Japan) and Dr T. Takahashi (Aichi Cancer Center, Nagoya, Japan) respectively.

LP-BM5 MuLV

A SC-1 clone chronically infected with LP-BM5 MuLV, termed the G6 cell line, kindly supplied by Dr H. C. Morse, III (National Institutes of Health, Bethesda, MD), has been maintained by Dr T. Mizuochi (Japanese National Institute of Health). The virus was prepared from the supernatant of G6 cells as previously described (3) and was kindly provided by Dr T. Mizuochi. The virus preparation (~5×10^4 p.f.u. ecotropic virus/ml) was stored at –70°C until use.

Antibodies for flow cytometry

The following mAb were used: RA3-6B2 mAb, which recognizes B220 (ATCC, Rockville, MD); 53-7.3 mAb, which recognizes Ly-1 (ATCC); and 2.4G2 mAb, which recognizes murine FcγR (ATCC). A37-501 mAb, which recognizes Ly5.1, and 104.2 mAb, which recognizes Ly5.2, were kindly provided by Dr K. Mitani (Meiji Institute of Health Science, Odawara, Japan) and Dr H. Yakura (Tokyo Metropolitan Institute for Neuroscience, Tokyo, Japan) respectively.

FACS analysis of CD5$^+$ B cells in B6 mice with MAIDS

For FACS analysis of CD5$^+$ B cells, cells in peritoneal washouts (PEC), peripheral lymph node or spleen were prepared from B6 mice infected with LP-BM5 MuLV at various times post-infection. These cells were stained with biotinylated anti-Ly-1 (CD5) mAb plus phycoerythrin (PE)-labeled streptavidin (Gibco/BRL, Gaithersburg, MD) and FITC-labeled anti-B220 mAb. Non-specific Fc-mediated binding of antibody was blocked by addition of unlabeled anti-FcγR mAb to cells before incubation with the labeled reagents. Analyses were carried out on a FACScan (Becton Dickinson, Mountain View, CA) equipped with an argon laser (480 nm), operating at 200 mW. Only cells within the lymphocyte gate were counted as previously described (12).

Reconstitution of XID mice with CD5$^+$ B cells

PEC (2×10^6) from non-infected B6 mice were transferred i.p. into XID mice. Nine weeks after the transfer, PEC from the recipient mice were stained with FITC-labeled anti-B220 mAb, biotinylated anti-Ly-1 mAb plus PE–streptavidin and analyzed with a FACScan.

Transfer of virus-infected cells

B6 (B6-Ly5.2, Igh^b), XID (B6-Ly5.2, Igh^b) and BALB.B (Igh^a), or B6-Ly5.1 mice were injected i.p. with 1×10^4 p.f.u. LP-BM5 MuLV. Single-cell suspensions were prepared from spleens and lymph nodes from infected mice 3–7 weeks after virus injection. The T cell-depleted, B cell-enriched population was prepared by treating lymph node cells with anti-Thy-1.2 mAb (Serotec, Oxford, UK) and guinea pig complement as previously described (20). The B cell-depleted, T cell-enriched population was obtained by passage over T cell enrichment immunocolumns (Biotex, Alberta, Canada). Contamination of T cells in the B cell-enriched population was <2% and that of B cells in the T cell-enriched population was ~12%. Cells obtained were transferred into XID or B6 mice i.p. and the development of MAIDS in the mice was followed.

Evaluation of MAIDS development

We examined for development of MAIDS on the basis of splenomegaly, hypergammaglobulinemia and suppressed proliferative responsiveness of lymphoid cells to concanavalin A (Con A) as previously described (3). The serum level of IgG2a was determined by isotype-specific ELISA according to the procedures described below. Con A response was assessed by [^{3}H]thymidine incorporation into spleen cells as described below.

ELISA

Serum concentrations of IgG2a of Igh^b and Igh^a allotypes in each group of mice were quantitated by ELISA using flat-bottomed flexible 96-well trays (Becton Dickinson) coated with anti-mouse IgG2a mAb (5.7; PharMingen, San Diego, CA) for Igh^b allotype and anti-mouse IgG2a mAb (Southern Biotechnology Associates, San Francisco, CA) for Igh^a allotype respectively (3). Sera were diluted 3-fold starting at 1:500 and incubated with antibody at room temperature for 2 h. After washing, biotinylated goat anti-mouse IgG mAb (PharMingen) was added to each well and the plates were incubated for another 2 h at room temperature. Then horseradish peroxidase–streptavidin (Zymed) was added to each well and incubated for 30 min at room temperature. Finally, *o*-phenylenediamine and hydrogen peroxide were added to each well. The enzyme reaction was stopped by adding sulfuric acid and the optical density at 495 nm was determined with a V-max Kinetic Micro Plate Reader (Molecular Devices, Menlo Park, CA). Using purified mouse IgG2a, (8.3 mAb, PharMingen), standard curves were generated and concentrations were determined with a computer program, SOFTmax (Molecular Devices).

Proliferative response to Con A

Proliferative responses of spleen cells to Con A were measured as previously described (3). Briefly, spleen cells (1×10^5/200 μl) were cultured in RPMI 1640 medium supplemented with 8% FCS, 5×10^{-5} M 2-mercaptoethanol, streptomycin (100 μg/ml) and penicillin (100 U/ml) in 96-well microplates for 3 days with Con A (2 μg/ml). Cultures were set up in triplicates. Proliferative response was assessed on the basis of [^{3}H]thymidine incorporation during the last 12 h of a 3 day culture.

Distinction of cell source by FACS analysis

PEC, lymph node cells and spleen cells were prepared from XID (B6-Ly5.2) mice that had been transferred with lymph node cells from B6-Ly5.1 mice with MAIDS. These cells were stained with FITC-labeled anti-Ly5.1 mAb and biotinylated anti-Ly5.2 mAb plus PE–streptavidin, and then analyzed by a FACScan.

Detection of Btk mutation

Lymph node cells from B6 mice infected with LP-BM5 MuLV 3–7 weeks before the experiment were transferred i.p. into

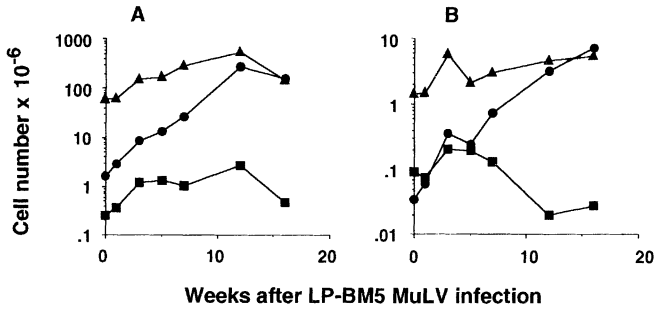

Fig. 1. Total cell number and number of $CD5^+$ B cells in various tissues of B6 mice along with LP-BM5 MuLV infection. Cells from peritoneal washouts (squares), lymph node (circles) and spleen (triangles) were prepared from B6 mice infected with LP-BM5 MuLV at various intervals post-infection. These cells were counted (A) and stained with biotinylated anti-Ly-1 (CD5) mAb plus PE–streptavidin and FITC-labeled anti-B220 mAb. Cell numbers of $CD5^+$ $B220^+$ cells are expressed (B).

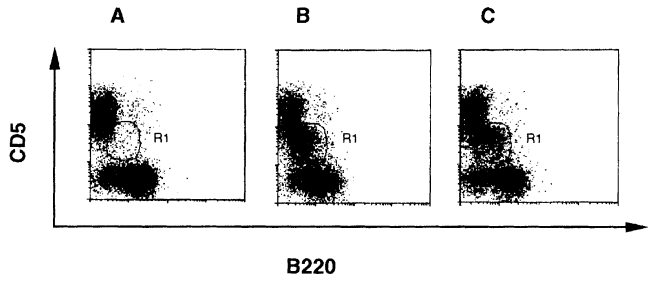

Fig. 2. Reconstitution of $CD5^+$ B cells in XID mice. Normal PEC (2×10^6) from B6 mice were transferred i.p. into XID mice. PEC from (A) XID, (B) B6 and (C) cell-transferred XID mice were stained according to procedures described in Fig. 1.

XID mice. Using the guanidinium thiocyanate method (24), total RNA was extracted from spleen and lymph node 12 weeks after the cell transfer. Total RNA was reverse-transcribed using random hexamers (Takara, Kyoto, Japan) and RNase H^- reverse transcriptase (Gibco/BRL) at 37°C for 60 min. The products obtained were amplified by PCR using a primer pair (forward primer, 5′-GGAAGCCATGGCTGCAGTG-3′; reverse primer, 5′-CCTCTCCTCGGAATCTGTCTTTC-3′) designed to generate a 255 bp fragment from the Btk cDNA including the XID mutation site. The PCR products were digested with *Hae*II (New England Biolabs, Beverly, MA) and separated electrophoretically on 1.5% agarose gels.

Table 1. Delayed progression of MAIDS in XID mice reconstituted with $CD5^+$ B cells

Mice	LP-BM5	IgG2a level (mg/ml)[b]	Lymphadenopathy[c]
XID reconstituted[a]	+	0.2–11	2/10
	−	0.05–0.11	0/4
XID	+	0.04–2.6	0/8
	−	0.006–0.095	0/4
B6	+	88–140	4/4
	−	0.1–1.5	0/4

[a]PEC (2×10^6) from naive B6 mice were transferred i.p. into XID mice 11 weeks before LP-BM5 MuLV inoculation.
[b]Serum IgG2a levels were determined 12 weeks after virus inoculation.
[c]The number of mice that showed lymphadenopathy within 12 weeks after virus inoculation.

Results

Delayed progression of MAIDS in XID mice reconstituted with $CD5^+$ B cells from wild-type mice

We have reported that XID mice show a delayed progression of MAIDS after LP-BM5 MuLV infection. As XID mice lack $CD5^+$ B cells which are the major target cells for defective LP-BM5 MuLV, our results suggested that $CD5^+$ B cells may play an important role in the progression of the disease (12). To evaluate this hypothesis, we asked whether the population of $CD5^+$ B cells is expanded in lymph node and spleen in B6 mice with MAIDS, and then examined whether susceptibility to MAIDS in XID mice can be restored by reconstitution of $CD5^+$ B cells from B6 mice. First, we monitored total cell numbers along with infection in various tissues. As shown in Fig. 1(A), total cell numbers of spleen, lymph nodes and PEC were increased by 5- to 100-fold during the infection period. Then we analyzed the proportion of $CD5^+B220^+$ cells in these tissues by flow cytometry using a FACScan and calculated the number of $CD5^+$ B cells. We found an increase in total numbers of $CD5^+$ B cells in lymph nodes after LP-BM5 MuLV infection (Fig. 1B), although the increase in the percentages of $CD5^+$ B cells in lymph nodes was marginal. In contrast, the number of $CD5^+$ B cells in the peritoneal cavity decreased and the total number of $CD5^+$ B cells in spleen remained in the normal range throughout the experimental period of 16 weeks.

To evaluate the role of $CD5^+$ B cells in the pathogenesis of MAIDS, we transferred PEC (2×10^6) from non-infected B6 mice into a group of 13 XID mice. Nine weeks after the cell transfer, three mice were sacrificed. Peritoneal washouts were harvested and examined for their cell surface markers to evaluate reconstitution of $CD5^+$ B cells. Results revealed that the proportion of peritoneal B cells expressing CD5 and B220 (R1) in the XID mice was significantly increased (Fig. 2C versus A) and was comparable to non-infected age-matched B6 mice (Fig. 2C versus B). We then inoculated LP-BM5 MuLV into 10 XID mice which had received the transfer of PEC. As controls, LP-BM5 MuLV was inoculated into a group of age-matched B6 and XID mice which had not received cells. The development of MAIDS was evaluated 12 weeks after the virus inoculation. As shown in Table 1, all B6 mice showed elevated levels of serum IgG2a and progressive lymphadenopathy after virus infection. Slightly increased serum IgG2a was observed in XID mice which had received the cell transfer. Only two of them showed a slight swelling of inguinal lymph nodes by palpation, while control XID mice did not show any swelling of inguinal lymph nodes by the virus inoculation. These results indicated that delayed progression of MAIDS

Table 2. Transfer of spleen cells from virus-inoculated XID mice induces development of MAIDS in B6 mice

Donor source[a]	Cell no.	Serum IgG2a (mg/ml)[b]	Weight of organ[c]		Response to Con A (c.p.m.)[d]
			Spleen (mg)	Lymph node (mg)	
XID	1×10^6	48.8	740	62	3948
	1×10^5	16.4	500	30	12,578
	1×10^4	5.0	266	8	14,144
B6	1×10^6	73.0	1186	309	5332
	1×10^5	37.9	914	44	4074
	1×10^4	11.6	441	32	10,300
LP-BM5[e]		37.6	648	30	6698
None[f]		0.26	86	4	21,519

[a]Spleen cells from XID or B6 mice were transferred i.p. into B6 mice 3 weeks after inoculation with LP-BM5 MuLV.
[b]Serum IgG2a levels were determined 3 weeks after the treatment.
[c]Weight of spleen and inguinal lymph nodes 9 weeks after the treatment.
[d]Proliferative response of spleen cells to Con A.
[e]Mice did not receive cell transfer, but were infected with LP-BM5 MuLV.
[f]Age-matched non-infected mice.

in XID mice was not significantly altered by the transfer of PEC containing $CD5^+$ B cells from wild-type mice.

Spleen cells from asymptomatic XID mice transmit MAIDS to B6 mice

As we reported in the previous paper (12), the resistance to LP-BM5 MuLV for MAIDS development in XID mice is not absolute. XID mice show apparent lymphadenopathy and the late onset of death. In order to confirm the virus infection of B cells from virus-inoculated XID mice, we performed RT-PCR to detect *gag* p12 mRNA of replication-defective MuLV, which is the causative agent of MAIDS. *gag* p12 mRNA expression in spleens of XID mice was detected at 6 weeks after the infection, although amplification of the gene from XID mice was weaker than that of the gene from B6 mice (data not shown).

Next we examined whether cells in asymptomatic XID mice were able to transmit MAIDS to B6 mice. Various numbers of spleen cells from asymptomatic XID or MAIDS-developed B6 mice were transferred into naive B6 mice. In two groups of B6 mice that received 1×10^6 spleen cells from XID and B6 mice all mice developed MAIDS-like symptoms represented by a vast elevation of serum IgG2a, an increase in the weight of spleen and lymph node and an impaired Con A responsiveness (Table 2). The severity of MAIDS developed depended on the number of cells transferred. Furthermore, cell numbers of XID mice required to transmit MAIDS were greater than that from B6 mice. B6 mice that had received 1×10^5 cells from virus-inoculated XID mice showed marginal MAIDS-like symptoms, while B6 mice that received 1×10^4 of cells from virus-infected B6 mice still showed significant immunodeficiency. B6 mice that received cells from non-infected XID or B6 mice did not develop MAIDS (data not shown). These results indicate that lymphoid cells from asymptomatic XID mice can transmit MAIDS to wild-type mice.

XID mice develop MAIDS after transfer of cells from B6 mice with MAIDS

We then asked whether XID mice fully develop MAIDS when cells from virus-infected B6 mice are transferred. When we transferred either spleen or lymph node cells (2×10^7) from virus-infected B6 mice into XID mice, mice developed MAIDS (Table 3). The XID mice which received lymph node cells from B6 mice with MAIDS exhibited elevated serum IgG2a and nine out of 10 mice showed lymphadenopathy within 12 weeks of cell transfer. Four out of 10 XID mice which had received spleen cells showed lymphadenopathy. We did not observe development of MAIDS in XID mice that received 1×10^6 lymph node cells from B6 mice with MAIDS (data not shown). Severity of the MAIDS developed in XID mice was similar to that observed in B6 mice which had received 1×10^6 cells from infected B6 mice. XID mice inoculated with LP-BM5 MuLV did not develop lymphadenopathy or splenomegaly 12 weeks after infection, although the serum level of IgG2a was slightly elevated.

Twelve weeks after the transfer of 2×10^7 lymph node cells

Table 3. Development of MAIDS in XID mice which had received cells from infected B6 mice

Recipient	Cells transferred	Serum IgG2a (mg/ml)[a]	Lymphadenopathy[b]
XID	lymph node[c]	2.3–41	9/10
	spleen[c]	0.4–13	4/10
	LP-BM5[e]	0.01–2.5	0/10
	none[f]	0.005–0.039	0/8
B6	lymph node[d]	54–73	4/4
	spleen[d]	68–73	4/4
	LP-BM5[e]	88–90	4/4
	none[f]	0.38–0.41	0/4

[a]Serum IgG2a levels were determined 9 weeks after the treatment.
[b]The numbers of mice that showed lymphadenopathy within 12 weeks after the treatment.
[c]Spleen or lymph node cells (2×10^7) from B6 mice infected with LP-BM5 7 weeks prior to the experiment were transferred i.p.
[d]One million spleen or lymph node cells from B6 mice infected with LP-BM5 were transferred into B6 mice.
[e]Mice did not receive cell transfer, but were infected with LP-BM5 MuLV.
[f]Age-matched, non-infected mice.

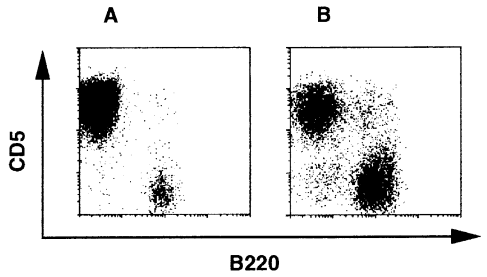

Fig. 3. Expansion of the B cell population in XID mice transferred with cells from MAIDS-developed B6 mice. (A) Lymph nodes from XID mice were stained with FITC–anti-B220 and biotin–anti-Ly-1 plus PE–streptavidin, and analyzed by flow cytometry. (B) Lymph node cells (2×10^7) from B6 mice with MAIDS were transferred i.p. into XID mice. Twelve weeks after the cell transfer, cells from enlarged lymph nodes of recipient mice were stained with FITC–anti-B220 and biotin–anti-Ly-1 plus PE–streptavidin, and then analyzed by flow cytometry.

Table 4. Transfer of B cells from LP-BM5 MuLV-infected B6 mice induces the development of MAIDS in XID mice[a]

Recipient	Cells transferred		IgG2a (mg/ml)[b]	Lymphadenopathy[c]
XID	total cells	2×10^7	21	4/4
		1×10^7	8.9	2/3
	B cells	1×10^7	33	4/4
	T cells	1×10^7	3.3	0/4
	LP-BM5[d]		3.0	0/4
	none[e]		0.02	0/4
B6	total cells	1×10^6	109	4/4
	B cells	1×10^6	93	4/4
	T cells	1×10^6	69	4/4
	LP-BM5[d]		73	4/4
	none[e]		0.42	0/4

[a]Lymph node cells from B6 mice 7 weeks after LP-BM5 MuLV infection were transferred i.p. into XID or B6 mice.
[b]Serum IgG2a levels were determined at 9 weeks after the treatment. Data are expressed as mean of treated mice.
[c]The number of mice that showed lymphadenopathy within 9 weeks after the treatment.
[d]Mice did not receive cell transfer, but were infected with LP-BM5 MuLV.
[e]Age-matched non-infected mice.

to XID mice, we prepared a cell suspension from enlarged lymph nodes of the mice with MAIDS, stained them with FITC-labeled anti-B220 and biotinylated anti-Ly-1 mAb plus PE–streptavidin, and analyzed them by a FACScan. The results revealed that the proportion of $CD5^-$ B cells was expanded, while the increase in the proportion of $CD5^+B220^+$ cells was marginal (Fig. 3).

B cells are responsible for the development of MAIDS in XID mice

To examine which cell population(s) are responsible for the development of MAIDS in XID mice, we prepared T cell-enriched and B-cell-enriched populations from lymph node cells of B6 mice with MAIDS and transferred them into XID or B6 mice. As shown in Table 4, XID mice which received 2×10^7 cells of the unfractionated and B-cell-enriched fractions

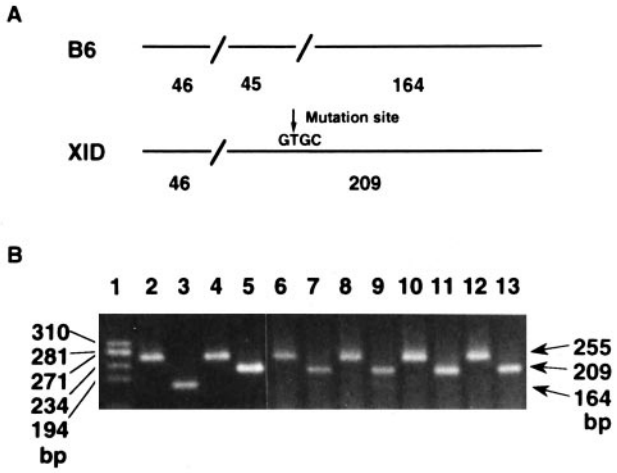

Fig. 4. PCR analysis of the *btk* gene. Lymph node cells from B6 mice infected with LP-BM5 MuLV were transferred i.p. after 3–7 weeks into XID mice. Twelve weeks after the cell transfer, total RNA was extracted from the spleen and lymph node of XID mice, and the 255 bp fragment of Btk cDNA including a XID mutation site was amplified by RT-PCR. The Btk mutation in XID was detected by *Hae*II restriction analysis. (A) Schematic representation of *Hae*II digestion for PCR products. (B) Detection of Btk mutation by *Hae*II digestion. DNA marker, lane 1; PCR products from spleen cells of B6 mice, lanes 2 and 3; from spleen cells of XID mice, lanes 4 and 5; from spleen cells of MAIDS-developed XID mice, lanes 6, 7, 10 and 11; from lymph node cells of MAIDS-developed XID mice, lanes 8, 9, 12 and 13. *Hae*II-digested PCR products were analyzed in lanes 3, 5, 7, 9, 11 and 13. Lanes 6–9 and 10–13 were derived from two individual mice.

of spleen cells from B6 mice developed lymphadenopathy and elevated levels of serum IgG2a. This was not observed by transfer of 1×10^6 cells of the B cell-enriched fraction (data not shown). Furthermore, 1×10^7 cells of the T cell-enriched fraction were hardly able to transmit the disease. In contrast, transfer of 1×10^6 cells of both the T and B cell-enriched populations from B6 mice with MAIDS to B6 mice resulted in lymphadenopathy and hypergammaglobulinemia. These data indicate that B cells, but not T cells, from B6 mice with MAIDS are able to induce MAIDS development in XID mice.

The massive lymphoproliferation and hypergammaglobulinemia expanded in XID mice are derived from XID mice

As described above, XID mice can develop lymphoproliferation when lymph node cells from virus-infected B6 mice are transferred. However, the question remained as to whether proliferated cells in the lymph node are of host (XID mice) origin or donor (B6 mice) origin. To address this question, total RNA was prepared from enlarged spleen and lymph nodes of XID mice with MAIDS, and a portion of the *btk* gene which included the mutation found in XID mice was amplified by RT-PCR. We detected the 255 bp PCR products of the *btk* gene from both B6 and XID mice (Fig. 4A and B, lanes 2, 4, 6, 8, 10, and 12). After *Hae*II digestion, the PCR product from the wild-type *btk* gene was cleaved into 45, 46, and 164 bp fragments (Fig. 4A and B, lane 3), while that of RNA from the XID-type *btk* gene was cleaved into 46 and 209 bp fragments (Fig. 4B, lane 5). As shown in Fig. 4, we observed a 209 bp fragment of the *btk* gene from MAIDS-developed

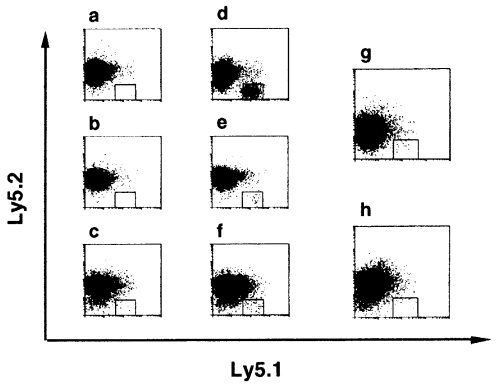

Fig. 5. Origin of cells from enlarged spleen and lymph nodes in XID mice which had received cells from virus-infected B6 mice. Lymph node cells from B6-Ly5.1 mice infected with LP-BM5 MuLV were transferred into XID mice 5 weeks after the infection. Ten weeks after the cell transfer, cells from enlarged spleens (g) and lymph nodes (h) were stained with FITC–anti-Ly5.1 and biotin–anti-Ly5.2 plus PE–streptavidin, and analyzed by flow cytometry. PEC (d), spleen cells (e) and lymph node cells (f) from XID mice 1 week after the cell transfer of infected B6-Ly5.1 cells. Those from normal XID mice (PEC, a; spleen, b; lymph node, c) were analyzed as a control by the same procedure.

XID mice (Fig. 4B, lanes 7, 9, 11 and 13). These results indicate that cells expanded from XID mice with MAIDS are derived from the XID mice (host origin).

Next, we used two kinds of MAIDS susceptible strains of mice, BALB.B (Igh^a allotype) and B6-Ly5.1 mice, as the donor of cells to confirm that cells from XID mice (Igh^b allotype/Ly5.2) can produce large amounts of IgG2a and proliferate following the transfer of cells from virus-infected B6 mice. Both BALB.B and B6-Ly5.1 mice developed MAIDS after LP-BM5 MuLV infection to a similar extent to B6 mice. We did not observe any mixed lymphocyte reaction responses of lymphocytes among B6, XID, BALB.B and B6-Ly5.1 mice (data not shown). After transfer of cells from BALB.B mice with MAIDS, XID mice developed lymphadenopathy, splenomegary and hypergammaglobulinemia, although the extent of MAIDS development was less than that of B6 mice infected with LP-BM5 MuLV (data not shown). XID mice which had received cells from BALB.B mice with MAIDS showed an elevation of IgG2a whose allotype was Igh^b.

Then we analyzed the expansion of donor-derived cells using a FACScan. We detected Ly 5.1^+ cells in the peritoneal washouts, spleen and lymph node (Fig. 5d–f) of XID mice 1 week after the cell transfer, while the percentages of $Ly5.1^+$ cells in XID mice before the transfer was $<0.3\%$. Ten weeks after the cell transfer, XID mice developed MAIDS as the same extent as B6 mice with LP-BM5 MuLV infection. We also found that $Ly5.1^+$ cells in enlarged spleen (Fig. 5g) and lymph node (Fig. 5h) of XID mice were $<0.3\%$. These results indicate that cells involved in hypergammaglobulinemia and lymphoproliferation in MAIDS-developed XID mice after the cell transfer are derived from XID mice.

Discussion

Although the presence of B cells is critical for the development of MAIDS, there are few reports about a causal relationship between B cells and the progression of the disease. We took advantage of the availability of XID mice which show a delayed progression of MAIDS and have a global defect in conventional ($CD5^-$) B and $CD5^+$ B cells. In this study, we report four major findings. (i) Susceptibility to disease induction is not restored by reconstitution of $CD5^+$ B cells from wild-type B6 mice. (ii) Spleen cells from asymptomatic XID mice can transmit MAIDS to wild-type mice by cell transfer. (iii) XID mice fully acquire the capability to develop MAIDS by the transfer of B cells, but not T cells, from B6 mice with MAIDS. (iv) The massive lymphoproliferation and hypergammaglobulinemia in XID mice are derived from host cells.

We have reported previously that XID mice are resistant to MAIDS, but the resistance in XID mice is not absolute (12). There are at least two possibilities to account for the delayed progression of MAIDS in XID mice. First, the delayed development of MAIDS in XID mice is due to failure of virus to expand as quickly as in wild-type mice. In fact, the amount of pathogenic virus in infected XID mice is vastly lower than that found in wild-type mice. Second, a subpopulation of B cells whose development and activation is impaired in XID mice may play an important role in MAIDS development. These possibilities are not mutually exclusive.

As we have shown, $CD5^+$ B cells are the major virus-infected B cell population (12). We inferred from these results that $CD5^+$ B cells might play an important role in the induction and progression of MAIDS and that the lack of $CD5^+$ B cells in XID mice might account for the delayed progression of MAIDS. We examined MAIDS development in XID mice that had been i.p. reconstituted with $CD5^+$ B cells from B6 mice. However, the delayed progression of MAIDS was not affected in these XID mice (Table 1). These results provide strong evidence that a lack of $CD5^+$ B cells is not the major reason for the delayed progression of MAIDS and that conventional B cells may play a more important role than $CD5^+$ B cells in the progression of MAIDS. This was ascertained in the present study by demonstrating that conventional B cells were expanded in XID mice that developed MAIDS (Fig. 2). Recently, Tang *et al*. reported similar findings to us (23) and suggested that resistance to MAIDS in *xid* mutants may be due to the effects of altered conventional B cell function. It still remains a possibility that interaction between $CD5^+$ and conventional B cells may be required for MAIDS development, and this interaction might be insufficient in XID mice. At this moment, we do not have evidence to support this possibility.

One of the most important observations in this study is that spleen cells from asymptomatic XID mice are as effective in transmitting MAIDS to B6 mice as those from B6 mice with MAIDS. These results indicate that asymptomatic XID mice have a sufficient amount of defective virus expression and integration to transfer MAIDS to B6 mice, although the detection of the defective virus is marginal. We detected expression of *gag* p12 mRNA in XID mice 6 weeks after virus inoculation. We also detected the defective virus genome by PCR using purified DNA from spleen cells of XID mice 3 weeks after infection, although the defective virus mRNA and DNA were not detected in XID mice 1 week after virus inoculation (data not shown). These results demonstrate that infected cells exist in the virus-inoculated XID mice. Thus, a small number of cells in XID mice may be infected with defective virus but

they may unable to expand in XID mice. This may be one of the main reasons for the delayed progression of MAIDS. Another possibility is that the amount of pathogenic and infectious virus present in cells from the virus-inoculated XID mice is low but may be sufficient to cause disease in B6 mice. The latter possibility should be re-evaluated in future studies.

The expansion of infected cells and the initial progression of disease may be suppressed in XID mice. XID mice may lack cells that are required for inducing the expansion of infected cells and the progression of disease. Our analysis revealed that B cells from B6 mice with MAIDS were effective in inducing MAIDS to XID mice (Tables 3 and 4). In addition, cells expanded in XID mice are of XID origin, but are not of B6 origin after the cell transfer (Figs 3 and 4). XID mice developed IgG2a b-allotype production after transfer of cells from BALB.B mice with MAIDS (data not shown). These observations demonstrate that the development of MAIDS in XID mice is not caused by massive growth of donor cells. In other words, transfer of B cells from B6 mice with MAIDS can induce the lymphoproliferation of B cells of XID mice and triggers the development of MAIDS in XID mice. We cannot exclude the possibility of expansion of B cells of B6 origin. The transferred virus-infected B6 cells may migrate progressively to lymph nodes and spleen in XID mice, and may trigger the expansion of host (XID) B cells. In such a case, vast expansion of the XID-derived cell population could prevent the detection of transferred B6-derived cells. In any case, we infer from the above results that B cells from B6 mice with MAIDS may play an important role to affect B cells in XID mice for initiation of MAIDS pathogenesis.

We report here that an XID-derived cell population, but not a B6-derived cell population, is expanded in XID mice by transfer of cells from B6 mice with MAIDS. We made several attempts to identify a B cell population of virus-infected B6 mice which is required for the progression of MAIDS in XID mice and have not yet succeeded. We will continue efforts to identify such a B cell population. A greater number of infected B cells from B6 mice is required to induce the progression of MAIDS in XID mice than in B6 (Tables 3 and 4). Currently, we do not have a good explanation for these observation. As XID mice have impaired activation of B cells via IL-5R (20,22), CD38 (25–27) and CD40 (28), B cells in XID mice may require stronger stimulation to trigger the progression of MAIDS than these in B6 mice. Alternatively, the number of infected B cells which triggers the progression of MAIDS may expand less easily in XID mice than in B6 mice, because of impaired Btk function in XID mice. B cells from XID mice might have impairment in the ability to present MAIDS-associated antigen to T cells. This might be caused by impaired activation of B cells of XID mice via CD38, CD40, IL-5R or other molecules expressed on activated B cells. In our preliminary experiments, LP-BM5 MuLV infection of B6 mice causes enhancement on B cells of various surface markers such as B7-1, LFA-1, ICAM-1, class I and class II MHC antigens, and Fas (S. Uehara *et al.*, unpublished observation).

In conclusion, we have provided evidence that (i) transfer of lymphoid cells from virus-infected XID mice into naive B6 mice can induce the development of MAIDS and (ii) transfer of B cells from B6 mice with MAIDS into XID mice efficiently induces the development of MAIDS. It is important to clarify in future why B cells in XID mice can be expanded in the presence of B cells from virus-infected B6 mice resulting in the development of MAIDS. The experimental system provided in this study will provide a useful tool for the delineation of the role of B cells in the development of MAIDS and for the understanding of the B cell abnormality in XID mice.

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Abbreviations

B6	C57BL/6
Btk	Bruton's tyrosine kinase
MAIDS	murine acquired immunodeficiency syndrome
MuLV	murine leukemia virus
PE	phycoerythrin
PEC	peritoneal washout cells
XID	X chromosome-linked immunodeficient.

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