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META-Controlled env-Initiated Transcripts Encoding Superantigens of Murine \textit{Mtv29} and \textit{Mtv7} and Their Possible Role in B Cell Lymphomagenesis\textsuperscript{1}

Namita Sen,\textsuperscript{2,*} William J. Simmons,\textsuperscript{2†} Rajan M. Thomas,\textsuperscript{*} Gregory Erianne,\textsuperscript{†} Da-Jun Zhang,\textsuperscript{*} Nelson S. Jaeggli,\textsuperscript{*} Ching Huang,\textsuperscript{*} Xiaozhong Xiong,\textsuperscript{†} Vincent K. Tsiaigbe,\textsuperscript{*} Nicholas M. Ponzio,\textsuperscript{†} and G. Jeanette Thorbecke\textsuperscript{3,*}

Spontaneous germinal center (GC)-derived B cell lymphomas of SJL mice (RCS) transcribe a 1.8-kb \textit{Mtv29} mRNA under control of the META-env promoter. The encoded vSAg29 stimulates syngeneic VBI6$^+$ CD4$^+$ T cells, thereby acquiring T cell help necessary for RCS growth. Other strains of B cell lymphoma-prone mice include \textit{Mtv29}* C57L and MA/MyJ, and the \textit{Mtv29}-\textit{Mtv7}-recombinant inbred strain, SW × J-1. The lymphomas of these mice produce similar mouse mtv-vSAg-encoding mRNA, as characterized by Northern blotting, PCR, and RNase protection. A 1.8-kb mRNA in C57L/J and MA/MyJ lymphomas hybridized with an \textit{Mtv7}-specific oligonucleotide, whereas SW × J-1 lymphomas produced 1.8-kb transcripts hybridizing with an \textit{Mtv7}-specific oligonucleotide. Similar META-env-initiated transcripts were absent from LPS-activated B cells from any strain examined but were detected in Peyer’s patch RNA from SJL mice. Like typical SJL-derived RCS, all these lymphomas stimulated syngeneic CD4$^+$ T cells and VBI6$^+$ T hybridoma cells. Immunohistochemical staining of primary tumors showed the presence of peanut agglutinin binding (PNA) charges. This article must therefore be hereby marked advertisement

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\textsuperscript{2} N.S. and W.J.S. contributed equally to this study.

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\textsuperscript{4} Abbreviations used in this paper: RCS, reticulum cell sarcoma; GC, germinal center; \textit{Mtv}, mammary tumor virus; RI, recombinant inbred; MMTV, mouse mtv; META, MMTV-env promoter/enhancer with associated transcription initiation site; LTR, long terminal repeat; ORF, open reading frame.

\textsuperscript{5} Female SJL/J (\textit{Mtv8} and \textit{Mtv29} positive), male SWR (\textit{Mtv7}, \textit{Mtv8}, \textit{Mtv14}, \textit{Mtv17}, and \textit{Mtv31} positive), female DBA/2 (\textit{Mtv1}, \textit{Mtv6}, \textit{Mtv7}, \textit{Mtv8},
Mtv11, Mtv13, Mtv14, and Mtv17 positive), CSTL (Mtv8, Mtv9, Mtv11, Mtv17, and Mtv29 positive), LAI, (Mtv6, Mtv8, Mtv9, Mtv11, Mtv13, Mtv17, Mtv23, and Mtv29 positive) MA/My (Mtv8, Mtv9, Mtv17, Mtv29, and Mtv42 positive), and female NOD mice (Mtv3 and Mtv17 positive; Ref. 14, 15) were purchased from The Jackson Laboratory (Bar Harbor, ME). (S/JL × SWR)F1 mice were bred in the animal facilities of New York University School of Medicine (New York, NY). Lymphoma cell lines were derived from primary tumors that developed in aging mice of different strains and designated as follows: SJL-derived, cRCS-2, cRCS-3, cNJ117, and cNJ1101; CSTL-derived, cNJ123 and cNJ120; MA/My derived, cNJ126 and cNJ124. In addition, primary tumors were studied from SW × J-1, lymphomas 326 and 340. The SW × J-1 strain was derived at The Jackson Laboratory, and the mice were generously donated by Dr. W. Beamer. These mice were studied previously for genetic markers (9, 16, 17). The lymphomas examined here were detected in another study in which the lymphoma incidences of the various SW × J RI mouse strains were examined (W. J. Siminovitch, N. M. Ponzo, V. K. Tsagbe, W. Beamer, G. Inghirami, and J. G. Thorbecke, manuscript in preparation). Unlike other SJL-derived lymphomas, NJ101 arose in a 2-year-old SJL mouse that was receiving chronic treatment with anti-CD4 mAb (18). It differs from other SJL tumors by its phenotype (IgM b), absence of vSAG expression, and an inability to stimulate syngeneic SJL T lymphocytes (2, 3).

TCR V b sequences were: L1, 5'-CGAGTCAAGGGACGAGGCAACTGC-3'; L2, 5'-GTTATTCGAGGGTTGAGTGAC-3'; 3'-GTAAAGTGCAATTCCACGG-3'; and 3'-CGCCCCATGAGTATCTCAG-3'. These mouse were studied previously for genetic markers (9, 16, 17). The strains and designated as follows: SJL-derived, cRCS-2, cRCS-3, cNJ117, and cNJ1101; CSTL-derived, cNJ123 and cNJ120; MA/My derived, cNJ126 and cNJ124. In addition, primary tumors were studied from SW × J-1, lymphomas 326 and 340. The SW × J-1 strain was derived at The Jackson Laboratory, and the mice were generously donated by Dr. W. Beamer. These mice were studied previously for genetic markers (9, 16, 17). The lymphomas examined here were detected in another study in which the lymphoma incidences of the various SW × J RI mouse strains were examined (W. J. Siminovitch, N. M. Ponzo, V. K. Tsagbe, W. Beamer, G. Inghirami, and J. G. Thorbecke, manuscript in preparation). Unlike other SJL-derived lymphomas, NJ101 arose in a 2-year-old SJL mouse that was receiving chronic treatment with anti-CD4 mAb (18). It differs from other SJL tumors by its phenotype (IgM b), absence of vSAG expression, and an inability to stimulate syngeneic SJL T lymphocytes (2, 3).

2–5 mCi [3H]TdR/well (spec. activity 2 Ci/mmol; DuPont/NEN, Boston, MA). Cells from triplicate cultures were harvested onto glass fiber filters (No. 934-AH; Whatman, Maidstone, CA) and covalently cross-linked to the membrane by UV irradiation. The membrane was prehybridized for 3 h at 42°C in prehybridization/hybridization solution (6× SSC, 10 mM EDTA, pH 7.5, 2× Denhardt's solution, 100 μg/ml sheared and denatured salmon sperm DNA, and 1% SDS). This was followed by overnight hybridization at 55°C with [32P]-labeled oligonucleotide probe. The filter was washed twice for 15 min at 45°C in prewarmed 6× SSC/0.1% SDS followed by two washes for 10 min in 2× SSC/0.1% SDS at room temperature. The filter was exposed to x-ray film at −70°C.

**DNA preparation and PCR**

Total RNA was isolated from cells by using the genomic DNA isolation kit of Promega (Madison, WI) according to the protocol provided by the manufacturer. PCR was performed with reagents provided in the PCR kit from Promega with a DNA thermal cycler (Perkin-Elmer, Foster City, CA). Reaction conditions were as follows: buffer containing 20 mM Tris-HCl, pH 8.4, 2.5 mM MgCl2, 50 mM KCl; 30 cycles of 45 s of 94°C, 1 min of 58°C, with a final extension step for 10 min at 72°C. Some PCR products were cloned into the PCR TM 2.1 vector (Invitrogen, San Diego, CA) and sequenced.

**RNA preparation and RT-PCR**

Total RNA was isolated from cells by using RNA Stat-60 (Tel Test, Friendswood, TX). Pretreatment of RNA with DNase 1 was performed to exclude DNA contamination. cDNA was synthesized by using the SuperScript II reverse transcriptase. cDNA synthesis was performed in 20 μl containing 50 mM KCl, 10 mM Tris-HCl, pH 8.4, 2.5 mM MgCl2, 50 mM KCl, 30 cycles of 45 s of denaturation at 94°C, annealing for 1 min at 60°C, and extension for 30 s at 72°C with a final extension step for 10 min at 72°C. Some PCR products were cloned into the PCR TM 2.1 vector (Invitrogen, San Diego, CA) and sequenced.

**RNase protection assay**

PCR was performed with the META-2 (7136–7160) and META-3 (7315–7339) primers with SJL liver DNA and the 204-bp product obtained was cloned into the pGEM-3 vector (Promega). The plasmid was linearized with HindIII, and radiolabeled RNA probe representing positions 7136 through 7339 was synthesized by using [32P]UTP and T3 RNA polymerase. The RNA protection assay was performed according to the manufacturer's protocol with the RNA protection kit (BioRad, Missis- (San Diego, CA). Briefly, 10–20 μg of total RNA was hybridized with 4 × 106 cpm of RNA probe overnight at 56°C. Nonhybridized probe was digested with 10 μg/ml RNase A and 65 U/ml RNase T1 for 30 min at 30°C. After extraction with phenol-chloroform and ethanol precipitation, the protected RNA fragments were separated on a 6% polyacrylamide/8 M urea gel and then subjected to PhosphoImaging (Molecular Dynamics, Sunnyvale, CA).

**Preparation of LPS-activated B cells**

Spleen cell suspensions at 106 cells/ml in 20-ml Falcon flasks were stimulated with 50 μg LPS/ml (Escherichia coli 055:B5; Difco Laboratories, Detroit, MI) for 48 h in IMDM (BioWhittaker, Walkersville, MD) containing 10% FCS (Life Technologies) and 0.05 mM 2-ME. After extraction with phenol-chloroform and ethanol precipitation, the protected RNA fragments were separated on a 6% polyacrylamide/8 M urea gel and then subjected to PhosphoImaging (Molecular Dynamics, Sunnyvale, CA).

**Mixed lymphocyte culture**

MLC were prepared in flat-bottom 96-well plates (Costar, Cambridge, MA) in RPMI 1640 medium (Mediatech, Springfield, NJ), supplemented with 10% FCS (HyClone, Logan, UT), 2 mM glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 0.05 mM 2-ME, as described (20). Responder cells (2 × 105/well) were mixed at various responder/stimulator ratios with γ-irradiated lymphoma cells (5,000–15,000 rad) in 0.2 ml. Cultures were incubated for a total of 96 h at 37°C, the last 16–18 h with 1 μCi [3H]Tdr/well (spec. activity 2 Ci/mmol; DuPont/NEN, Boston, MA). Cells from triplicate cultures were harvested onto glass fiber filters (No. 934-AH; Whatman, Maidstone, U.K.) and counted in a scintillation counter. Data for MLC cultures were expressed as Δcpm, calculated as cpm of MLC – (cpm of responder cells alone + cpm of stimulator cells alone).

**Stimulation of T cell hybrids**

Under similar culture conditions as for MLC, lymphoma cells were cocultured with an extensive panel of T-T hybridoma cell lines (105 cells/well), each expressing a different TCR V β. Plate-bound anti-TCR mAb (H57-597) or anti-CD3 mAb (145-2C11), and/or PHA (5 μg/ml), were used as positive control stimuli. Supernatants from replicate cultures were harvested 24 h after initiation of culture and stored at −20°C. IL-2 concentrations were measured with an ELISA kit, with the recombinant mL-2 standard provided (MiniKit KM-II-2; Endogen, Woburn, MA).
Results

Characterization of MMTV transcripts in lymphoma cells from different strains

The RT-PCR products from various lymphomas are represented in Table I. Some 3’-primers were specific for Mtv29 (R29), for Mtv7 (R7), for Mtv8 (R8), and for Mtv17 (R17), whereas the RLTR represents a common region of the 3’ LTR sequences. Control PCR done without reverse transcription were invariably negative. Control PCR done without prior reverse transcription were invariably negative. 

The sequence of the 3’ LTR of Mtv8 is exactly like that of Mtv11; the 3’ LTR region of Mtv43 is very similar to Mtv7 (19).

This PCR product was cloned and sequenced; it is encoded by Mtv8 or Mtv11 (not Mtv9).

As the RNase protection assay uses probes for an env region that is quite similar in sequence for all these MMTVs, the META initiation could be for any of the transcripts detected by PCR using L3.

Table I. MMTV RT-PCR product sizes from lymphomas in different mouse strains

<table>
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<tr>
<th>PCR Primersa</th>
<th>SJL/J cRCS-X</th>
<th>cNJ117 cNJ101</th>
<th>C57L/J cNJ123</th>
<th>cNJ120</th>
<th>MA/Mj/J cNJ126</th>
<th>cNJ124</th>
<th>SW × J-1 326</th>
<th>340</th>
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METAd Initiation + + + + + + + + +

* L1 hybridizes immediately adjacent to the 5’ LTR P1 promoter, L2 in the env region upstream from the META initiation site, identified for vSAg29 transcripts, and L3 in the env region immediately downstream from this initiation site. RLTR detects a common, whereas other R primers detect Mtv-specific regions of the 3’ LTR sequences.

** The sequence of the 3’ LTR of Mtv8 is exactly like that of Mtv11; the 3’ LTR region of Mtv43 is very similar to Mtv7 (19).

*** This PCR product was cloned and sequenced; it is encoded by Mtv8 or Mtv11 (not Mtv9).

As the RNase protection assay uses probes for an env region that is quite similar in sequence for all these MMTVs, the META initiation could be for any of the transcripts detected by PCR using L3.

FIGURE 1. Schematic representation of MMTV provirus. Two MMTV LTRs, consisting of the U3 region containing the RCS-orf, the R and U5 regions, flank the viral structural genes gag, pol, and env. The position of a MMTV-LTR promoter (10, 11, 38) and the META (39, 40) are identified. Also shown are the previously mapped splice donor (SD1) and splice acceptors for regular env (SAenv) and ORF (SAAorf) mRNAs. As also shown previously in SJL cells (13), there is another shortened env mRNA (2.9 kb) that uses another splicing mechanism and splicing donor site (SD2), but the same SAAorf. Moreover, in RCS cells, the novel promoter (META) within the env initiates the expression of endogenous vSAg29 mRNA (1.8 kb) in which there is the same deletion as in the 2.9-kb shortened env transcripts (13). Based numbering corresponds to the analogous sequence of a milk-transmitted MMTV (41). The positions of the probes used as well as the forward and reverse primers used for RT-PCR are indicated.
of $Mtv7$ in the RNA prepared from SW × J-1 lymphomas 326 and 340 (Fig. 2, A and B). Thus, all the 1.8-kb transcripts in lymphoma cells contain part of the env region, in agreement with initiation in the env rather than in the 5′ LTR. In contrast, the 1.8-kb band of the RNA from LPS-activated DBA/2 B cells, DBA/2, hybridizes with the LTR, but not with the META-env probe (Fig. 2). The $Mtv29$-LTR-specific oligonucleotide probe hybridizes with each of the 3 $Mtv$ transcripts in the SJL lymphomas cNJ117 and cRCS-X and the C57L lymphoma cNJ123.5 (Fig. 2C), but not with RNA from lymphomas 340 and 326 (not shown), whereas the $Mtv7$-LTR-specific oligonucleotide probe hybridizes with RNA from the SW × J-1 lymphomas but not with RNA from cRCS-X (Fig. 2D). Thus, the SW × J-1 lymphomas exhibit the characteristic mRNAs for $Mtv7$ of 4.1-, 2.9-, and 1.8-kb lengths.

Only RNase protection assays can firmly establish the presence of transcripts initiating within the env region. This assay should show protected fragments of ~92 bp, starting at the initiation site at position 7247 (13) and ending at the splice donor site (SD2, 7338; see Fig. 1). The protected bands of ~204 bp most likely detect env transcripts initiating in the 5′ LTR. Consistent with the presence of transcripts initiated at position 7247 and spliced at position 7338 is the detection of a 1.1-kb RT-PCR product when the L3 primer is used in combination with either R29 or R7 (Table I). The L3 primer binds within the env, downstream from the initiation site of the vSAg mRNA identified for $Mtv29$ (13). The unspliced 2.3-kb RT-PCR products, amplified with these primer pairs, contain the complete 3′-env region and are not likely to encode the vSAg mRNA.

vSAg mRNA initiating immediately downstream from the META-env region was detected by RNase protection assay (Fig. 3A). Except for cNJ101 (the atypical IgM+ SJL lymphoma that does not stimulate T cells; Refs. 3 and 18) and one MA/My lymphoma (cNJ124), all lymphomas examined contained at least one $Mtv$ that used this initiation site. Comparison of the results for RNase protection and RT-PCR shows that in SJL lymphomas and in the C57L lymphoma cNJ123, $Mtv29$ was using this site. However, considering the detection of more than one 1.1-kb RT-PCR product with L3 in another C57L lymphoma (cNJ120) and in the MA/My lymphoma (cNJ126), it was not certain whether $Mtv29$ or one of the other $Mtv$s, $Mtv8$, $Mtv9$, and/or $Mtv17$, was responsible for the use of the META-env initiation site.

Of great interest is the use of this META-env initiation site for vSAg transcription in the SW × J-1 lymphomas, 340 (Fig. 3A) and 326, where $Mtv7$ is the only candidate, as judged by the RT-PCR.

![FIGURE 2. Northern blots of lymphoma RNA with different MMTV probes. The patterns obtained with the LTR probe (A) and the env (7136–7339) probe (B) demonstrate the presence of both LTR and env sequences in the 1.8-kb mRNA from the RI strain 1 lymphomas (326 and 340), MA/My lymphoma NJ126, and from the C57L lymphoma (NJ123), similar to the 1.8-kb transcripts of SJL lymphomas (i.e., cNJ117), as also was shown previously (13). The env sequences are not present in the 1.8-kb transcripts detected with the LTR probe in LPS-activated DBA/2 B cells. Probing with the $Mtv7$-specific oligonucleotide shows that these 1.8-kb transcripts in lymphomas 326 and 340 are produced by $Mtv7$ (D). The 1.8-kb mRNAs for MMTV-LTR in SJL (cNJ117 and cRCS-X) and C57L (cNJ123.5) lymphomas, hybridize to the $Mtv29$-specific oligonucleotide probe (C). Specificity of the oligonucleotide probes is shown by the lack of hybridization of RNA from cRCS-X with the $Mtv7$ oligonucleotide (D) and of RNA from lymphomas 326 and 340 with the $Mtv29$ oligonucleotide probe (not shown).](http://www.jimmunol.org/)

![FIGURE 3. RNase protection assay for detection of the META-env initiation site of MMTV transcripts. Aliquots (20 μg) of total RNA isolated from the indicated tissues were hybridized in vitro to a labeled RNA probe made by transcribing an RCS cDNA fragment of the sequence 7136–7339 using [α-32P]UTP and T3 RNA polymerase. RNA-RNA hybrids were digested by RNase A and RNase T1, and double-stranded RNase-resistant fragments were separated on a 6% denaturing polyacrylamide sequencing gel. Arrows indicate the predominant fragments; the lengths of the fragments in nucleotides are indicated. The shorter fragments, seen with RNA from the RCS cell lines cRCS-X and cNJ117 but not NJ101, the C57L cell line, cNJ123.5, the MA/My cell line cNJ126 but not cNJ124, and the RI strain 1 lymphoma 326 (A), are the products associated with the initiation sites within env, the longer ones seen with all the lymphomas and also with RNA from LPS-stimulated B cells (B) are the products protected by the env mRNAs that initiate in the 5′ LTR. The shorter fragment also is detected in RNA from SJL Peyer’s patches (B).](http://www.jimmunol.org/)
products detected. These results clearly indicate that the Mtv7 transcripts in the lymphomas from SW × J-1 mice are analogous to those for Mtv29 in the other lymphoma prone strains studied here.

Characterization of MMTV transcripts in normal B cells

Normal LPS-stimulated B cells never used the env initiation site for Mtv transcripts (Fig. 2B) and also failed to show a 5′ LTR-initiated vSAg mRNA, as judged by the RT-PCR product for either Mtv8 in normal B cells in experiments with Mtv8 (1.1-kb product with L1 and R8) was seen for Mtv in LPS-activated B cells from some strains, including DBA/2 and BALB/c, but not from SJL mice (Table II). Indeed, although normal lymphomas from different strains were used during the typical lymphomas arise in this strain, already showed 5′ LTR-initiated vSAg mRNA, as judged by the RT-PCR product for either Mtv or Mtv7 (Table II). However, the 5′ LTR-initiated vSAg mRNA (1.1-kb product with L1 and R8) was seen for Mtv in LPS-activated B cells from some strains, including DBA/2 and BALB/c, but not from SJL mice (Table II). LPS-stimulated B cells from SJL mice showed an unidentified vSAg mRNA that gave a 1.1-kb product on RT-PCR with L3 and RLTR but not with any other reverse primer used, in agreement with the known absence of these MMTVs in this strain. RNase protection assays did not show META env-initiated vSAg transcripts in the RNA from LPS blasts. However, it is interesting that the RNA isolated from Feyer’s patches taken from SJL mice aged 3–6 mo, i.e., several months before the typical lymphomas arise in this strain, already showed evidence of this initiation site (Fig. 3B). Indeed, although normal lymphoid tissue from other sites in SJL mice, such as spleen and bursal lymph nodes, failed to show these transcripts, Feyer’s patches consistently contained them.

Stimulation of syngeneic T cells and of T hybridoma cells by lymphomas from different strains

It was shown previously that unprimed syngeneic T cells are stimulated to proliferate in vitro by γ-irradiated cells from SJL lymphomas (21, 22). The same appears to be true for lymphomas of C57L, MA/My, and SW × J-1 origin (Table III). In the case of SJL lymphomas, this was shown previously to be primarily attributable to the specific stimulation of Vβ16+ CD4+ T cells and inhibited by anti-I-1A8 (2). Therefore, the ability of C57L and MA/My lymphoma cell lines to stimulate T hybridoma cells bearing various individual Vβ-chains in their TCRs was compared with that of the SJL lymphoma line cNJ117 (Table IV). The in vitro C57L lymphoma line, cNJ123.5, strongly stimulated Vβ16+ T hybridomas, as did cNJ117, whereas weak stimulation of the Vβ8.1- and the Vβ11-bearing T hybridomas by cNJ123.5 also was observed. This finding suggested the presence of vSAg29 in both cNJ117 and cNJ123.5, as well as that of an additional vSAg (Mtv8, Mtv9 and/or Mtv17) in the C57L line that was absent from the SJL line. The MA/My line, cNJ126, also caused detectable stimulation of Vβ16+ T hybridoma cells, and strongly stimulated Vβ11+—Vβ12+ and Vβ17a+ T cells (Table IV).

In view of the expression of mRNA encoding the vSAg7, the ability of the SW × J-1 lymphomas, 326 and 340, to stimulate T hybridomas also was examined. To our surprise we found that among several Vβ16+ T hybridoma cells examined, all responded strongly, whereas no stimulation of Vβ6+ T cells was obtained (Table IV). The stimulation of Vβ16+ T hybridoma cells by both tumors 326 and 340 was abolished by inclusion of anti-I-1A+ in the medium. Stimulation of Vβ6+ T cells by vSAg7 is known to be strongly dependent on the expression of I-E in the presenting cells (23). Thus, in view of the fact that SW × J-1 is H2b, it seemed likely that vSAg7 could not properly be presented by the lymphoma cells (24). Indeed, when the abilities of LPS-stimulated B cells from SWR (H2q, Mtv7+) and BALB.D2 (H2d, Mtv7+) to stimulate these Vβ6+ T hybridoma cells were compared, the SWR cells failed to stimulate (< 0.15 pg IL-2/ml), whereas the BALB.D2 cells did (1.5 pg IL-2/ml).

Histological features of the SW × J-1 lymphomas

In previous studies, the characteristics of C57L lymphomas were described as very similar to those of SJL primary lymphomas (25).

Table II. MMTV RT-PCR products from LPS-activated B cells of different mouse strains

<table>
<thead>
<tr>
<th>PCR Primers</th>
<th>SJL/J</th>
<th>LAF1</th>
<th>BALB/c</th>
<th>DBA/2</th>
<th>SWR × SJL</th>
<th>NOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1-RLLTR</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.1</td>
<td>1.1</td>
<td>1.8</td>
</tr>
<tr>
<td>L1-R29</td>
<td>1.8</td>
<td>1.8</td>
<td>None</td>
<td>None</td>
<td>1.8</td>
<td>None</td>
</tr>
<tr>
<td>L1-R8</td>
<td>1.8</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>L1-R7</td>
<td>None</td>
<td>ND</td>
<td>None</td>
<td>None</td>
<td>1.8</td>
<td>None</td>
</tr>
<tr>
<td>L2-RLLTR</td>
<td>1.5; 2.7</td>
<td>1.5; 2.7</td>
<td>1.5; 2.7</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>L2-R8</td>
<td>1.5</td>
<td>1.5</td>
<td>None</td>
<td>None</td>
<td>1.5</td>
<td>None</td>
</tr>
<tr>
<td>L2-R7</td>
<td>None</td>
<td>ND</td>
<td>None</td>
<td>None</td>
<td>1.5</td>
<td>None</td>
</tr>
<tr>
<td>L3-RLLTR</td>
<td>1.1; 2.3</td>
<td>1.1; 2.3</td>
<td>1.1; 2.3</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>L3-R29</td>
<td>1.1; 2.3</td>
<td>1.1; 2.3</td>
<td>1.1; 2.3</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>L3-R8</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>L3-R7</td>
<td>None</td>
<td>ND</td>
<td>None</td>
<td>None</td>
<td>1.1</td>
<td>None</td>
</tr>
</tbody>
</table>

* To see these products, 40 PCR cycles were required, whereas for all the other products in this table 30 cycles sufficed.

Table III. B lymphoma cells stimulate proliferative responses in syngeneic lymph node cells

<table>
<thead>
<tr>
<th>Source of Responder Cells</th>
<th>γ-Irradiated Lymphoma Cells</th>
<th>∆ cpm&lt;sup&gt;a&lt;/sup&gt; for Responder/Stimulator Ratios of 2:1 or 4:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>SJL/J</td>
<td>SJL-derived cNJ117</td>
<td>74,427</td>
</tr>
<tr>
<td>C57L/J</td>
<td>C57L-derived cNJ123.5</td>
<td>85,006</td>
</tr>
<tr>
<td>MA/My/J</td>
<td>MA/My-derived cNJ126</td>
<td>73,405</td>
</tr>
<tr>
<td>(SJL × SWR)F&lt;sub&gt;3&lt;/sub&gt;</td>
<td>SW × J-1 primary lymphoma 340&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23,122</td>
</tr>
<tr>
<td>(SJL × SWR)F&lt;sub&gt;5&lt;/sub&gt;</td>
<td>SW × J-1 primary lymphoma 326&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51,682</td>
</tr>
</tbody>
</table>

<sup>a</sup> ∆ cpm = cpm of mixed cell cultures − (cpm (responders) + cpm (stimulators) alone).

<sup>b</sup> These cells were stored frozen. They were thawed and cultured overnight before use as stimulator cells. These cells also strongly stimulated Vβ16+ T hybridoma cells.
Like the GC cells from which they are derived, the majority of the primary tumor cells in both SJL and C57L mice also stain with PNA-peroxidase (see also Ref. 1). Therefore, in the present study we examined sections of primary lymphomas from SW × J-1 mice. Again, a very similar morphology and PNA− staining of the tumor cells in both Peyer’s patches and mesenteric lymph nodes was seen, entirely comparable to SJL primary lymphomas (Fig. 4).

Discussion

The present results suggest that expression of META-env-initiated vSAgs encoded by endogenous proviral MMTV is common in the B cell lymphomas of the mouse strains studied here. In the lymphomas originating in Mtv29 1 strains, the same vSAg 29 that permits SJL lymphomas to stimulate Vβ16+ CD4 T cells also is expressed. This observation is supported by the presence of the typical RT-PCR products, use of the env initiation site, and the presence of prominent Mtv29 1.8-kb mRNA. However, what is more striking is the observation that the RNA from lymphomas arising in the Mtv29 1 strain, SW × J-1, exhibits identical RT-PCR products, bands hybridizing with the LTR probe on Northern blots, and META-env initiation of Mtv29 1 transcripts. These transcripts clearly come from Mtv7, because Mtv8, the only other MMTV in that strain, does not show any RT-PCR products in either of the two lymphomas examined in detail. In a previous study in which a quantitation was made of Real-digested RT-PCR products obtained from 5′ LTR initiated (presumably vSAg) transcripts from Mtv6, Mtv7, Mtv8, and Mtv9 in normal B and T cells from BALB-D2.Mls-1a mice, such transcripts for Mtv7 were found to be highly expressed, particularly in B cells (26). These LTR-initiated transcripts were thought to encode vSAg7, which is known to be highly expressed in B cells and to cause efficient deletion of Mtv7-responsive Vβ6+ , Vβ7+ , Vβ8.1+ , and Vβ9+ T cells in I-E+ strains that possess Mtv7 in their genome (27–29). However, the present results suggest that LPS-activated B cells do not produce a typical LTR-initiated vSAg for Mtv7, neither in DBA/2 nor in SWR × SJL F1, B cells, but that they also fail to produce META-env-initiated transcripts. Therefore, these data do not describe the mRNA encoding the potent vSAg7 in normal B cells. It is likely that the 3′-env promoter (P2env), described by Arroyo et al. (30), is used for vSAg7-encoding transcripts in Mtv7 1 LPS blasts. We have not yet examined whether the lymphoma cells studied here also use, in addition to the META, the P2env promoter for vSAg7-encoding transcripts; the prominence in Northern blots of an additional smaller mRNA for Mtv7 in lymphoma 340 suggests this possibility.

The sequence of Mtv29 differs by only a single base in the regions found important for promoter activity in the 3′-env of Mtv7 (30). However, with respect to the possibility that Mtv29 might also use a 3′-env promoter and initiation site in LPS blasts, it should be mentioned that in previous studies we have not found syngeneic T cell stimulation by LPS blasts from SJL mice. Moreover, RNA prepared from LPS blasts from SJL mice does not exhibit a 1.8-kb band on probing with an LTR probe, suggesting that, unlike the SJL lymphomas, they fail to express vSAg29 (or vSAg8).

The early histology associated with lymphoma development strongly indicates that SJL lymphomas usually arise in GCs from gut-associated lymphoid tissue, as originally shown by Siegler and Rich (31) and, more recently, by Secord et al. (1) in both normal and Bcl2-transgenic SJL mice. Similarly, all the primary lymphomas from the other strains studied here have a similar morphology, frequently involve Peyer’s patches, and are PNA+. If the META-env-initiated transcripts of Mtv29 play a causal role in the induction of these lymphomas by eliciting a response from Vβ16+ CD4 T cells that then causes a chronic stimulation of B cells, one would expect to see such Mtv29 transcripts in Peyer’s patches before the lymphomas arise. As shown in the present study (Fig. 3B), this was indeed found. At this point, it cannot formally be excluded that some lymphoma cells are already present in the Peyer’s patches from SJL mice months before significant lymphadenopathy occurs and that this early expression of mRNA for vSAg29 is attributable to them rather than to normal GC cells. In preliminary studies, it was found that Peyer’s patch cells from 6-mo-old but not from 3-mo-old SJL mice or from spleen were able to stimulate syngeneic lymph node cells. A more complete study of the stimulatory ability of these cells is underway. We also observed that similar transcripts are found in Peyer’s patches from SJL × BALB/c mice that lack Vβ16+ T cells and do not develop lymphomas. This suggests that the expression of vSAg29 is in normal B cells from Peyer’s patches rather than in lymphoma cells.

Expression of Mtv8 may be regulated differently in various strains of mice (32). Therefore, it is of interest that Mtv8, which encodes a relatively potent vSAg in many strains, exhibits vSAg8 transcripts that clearly initiate in the 5′ LTR, does not exhibit such transcripts in either SJL lymphomas or LPS-induced B cell blasts from SJL mice. Lymphoma cells from the RI strains SW × J-1 also lack such Mtv8 transcripts. In contrast, the lymphoma cell lines derived from MA/My mice clearly exhibit 5′ LTR-initiated Mtv8 and Mtv17 encoded vSAg transcripts as well as proteins, as shown by their ability to stimulate Vβ11, Vβ12, and Vβ17a bearing T hybridoma cells (15). These lymphoma cells weakly stimulate Vβ16+ T hybridoma cells. In contrast, although both vSAg29 and vSAg8 transcripts also are present in the C57L lymphoma lines, the ability of C57L lymphomas to stimulate Vβ16 T hybridomas was significantly greater than their stimulatory activity for Vβ8.1+, Vβ11-, or Vβ12-bearing hybridomas. It is
Within the lymphoma cells, clear stimulation of syngeneic T cells is observed, reflecting the nature of the most important T cell subset in this response. This is particularly true for the I-E\(^b\) MA/My mice, where V\(\beta\)16\(^b\) T cells (unpublished observation), in addition to V\(\beta\)5.1\(^b\), V\(\beta\)11\(^b\), and V\(\beta\)12\(^b\) T cells, are required. Moreover, the need for CD4 T cells in promoting in vivo growth of SJL and C57L lymphomas was clearly established in previous studies (7, 20, 25, 37). This information is not yet available for the MA/My or the SW \(\times\) J-L lymphomas studied here.

It is possible that differential methylation of the LTR and env promoter regions of various Mtvs in different strains, as well as in different tissues of mice, influences the transcription patterns seen in the present study. Preliminary data indicate that significant differences exist between SJL liver and lymphoma DNA in the degree of methylation present in the META-env promoter region of Mtv29 (R. M. Thomas, N. Sen, D. J. Zhang, V. K. Tsiagbe, and G. J. Thorbecke, manuscript in preparation). Further studies are needed to evaluate whether such variations in DNA methylation patterns and/or differences in the presence and recruitment of transcription factors may explain the production of META-env-controlled transcripts in Mtv29 in GC cell-derived lymphomas (and perhaps also normal GC cells), but not in LPS-activated B cells.

References
mRNA for the long terminal repeat gene product. J. Virol. 46:42.


25. Erianne, G. S., J. Wajchman, R. Yauch, V. K. Tsaijbe, B. S. Kim, and


eliminates T cells specific for Mls-modified products of the major histocompat-


moter elements between a viral superantigen and the major histocompatibility complex class II-associated invariant chain. J. Virol. 71:1237.


34. Woodland, D. L., H. P. Smith, S. Surman, P. Le, R. Wen, and M. A. Blackman. 1993. Major histocompatibility complex-specific recognition of Mls-1 is medi-
ated by multiple elements of the T cell receptor. J. Exp. Med. 177:433.

35. Tsaijbe, V. K., S. Chickramane, A. R. Amin, J. M. Edington, and


