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Clonal Expansion of Double-Positive Intraepithelial Lymphocytes by MHC Class I-Related Chain A Expressed in Mouse Small Intestinal Epithelium

Eun Jeong Park,†‡# Ichiro Takahashi,*† Junko Ikeda,† Kazuko Kawahara,‡ Tetsuji Okamoto,‡ Mi-Na Kweon,*‡ Satoshi Fukuyama,*# Veronika Groh,* Thomas Spies,† Yuichi Obata,§ Jun-Ichi Miyazaki,¶ and Hiroshi Kiyono2*#

Expression of a distant homologue MHC class I molecule, MHC class I-related chain A (MICA), has been found to be stress inducible and limited to the intestinal epithelium. This nonclassical MHC molecule is associated with various carcinomas in humans. To understand the biological consequences of MICA expression in the gut, we generated transgenic (Tg) mice (T3b-MICA Tg) under the control of the T3b promoter. The T3b-MICA Tg mice expressed MICA selectively in the intestine and had an increased number of TCRαβ CD4CD8αα double-positive (DP) intraepithelial lymphocytes (IELs) in the small bowel. These MICA-expanded DP IELs exhibited a bias to Vβ8.2 and overlapped motifs of the complementarity-determining region 3 region among various Tg mice. Hence, the overexpression of MICA resulted in a clonal expansion of DP IELs. Studies in model of inflammatory bowel disease showed that transgenic MICA was able to attenuate the acute colitis induced by dextran sodium sulfate administration. Therefore, this unique in vivo model will enable investigation of possible influences of stress-inducible MICA on the gut immune surveillance. The Journal of Immunology, 2003, 171: 4131–4139.

Intestinal epithelia contain a developmentally and functionally specialized T cell pool, the so-called intraepithelial lymphocyte (IEL)3 (1, 2). Because of their specific and unique location in the mucosal epithelium, IELs are often regarded as a first line of mucosal barrier against enteric flora (3). With respect to IEL ontology, it can be divided into αβ T cells bearing CD4 or CD8αβ coreceptors (thymus dependent) and γδ or αβ T cells bearing CD8αα coreceptors (thymus independent) (4–8). However, a recent report suggests that the thymus is critical for generation of TCRαβ CD8αα IELs also (9). Little is known about locally expressed key molecules that may be involved in the selection and maturation of extrathymic IEL; reportedly though, some integrins or chemokine receptors facilitate the migration of TCRγδ IELs or their precursors (10).

A nonclassical MHC class I chain A (MICA) molecule is stress inducible and mainly expressed on intestinal epithelium and various epithelial tumors (11–13). Because of the discovery that intraepithelial Vβ8 T cells recognize MICA (14), interaction of the NKG2D receptor on these T cells with MICA has been suggested to be important for activation of NK and T cell responses against MICA-bearing tumors (15). In addition, bacterial infection has enhanced the expression of MICA on target cell surface and up-regulated Vγ2Vδ2 T cell activation by nonpeptide Ags (16).

The increase in MICA expression induced by various stresses, including heat shock, oxidative stress, and virus or bacteria, together with the expression of MICA locally in gut epithelium prompted us to consider the possibility that interaction of MICA with IEL is important for the development and effector functions of IEL. In particular, because the gut is in a permanent state of mild inflammation or immunological stress due to exposure to commensal microflora and food Ags, lymphocytes struggling against damaged cells on the frontline may be necessary for maintaining host homeostasis (17, 18). However, the function of MICA expressed in the human intestinal tracts and consequences of the increase in MICA observed in vivo are undefined.

To evaluate these issues, in this work, we generated a transgenic model ectopically expressing human MICA, under the control of the T3b promoter in the mouse intestine. The transgenic mice expressed human MICA specifically on their intestinal epithelium and possessed numerous CD4CD8αα (double-positive (DP)) IELs in their small intestine. We thus inquired into the characteristics of this expanded DP subset, examining the clonotype and DNA sequence of complementarity-determining region 3 (CDR3) to determine whether MICA exposure biased these cells toward a unique Vβ repertoire. Moreover, we introduced an experimental inflammatory bowel disease model into the transgenic (Tg) mice.
and showed a substantial attenuation of the development of intestinal disorder.

Materials and Methods

DNA construct and the generation of T3b-MICA Tg mice

The 2.8-kb promoter region of the T3b gene was shown to direct transgene expression exclusively in the epithelial cells of the small and large intestines (19). The upstream end of the T3b promoter region in the T3b transgene vector was originally an Sp6I site, but was changed to a KpnI site using a linker, because of the presence of an Sp6I site in the MICA cDNA.

The human MICA cDNA, including 1.2 kb of the whole MICA coding sequence, was cloned by PCR, and its sequence was confirmed (11). The T3b–MICA transgene was constructed by inserting this MICA cDNA into the unique EcoRI site of the T3b transgene vector, which contains the T3b promoter and the rabbit β-globin gene sequences from the second exon to the third exon, including the polyadenylation signal. The transgene vector was digested with KpnI and XhoI, and the resulting 5.5-kb fragment of the T3b–MICA transgene was isolated and used for microinjection into the pronuclei of one-cell embryos of BDF1 mice, to produce T3b–MICA transgenic mice, as described previously (20).

The transgene expression, total RNA was isolated from various tissues by using TRIzol reagent (Invitrogen, Carlsbad, CA), according to the manufacturer’s protocol.

RT-PCR

Total RNA was extracted from various tissues by using TRIzol reagent (Invitrogen, Carlsbad, CA), according to the manufacturer’s protocol. DNase digestion of extracted RNA was performed before cDNA synthesis. A total of 1 μg of total RNA was reverse transcribed into cDNA using Omniscript reverse transcriptase (Qiagen, Valencia, CA). PCR amplification (GeneAmp PCR system 9700) (PerkinElmer, Foster City, CA) was performed initially at 95°C for 5 min and then in sequential cycles at 95°C for 30 s, 61°C for 30 s, and 72°C for 40 s, followed by an extension for 10 min at 72°C. The oligonucleotide primers used for the determination of MICA expression were 5'-CTCGAGGAGCCCCCAGCTCCTGTATATA-C3' as a forward primer, and 5'-GGATCTCAGACAGCTTAATC-3' as used as a backward primer.

Immunoblotting

Whole cell lysates (20 μg of proteins) were separated on 1-mm-thick 4–20% Tris-glycine gels and then transferred to nitrocellulose. Equal protein loading in each of the lanes was confirmed by staining the same gel with GelCode Blue Stain Reagent (Pierce, Rockford, IL). The filters were blocked with 5% (w/v) nonfat dry milk powder in TBST (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, and 0.05% Tween 20). Anti-MICA mAb 2C10 (mAb IgG3) (13) was diluted 1:2000 in TBST containing 3 mg/ml BSA and incubated with the filter at 2 h. As a loading control, anti-β-actin mAb (Sigma-Aldrich, St. Louis, MO) was also incubated with a separate filter. After washing with TBST, the filters were incubated for 1 h with HRP-conjugated goat anti-mouse IgG3 (1/3000 in TBST) (Southern Biotechnology Associates, Birmingham, AL). Filters were washed extensively with TBST, and immunoreactive bands were visualized by chemiluminescence reagent (NEN Life Science, Boston, MA).

Immunohistochemical assay

Blocks of intestine, spleen, and thymus were removed, fixed in 4% paraformaldehyde, embedded, and snap frozen in OCT compound (Tissue-Tek, Torrance, CA). Sections (5 μm) were cut in a cryostat and air dried. The sections were weathered with H2O2, treated with 10% PBS, and then incubated with a titrated dilution of anti-MICA mAb 6D4 (mouse IgG2a) (21). For the detection of bound Abs, Vectorstain ABC kit (Vector Laboratories, Burlingame, CA) and diaminobenzidine substrate kit (Funakoshi, Tokyo, Japan) were used. Slides were then counterstained with hematoxylin. Control sections without the primary Ab or with an isotype control were run in parallel.

InTESTINAL MICA TRANSGENE INDUCES CD4CD8 IELs

Lymphocytes were isolated from spleens and IELs, as described previously (21). The use of IELs, after Peyer’s patches and fatty tissues were removed, a standard mechanical dissociation method was performed and followed by a Percoll discontinuous density gradient separation. After blocking with anti-CD16/CD32 FcR mAb (2.4G2), the cells were stained using following labeled mAb conjugates: FITC-conjugated anti-CD4 mAb (LT34; MR5-4); anti-CD8 mAb (KT4, Vβ1.5, 5.2 (MR9-4); Vβ6 (RR4-7); B7 (TR310), Vβ8.1, 8.2 (MR5-2); Vβ11 (RR3-15); Vβ12 (MR11-1), Vβ13 (MR12-3), Vβ15 (MR14-2), TCRγδ (GL3), PE-conjugated anti-TCRβ mAb (H57-597), TCRγδ (GL3), CD69 (H1.2F3), CD44 (Ly-2; IM7), CD62L (MEL-14), and allophycocyanin (APC)-conjugated anti-CD16 (Ly-2; 53-6-7) mAb. All mAbs were purchased from BD PharMingen (San Diego, CA). The stained cells were then washed and analyzed with a FACS caliber flow cytometer (BD Biosciences, San Jose, CA).

Immunoassay analysis for Vβ repertoire

As previously described, cDNA synthesized from total RNA of the sorted cells was used for Vβ-Cβ amplification (23). The oligonucleotide primers used for these reactions were: forward (Vβ8.2), 5'-CATTATCTATAGTGGCTGCCC-3'; reverse (Cβ145), 5'-CAGCTGATGTCGTGGACAC-3'. The amplified products were then used to run off reactions with an oligonucleotide primer labeled with a fluorescent tag (Cβ5-6-carboxyfluorescein; 5'-6-carboxyfluorescein-CTTGCTGAGGAGATCACATTCTC-3'). The runoff products were subjected to capillary electrophoresis in an automated DNA sequencer (PE Applied Biosystems, Foster City, CA), and CDR3 size distribution and signal intensities were analyzed with GeneScan software (PE Applied Biosystems). Cloning and sequencing of selected Vβ-β rearrangements

Each Vβ-β-amplified product was shot gun cloned with the pGEM-T Easy TA cloning kit (Promega, Madison, WI) (23). Resulting colonies were randomly selected for plasmid DNA isolation by using ABI Prism MiniPrep kits (PE Applied Biosystems). Sequencing reactions were performed with an IRDye AFLP Kit (LI-COR, Lincoln, NE) and analyzed on an LI-COR4000 sequencer (LI-COR).

Animal experiment for induction and analysis of dextran sodium sulfate (DSS)-induced colitis

Colitis was induced by the administration of DSS (2.5% w/v; m.w., 40,000; ICN Biomedicals, Irvine, CA) in the drinking water for 5 days (24). DSS water consumption and weight were recorded daily. For the assessment of the severity of colitis, animals were sacrificed on days 5, 9, and 17 after beginning of the DSS treatment, and the colons of the Tg mice and C57BL/6 mice were examined histologically. Tissue samples obtained from the proximal and distal colon were fixed in 4% paraformaldehyde in PBS, embedded in paraffin, and sectioned at a thickness of 6 μm. The tissue sections were stained with H&E. Severity of colitis was evaluated by the standard scoring system, as described previously (25). Each region of the colon (ascending proximal and descending distal colon) was graded semiquantitatively from 0 (no change) to 3 (most severe change) per examination item. The grading represents a degree of monocyte and/or neutrophil infiltration, goblet cell and/or mucous loss, epithelial erosion, and ulceration. The scoring was performed by a blinded manner.

Statistical analysis

Significant differences between mean values were determined by Student’s t test. p < 0.05 was considered to be statistically significant.

Results

Selective expression of human MICA in the intestine of T3b-MICA Tg mice

Human MICA cDNA, expressed under the control of the T3b promoter, was specifically expressed in intestinal epithelial cells (Fig. 1A). From 10 founder mice, three representative lines (Tg-07-5, Tg-07-6, and Tg-09-4) were maintained by mating to C57BL/6 mice. We mainly used F2 and F3 MICA Tg mice in this study. Thus, in addition to the background effect of backcross partner, C57BL/6 strain, the effect of the DBA2 strain that was used to make a donor for BDF1 embryos also might have been involved in the phenotype of the Tg mice. To determine the tissue specificity of the transgene expression, total RNA was isolated from various tissues of the 17-day-old mice.
FIGURE 1. Selective expression of MICA in the intestinal tracts of T3b-MICA Tg mice. A, DNA construct. Human MICA cDNA was designed to be expressed in mouse intestinal tracts under the control of the T3b promoter. B, RT-PCR. MICA expression in intestinal tissue of the T3b-MICA Tg mice was determined by standard RT-PCR, as described in Materials and Methods. The expected size of MICA-specific cDNA (0.8 kb) was shown in the lanes of small and large intestines. C, Immunoblotting. Left, Matured MICA proteins were expressed in small (SI) and large intestinal (LI) tissues, but not in spleen, thymus, and mesenteric lymph node of T3b-MICA Tg mice. As a loading control, β-actin blot was also used in a separate membrane containing the same amount of whole cell lysates as those of MICA blot. Right, MICA was specifically expressed only in small and large intestines from all three lines of Tg mice, but not in non-Tg C57BL/6 mice. D, Immunohistochemical assay. The analyses by using anti-MICA mAb (6D4) exhibited that MICA resides in villous of the small intestine (SI) and tip and villous regions of the large intestine (LI). a, Tg-09-4; b, Tg-07-5; c, magnified photographs of Tg-09-4 (upper, SI; lower, LI; left, proximal; right, distal region). d, C57BL/6 (left, SI; right, LI). e, Tg-09-4 (left, spleen; right, thymus). MICA protein was expressed both in proximal and distal regions, although the differential pattern of transgenic expression showed large intestine and proximal region were slightly more than small intestine and distal region, respectively. Bars indicate 100 μm.
tissues of the T3b-MICA Tg and wild-type (WT) mice and subjected to MICA-specific semiquantitative RT-PCR analysis (Fig. 1B). Transgenic MICA mRNA was expressed selectively in the gastrointestinal tract, i.e., small and large intestine, but not in other tissues, i.e., spleen, thymus, and mesenteric lymph node, and in none of the same tissues of WT mice. MICA production by intestinal epithelial cells was confirmed also at the protein level by immunoblotting analysis (Fig. 1C); by use of anti-MICA mAb 2C10, MICA protein was found in the small and large intestines of T3b-MICA Tg, but not in those tissues of WT mice. In situ expression of MICA protein was documented also by immunohistochemical analysis using anti-MICA mAb 6D4, in both the small and large intestine of the T3b-MICA Tg mice. In Fig. 2B, representative flow cytometry analysis of the TCR Vβ-gated IEL populations in the small intestines of WT mice and the Tg line (Tg-07-6) is illustrated; the population of DP IEL was markedly increased in all the Tg animals examined. In contrast, despite strong expression of MICA in the epithelium of the large intestine, no change in the colonic IEL population was seen (Fig. 2C). Splenic cells isolated from the Tg line had the same distribution of CD4 and CD8 T cell populations as that of WT mice (Fig. 2D). Thus, locally expressed MICA resulted in increased numbers of TCRαβ CD4CD8αα (DP) IELs selectively in the small intestine.

**Table I.** Enumeration of cells for each SI-IEL population in T3b-MICA Tg lines

<table>
<thead>
<tr>
<th>Line</th>
<th>Total IEL</th>
<th>TCRγδ</th>
<th>CD4</th>
<th>CD8</th>
<th>CD4CD8αα (DP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT (n = 7)</td>
<td>12.3 ± 2.3</td>
<td>4.4 ± 1.5</td>
<td>0.9 ± 0.3</td>
<td>3.9 ± 1.0</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>Tg-07-5 (n = 5)</td>
<td>15.2 ± 4.5</td>
<td>4.1 ± 1.0</td>
<td>1.3 ± 0.6</td>
<td>2.7 ± 1.4</td>
<td>3.9 ± 1.3</td>
</tr>
<tr>
<td>Tg-07-6 (n = 3)</td>
<td>12.0 ± 4.2</td>
<td>2.2 ± 1.0</td>
<td>1.5 ± 0.6</td>
<td>2.8 ± 1.6</td>
<td>2.9 ± 0.9</td>
</tr>
<tr>
<td>Tg-09-4 (n = 8)</td>
<td>17.5 ± 6.7</td>
<td>5.8 ± 2.5</td>
<td>1.3 ± 0.5</td>
<td>3.4 ± 1.6</td>
<td>4.0 ± 2.9</td>
</tr>
</tbody>
</table>

*The numbers of cells are indicated as × 10⁶ ± SD. n, Number of mice tested. The total cells were prepared from the Percoll gradient interface, as described in Materials and Methods. The total number of lymphocytes in each population was calculated by multiplying the percentage of each population determined in Fig. 2 by the total number of lymphocytes.

Increase in TCRαβ CD4CD8αα (DP) IELs in the small intestine IELs from the WT mice and three Tg lines were isolated and analyzed for possible alterations induced by MICA expression in the intestinal epithelium. In the presence of the MICA, the total number of IEL and the numbers of γδ T cells and the CD4 and CD8αβ subsets were not changed (Table I). However, the absolute number as well as the percentage of TCRαβ CD4CD8αα (DP) IELs in the total IEL population were increased 7–10 times, whereas the number of TCRαβ CD8αα IELs was decreased by about one-half (Table I and Fig. 2A).

Because MICA expression affected the TCRαβ fraction of IEL, we next compared the changes in these cells between the small and large intestine of the T3b-MICA Tg mice. In Fig. 2B, representative flow cytometry analysis of the TCR Vβ-gated IEL populations in the small intestines of WT mice and the Tg line (Tg-07-6) is illustrated; the population of DP IEL was markedly increased in all the Tg animals examined. In contrast, despite strong expression of MICA in the epithelium of the large intestine, no change in the colonic IEL population was seen (Fig. 2C). Splenic cells isolated from the Tg line had the same distribution of CD4 and CD8 T cell populations as that of WT mice (Fig. 2D). Thus, locally expressed MICA resulted in increased numbers of TCRαβ CD4CD8αα (DP) IELs selectively in the small intestine.

**Maturity of the DP IELs by expression of other surface markers**

DP IELs have been proposed to have a distinct origin and to use unique maturation pathways, unlike their thymus counterpart...
(CD8β positive), which expresses low TCR levels and mediates the maturation of CD4 or CD8 T cells (26, 27). When the selectively propagated DP IELs in the T3b-MICA Tg mice were analyzed for the expression of other surface markers, the cells were shown to exclusively express CD69 and CD44, but not CD62L (data not shown) like those of normal C57BL/6 mice (Fig. 3A). When we analyzed the NKG2D expression in DP IELs isolated from Tg and C57BL/6 mice by RT-PCR, the expressions were detected in DP IELs from both mice, even though the expression levels were lower than those of CD8αα IELs (data not shown).

In contrast, there was no change in γδ IEL repertoire in the Tg mice when compared with normal C57BL/6 mice in terms of usage of TCR Vγ and TCR Vδ mRNA, as shown by RT-PCR analysis (data not shown). Moreover, when the phenotype of γδ IELs was examined with respect to CD4, CD8α, and CD8β, the cells prominently expressed CD8αα homodimers, but not CD4δ or CD8δβ subsets. The γδ IELs also expressed a high level of CD69 and CD44 (Fig. 3B). Thus, the pattern of cell surface markers indicates that the DP IEL and γδ IEL subpopulations have a characteristic of activated T cells (28) and, especially, that the propagated DP IELs are fully mature.

Expansion of the DP IELs expressing Vβ8.1, 8.2 repertoire
To further investigate the nature of the expanded DP IELs, we next characterized the cells’ usage of Vβ chains. The total numbers of small intestinal IELs from WT and Tg mice (Tg-09-4 line) used for the Vβ usage analysis were 1.6 × 10⁷ ± 0.4 × 10⁶ (n = 4, on average) and 1.9 × 10⁷ ± 0.7 × 10⁶ (n = 3, on average), respectively. The numbers of CD4 and DP IEL were 0.98 × 10⁶ ± 0.08 × 10⁶ and 0.84 ± 0.08 (n = 4, on average), respectively. The DP IEL numbers were similar in WT and Tg mice.

A, Comparison of the frequency of different Vβ chains expressed by DP T cells in SI-IEL of T3b-MICA Tg mice. Three WT mice and four mice of the Tg-09-4 line were explored in this flow cytometry analysis. IELs were stained with FITC anti-Vβx, PE anti-CD4, and APC CD8α mAbs. All the subsets were determined after gating on CD4 or CD4 plus CD8α IELs. The total number of lymphocytes in each population was calculated by multiplying the respective percentage of each population. The number of each subset or each Vβ repertoire was calculated by multiplying the respective percentage of each repertoire by the total number of each subset. When splenocytes isolated from the mouse showing the greatest increase in DP IELs (4917 ± 10⁶) among four Tg mice were also analyzed by the same method, no significant differences were found between WT and Tg mice (data not shown). B, Immunoscope analysis of Vβ8.2 clonotype of CD4 or CD8 T cells from splenocytes and CD4 or DP T cells from SI-IELs of WT or T3b-MICA Tg mice. Individual populations were purified by cell sorting using FACSVantage. cDNA from each FACSorted subset was subjected to PCR using Vβ8.2- and CB-specific primers, followed by a runoff with a nested fluorescent CB-specific primer (23). The CDR3 size distribution was analyzed with the GeneScan software program. Arrows indicate expansions discussed in the text. The intensity of fluorescence is presented in arbitrary units as a function of CDR3 length in amino acids. The results shown are representative of five individual mice of T3b-MICA Tg (Tg-09-4 line) and WT mice.
0.32 × 10^6 and 0.62 × 10^6 ± 0.2 × 10^6 in WT mice, and 1.64 × 10^6 ± 0.64 × 10^6 and 5.13 × 10^5 ± 1.85 × 10^5 in T3b-MICA Tg mice. As illustrated in Fig. 4A, changes were detected in the numbers of CD4 and, especially, DP IELs in the small intestine of T3b-MICA Tg mice compared with those of WT mice; DP IELs harboring TCR Vβ8.1, 8.2 were markedly expanded (3.74 × 10^7 ± 1.77 × 10^6 Tg mice; 0.78 × 10^6 ± 0.73 × 10^5 WT mice). The increased DP subset compared with that from WT mice was accounted for by this propagated repertoire. We also compared the CD4 and CD8 subsets for Vβ repertoire usage in splenic cells from the Tg mouse line (Tg-07-6) that had the most plentiful Vβ repertoire usage in splenic cells from both the WT and Tg mice. In the absence of MICA, no propagation of the unique T cell clone having the Vβ8.1, 8.2 chain was observed in the splenocytes of the T3b-MICA Tg mice (data not shown). These results are additional evidence that MICA mediated the clonal expansion of DP IELs expressing the TCR Vβ8.1, 8.2 repertoire in a small intestine-restricted manner.

Clonality of DP IELs expressing TCR Vβ8.2

Using immunoscope analysis associated with DNA sequencing of the CDR3, we next determined the clonality or redundancy of the TCR Vβ8.1, 8.2 chain repertoire used by the DP IELs. By Vβ chain-specific semiquantitative RT-PCR, we found that the expanded repertoire of DP IEL was preferentially associated with the Vβ8.2 chain (data not shown). As shown in Fig. 4B, a typical Gaussian distribution of CDR3 lengths of Vβ8.2-Cβ PCR products was observed in the CD4 and CD8 splenocytes from both the WT and Tg mice. The CD4 IEL of the Tg mice had some oligoclonality for the Vβ8.2 chain, compared with that of WT mice (Fig. 4A). The DP IEL of interest exhibited a bias of a single peak of CDR3 length for the Vβ8.2 chain in the Tg line, but not in WT mice (Fig. 3B). This skewing suggested a clonal expansion of the Vβ8.2-restricted DP IEL induced by MICA. We next analyzed the DNA sequence of the CDR3 region for each amplified Vβ-1β combination. As shown in Table II, the Vβ-1β pair had a tendency toward the Vβ8.2-Jβ2.7 or Vβ8.2-Jβ1.6 combinations, although this trend was not observed in all the Tg mice. Among the other T3b-MICA Tg mice examined, Tg-07-5-b mouse showed a Vβ8.2-Jβ2.7 combination containing a major CDR3 sequence (1/6). Also, Tg-07-5-d, Tg-07-6-a, and Tg-09-4-a showed a Vβ8.2-Jβ2.4 combination containing a major CDR3 sequence (SDWGGQNTL) (6/8). Furthermore, restricted motifs for the CDR3 region in the Vβ8.2-Jβ2.7 and Vβ8.2-Jβ1.6 combination were found to possess a sequence GDRQGEFQ in individual mice in two different lines (Tg-09-4-a and Tg-07-5-a) and SDRGHNSPL in two mice of Tg-09-4 (-b and -c), respectively (data not shown). These data imply that MICA-induced clonal expansion of Vβ8.2-harboring DP IEL developed a tendency to restrict limited sequences in the CDR3 region.

Effect of MICA transgene against DSS-induced colitis

It was important to examine physiological and immunological contribution of transgenic MICA in vivo. The T3b-MICA Tg mice were subjected to the experimental disease-inducing protocol of DSS colitis. The onset of colitis in DSS-treated MICA Tg mice was substantially delayed when compared with that of DSS-treated non-Tg C57BL/6 mice. The DSS-treated MICA Tg mice lost significantly less body weight during the period of the observation.

Table II. Vβ8.2-CDR3 sequence-Jβ chain combination of CD4 and DP subset in SI-IELs

<table>
<thead>
<tr>
<th>CDR3 Sequences for Vβ8.2 Chain</th>
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<tbody>
<tr>
<td>CD4 WT-a</td>
</tr>
<tr>
<td>GQAQTEV-Jβ1.1</td>
</tr>
<tr>
<td>GDEPGQTGQL-Jβ2.2</td>
</tr>
<tr>
<td>GVRGGAJTL-Jβ2.4</td>
</tr>
<tr>
<td>GDWGNQNDTG-Jβ2.5</td>
</tr>
<tr>
<td>Tg-07-5-a</td>
</tr>
<tr>
<td>SDPNYAEQ-Jβ2.1</td>
</tr>
<tr>
<td>GDGGGAETL-Jβ2.3</td>
</tr>
<tr>
<td>GLGWAETL-Jβ2.3</td>
</tr>
<tr>
<td>QRVRQQTQ-Jβ2.3</td>
</tr>
<tr>
<td>GAGLQRTQ-Jβ2.5</td>
</tr>
<tr>
<td>GDADYEQ-Jβ2.7</td>
</tr>
<tr>
<td>GEGGRQEQ-Jβ2.7</td>
</tr>
<tr>
<td>GDRQGEFGQ-Jβ2.7</td>
</tr>
<tr>
<td>GDRQGEFGQ-Jβ2.7</td>
</tr>
</tbody>
</table>

* The predominant or expanded sequences are indicated (bold letters). The detection frequencies are determined by the number of sequences with the listed CDR3 sequence of the total number of sequences generated for the particular Vβ8.2-1β combination in the mouse indicated.
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FIGURE 5. The suppressive effect of transgenic MICA for the development of DSS-induced colitis. A. Body weight was measured daily. Data shown represent the mean ± SD of mice in each group (n = 5) (*, p < 0.05). B. Histological analysis of colons in DSS-treated MICA Tg mice. The severity of the colitis was also determined in each group of mice (n = 3) by using the histological disease-scoring system (25). The transgenic MICA ameliorated the severity of the colitis. Bars indicate 50 μm (proximal colon) and 100 μm (distal colon).

Discussion

In this study, we report the generation of a Tg model expressing human MICA selectively in mouse intestine. The T3b-MICA Tg mice possess some interesting properties. First, the mice expressed the MICA only in the intestinal tract (small intestinal villi and large intestinal tips). Second, the mice contained a greatly increased CD4CD8α (DP) IEL in the small, but not the large intestine, and this population was present only in the IEL, not in lamina propria lymphocyte. Third, the increased DP subset was almost entirely biased toward the Vβ8.2 chain repertoire, with some restricted CDR3 sequences. Based on these results, it is evident that the overexpression of intestinal MICA specifically promoted the development of clonally expanded DP IELs in the small intestine.

A major reason we chose to express intestinal MICA by the use of the T3b promoter was that both the T3b (a thymic leukemia Ag) and the MICA are nonclassical MHC class Ib molecules, and it has been reported that both of them are preferentially expressed in the intestinal epithelial cells (14, 19, 29). Thus, it could be expected that the T3b-driven MICA molecule expressed in the murine intestinal immune system would be represented and behave physiologically in the in vivo situation. In addition, our recent separate results showed that transgenic MICA is preferentially expressed in the basolateral side of the intestinal epithelial cells (data not shown), as previously reported (30). Thus, MICA and its derived mucosal T cells may be important components of the innate regulatory network that maintains immunologic homeostasis in the harsh environment of the intestinal tract. In this regard, T3b promoter-driven, transgenic MICA was able to attenuate the DSS-induced intestinal inflammation (Fig. 5), indicating the basolateral expression of MICA in intestinal epithelia be appropriate for the immunological surveillance against mucosal inflammation.

CD4CD8α (DP) lymphocytes in mice are present mainly in the small intestine and, unlike thymic DP T cells, highly express CD3 on their surfaces (31). This interesting subset, harboring mutually exclusive coreceptors, is proposed to be involved in intestinal T cell maturation and development, and possibly to function in both innate and adaptive immunity (32). DP IELs have been shown capable of secreting Th2-type cytokines and providing help to B cells for the secretion of IgG (33). Also, TCRαβ-mediated signaling initiated cytotoxic function, but did not induce proliferation of the DP IELs (34), and murine CD4 IELs were found to give rise to DP IELs in an inflammatory bowel disease model (35). Recently, it was shown that CD4⁺CD8α T cells in the intestinal epithelium were functioning as regulatory T cells for the prevention of inflammatory bowel disease in an IL-10-dependent fashion (36). Together with this report (37) and our present finding that T3b-MICA Tg mice are resistant to the development of DSS-induced colitis, an interesting scenario would be that a stress-associated nonclassical MHC class I molecule participates in the preferential induction of DP IELs with a regulatory function for the maintenance of host immune homeostasis from the development of the intestinal inflammation. Although the ontogeny, function, and precise reason for the propagation of DP IELs remain to be clarified, our unique T3b-MICA Tg mouse model most likely will be useful for this purpose.

There is evidence that DP IELs arise from thymus-derived CD4 T cells, which migrate into the epithelium and express CD8 (32); a report that the transfer of CD4 peripheral T cells into SCID mice
reconstituted DP IELs also supports this view (35). Furthermore, both CD4 and DP subsets of rat IELs reportedly showed oligo-
clonality and overlapping β-chain repertoires, and the DP subset contains a considerably more restricted repertoire than do CD4+ IELs (37). Our data from immunoscope and CDR3 sequence analy-
ses also reveal that DP IELs have more restricted oligoclonality than do CD4 IELs in T3β-MICA Tg mice (Fig. 3 and Table II). How-
ever, in addition to the possibility that DP IELs mature from CD4 IELs, it is possible that CD8α IELs transform into DP IELs by ac-
quisition of the CD4 coreceptor under the influence of MICA in
the intestinal epithelium. As depicted in Fig. 2A, contrary to
TCRaβ CD4 or CD8αβ population exhibiting no quantitative al-
teration, the percentage of CD8α IELs was no more than 50% of
those in WT mice, while CD4CD8α (DP) IELs were concurren-
tly increased in all Tg lines; perhaps this result reflects matu-
ration of CD8α IELs into CD4CD8α IELs.

An intriguing question is what is the driving force responsible for human MICA accelerating a bias to clonal selection of DP IELs and their propagation? Some interesting possibilities can be enter-
tained. First, the transgenic MICA molecules could be recognized by an NKG2D-like or -related receptor for MICA, expressed on the DP IELs. In this regard, in a separate study, we are assessing the reactivity of the NKG2D tetramer with intestinal epithelial cells (IECs) isolated from the small intestine of the T3β- MICA Tg mice by flow cytometric analysis and immunohistochemical analy-

We have found that IECs isolated from the MICA Tg, but not WT, mice bind strongly to the NKG2D tetramer, and an intense signal is present in the basolateral portions of IECs (data not shown). In contrast, splenocytes isolated from both T3β- MICA Tg and WT mice did not react with the NKG2D tetramer. These re-

results suggest that ectopically expressed MICA molecules in the Tg mice can interact specifically with NKG2D molecules on neighboring IELs in a physicochemical fashion. Second, CD8α corec-

ceptors on DP IEL could directly bind to MICA independent of
TCR specificity, similar to the reported interaction between
CD8α and the thymic leukemia Ag, which is another nonclassical
MHC class I molecule expressed on intestinal epithelial cells,
modulating T cell responses (38). And the lack of expansion of
MHC class I molecule expressed on intestinal epithelial cells,
contains a considerably more restricted repertoire than do CD4
IELs, with a lower frequency of DP T cells than do small intestinal IELs
(39, 40). Collectively, we emphasize that our T3β-MICA Tg mice are
a unique in vivo model that can be used to elucidate the biolog-
ical role of the stress-inducible nonclassical MHC molecules for
the regulation of gastrointestinal immune surveillance and homeostasis.

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