A Mutant Collagen XIII Alters Intestinal Expression of Immune Response Genes and Predisposes Transgenic Mice to Develop B-Cell Lymphomas

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Abstract

Epithelial cells of mucosal surfaces are critical for maintaining immune homeostasis by aiding in the discrimination of pathogenic and commensal microorganisms and modulating the activities of antigen-presenting cells and lymphocytes. Functional breakdowns resulting in chronic infection and inflammation are associated with the development of hemato-logic and solid neoplasms for which detailed pathogenic mechanisms are poorly understood. Mice heterozygous for a transgene Col13a1del expressing a mutant collagen XIII developed clonal mature B-cell lineage lymphomas originating in mesenteric lymph nodes (MLN). The tumors were associated with T cells and macrophages. The incidence of disease was reduced 2-fold in transgenic mice raised under specific pathogen-free conditions, suggesting a role for infectious agents. The lymphomas did not express the mutant collagen XIII, indicating that its influence on tumorigenesis was B-cell extrinsic and likely to be associated with collagen XIII–positive tissues drained by the MLN. Studies of the small intestines of transgenic mice showed that the subepithelial basement membranes (BM) were highly abnormal and that they exhibited heightened expression of genes involved in immune responses. These results define collagen XIII–dependent maintenance of the intestinal BM as a previously unappreciated component of immune responses and a critical determinant of cancer susceptibility. [Cancer Res 2008;68(24):10324–32]

Introduction

The last 20 years have witnessed remarkable progress in understanding the cellular origins and molecular mechanisms involved in the development of B-cell lineage neoplasms. Reciprocal chromosomal translocations that aberrantly juxtapose regulatory sequences of immunoglobulin (Ig) genes with proto-oncogenes are the hallmark of many types of human B-cell lymphomas (1, 2) and subsets of mouse B lineage tumors, including most plasmacytomas (3) and some diffuse large B-cell lymphomas (DLBCL; ref. 4). It is well established that these B-cell–intrinsic molecular changes are not sufficient to fully induce transformation (5) and that secondary genetic mutations and other factors have important roles in the pathogenesis of B-cell malignancies. It has been suggested that nongenetic B-cell–extrinsic contributions to lymphoma induction, progression, and survival may be multiple. They include engagement of the B-cell receptor (BCR) by self or microbial antigens (6), influences mediated by T cells activated by microbial pathogens (7), host inflammatory responses (8–10), and the interface between tumor cells and stromal elements such as collagen interactions with integrins and other receptors (11, 12). Fifteen percent to 20% of all cancers are estimated to be linked to underlying infections and inflammatory responses (13). For example, inflammatory bowel diseases are strongly associated with colon carcinogenesis, and hepatitis B and C infections are well-known risk factors for liver carcinoma (14).

The transmembrane collagen XIII is localized in cell-cell and cell-matrix junctions (15, 16). In previous studies, mice that were homozygous for a transgene (Col13a1del) expressing mutant collagen XIII with a partial deletion of the collagenous ectodomain died in utero of cardiovascular defects (17, 18). The studies to be presented here were prompted by the unanticipated findings that mice heterozygous for the Col13a1del allele developed T-cell–rich, histiocytic-rich B-cell lymphomas appearing in mesenteric lymph nodes (MLN) and that the incidence of lymphomas was significantly reduced in mice raised under specific pathogen-free (SPF) conditions. This suggested that collagen XIII might normally function as a novel tumor suppressor gene for intestinal microbe-dependent lymphomas. We suggest that this effect is mediated through structural contributions to the subepithelial basement membrane (BM) as a previously unappreciated component of immune responses mediated by cells of the intestinal epithelium and lamina propria.

Materials and Methods

Generation of collagen XIII Col13a1del transgenic mice and their health monitoring. Col13a1del mice were prepared and genotyped as previously described (17). The health monitoring in the SPF barrier unit was performed every 4 mo according to Federation of European Laboratory Animal Science Associations (FELASA) recommendations (19). Mice reared in a conventional animal facility were tested for all viruses recommended by FELASA and for several bacteria known to commonly afflict mouse colonies. Mice reared in the conventional unit were positive for the mouse hepatitis virus, the Theiler’s murine encephalomyelitis virus, Helicobacter pylori, and Pasteurella pneumotropica, whereas mice in the SPF conditions...
were negative for these and other microbes specified by FELASA. All of the animal experiments were approved by the Animal Care and Use Committee of the University of Oulu.

**Tissue preparation.** Heterozygous mice and wild-type (wt) littermates were studied for the occurrence of tumors at 18 mo of age. Tissue samples were either used fresh or rapidly frozen in liquid nitrogen and stored at −70°C until used for immunofluorescence staining and RNA extraction or fixed in 10% buffered formalin and embedded in paraffin for histologic and immunohistochemical analysis.

**Histologic and immunohistochemical analysis.** For general histology, 5-μm-thick paraffin or frozen specimens were stained with H&E according to standard procedures. Antibodies against CD3/PECAM (Pharmingen), CD45 (Pharmingen), CD68 (Serotec), type IV collagen (Chemicon), desmin (Sigma), and collagen XIII (15) were used.

**Flow cytometry (fluorescence-activated cell sorting).** Tumor tissues were homogenized in a Medimachine homogenizer (DAKO) and the cells were counted and analyzed on a FACSCalibur flow cytometer (Becton Dickinson) using CellQuest software (Becton Dickinson). Forward and side-scatter gating was used to exclude dead cells from the analysis. FITC- or PE-conjugated monoclonal antibodies (Becton Dickinson) against CD3, CD45 (Pharmingen), CD68 (Serotec), type IV collagen (Chemicon), desmin (Sigma), and collagen XIII (15) were used.

**Molecular analysis.** Total RNA was isolated from tumors and used as a template for the reverse transcription (RT) reaction followed by PCR as previously described (17). High molecular weight DNA was prepared from snap-frozen mesenteric lymphomas and studied by Southern blotting. The DNA was digested with EcoRI or HpaI and hybridized with the Ig probe for IgH rearrangements and digested with restriction endonuclease BamHI or HhaI for T-cell receptor β chain (TCRβ) rearrangements. The probes were provided by Dr. Terence Rabbitts (MRC Laboratory of Molecular Biology, Cambridge, United Kingdom).

**Electron microscopy.** Biopsies from the small intestine of control and transgenic mice were fixed in a mixture of 1% glutaraldehyde and 4% formaldehyde in 0.1 M phosphate buffer, postfixed in 1% osmium tetroxide, dehydrated in acetone, and embedded in Epon EMbed 812. Thin sections were cut with a Reichert Ultracut ultramicrotome and examined in a Philips CM100 transmission electron microscope (FEI). Images were captured with a charge-coupled device camera equipped with Tietz EM-Menu, version 3, from Tietz Video and Image Processing Systems GmbH.

**Microarray analysis.** The separated tissues were fixed immediately and stored at −70°C until used for the extraction of the RNA. Total RNA was purified using the RNeasy kit (Qiagen). RNA (5 μg) was used as a template for synthesizing the cDNA and making biotinylated cRNA, according to the manufacturer’s instructions (Affymetrix). The biotinylated cRNA was hybridized on the GeneChip Mouse Expression Set 430 2.0 Array, which represents ~45,000 mouse transcripts. The arrays were scanned with a GeneChip Scanner 3000 and the resulting expression data were analyzed with the DNA-Chip analyzer (dChip; ref. 20) and the Affymetrix GeneChip Operating System. The intensities of the signals for all probe sets were scaled to a target value of 500. The genes in which altered expression was observed in three of the four small intestines of the transgenic mice compared with two small intestines of their control littermates are shown in Table 1.

**Results.**

**Occurrence of lymphomas in Col13a1<sup>del</sup> mice.** Mice heterozygous for the Col13a1<sup>del</sup> transgene raised in a conventional colony exhibited no abnormalities through a year of age. Necropsies performed on a cohort of mice 18 months of age revealed that 33 of 209 (15.8%) of the Col13a1<sup>del</sup> mice had changes suggestive of lymphomas. They exhibited prominent enlargement of the MLN (Fig. 1A) that was associated with marked splenomegaly in 15 of these cases. In contrast, only 3 of 146 (2.1%) of littermate control mice exhibited similar changes, a highly significant difference (P = 0.002). Both control and mutant mice are of the B6D2F1 strain and they were housed under identical conditions.

Our health monitoring data indicated occurrence of several pathogenic microbes in the conventional facility that were not present in the SPF-reared mice (see Materials and Methods). To assess if environment might influence the development of lymphomas in our mice, we necropsied mice from a barrier colony. We found that 14 of 190 (7.4%) of Col13a1<sup>del</sup> but only 1 of 118 (0.8%) of wt mice exhibited enlargement of the MLN. Splenomegaly was seen in four of the Col13a1<sup>del</sup> mice, although it was not as marked as in affected conventionally reared transgenics. Moreover, in mice from the conventional space, the total length of the lymphomas often reached 4 to 6 cm, whereas the lymphomas developed in mice from the barrier were usually <2 cm. Thus, the lymphoma susceptibility in Col13a1<sup>del</sup> mice may be driven by antigenic or inflammatory influences of the intestinal environment.

To determine the cellular origin of the tumors and their relation to collagen XIII expression, we first examined single-cell suspensions from seven MLNs for expression of cell surface antigens by flow cytometry. In a typical experiment, the lymphoma contained 53% of T cells and 32% of B cells of total viable lymphocytes (Fig. 1B and C). Immunohistochemical studies showed that the tumors often contained substantial populations of cells expressing CD68, a cell surface marker for macrophages and activated dendritic cells (data not shown). Moreover, we analyzed naive and activation markers on purified CD4<sup>+</sup> and CD8<sup>+</sup> T cells and found that lymphomas were populated by activated CD4<sup>+</sup> cells (Supplementary Table S1).

To determine if the T-cell and/or B-cell populations present in the tumors were clonal, we examined DNA from the tumors for the organization of IgH and TCRβ loci by Southern blot method (Fig. 1D). The results revealed clonal rearrangements of the Ig gene in 5 of 11 cases examined, whereas clonal rearrangements of TCRβ were not observed. According to the Southern blot analysis, rearranged bands in lymphomas at lanes 1 and 2 are more intense than the germ-line band, indicating high proportion of tumor cells within these lymphoma tissues. In lymphoma case 3, the rearranged bands are equally intense, suggesting a single clone with a productive and nonproductive rearrangement.

**Histologic studies.** We performed extensive histologic analyses of tissues from mice 4 or 12 months of age without visible evidence of lymphoma at necropsy and 18-month-old mice with clinically obvious tumors. The earliest histologic changes were seen in a few of the 4-month-old and in a higher proportion of the 12-month-old Col13a1<sup>del</sup> mice without substantial lymphadenopathy or splenomegaly. The affected areas included the paracortical regions of MLNs and the splenic periarteriolar lymphoid sheaths (PALS) and, less often, germinal centers (GC) in the nodes and spleens. Areas of the lymph node paracortex (data not shown), and as shown at lower power the splenic PALS (Fig. 2A), were populated by cells with cytology more similar to that of centroblasts and others having features of lymphoblasts. Small lymphocytes with dense nuclei that usually populate the PALS were pushed to the periphery of the follicle. The blast-like cells were sometimes seen to have fingered their way through the PALS to the outer limits of the follicle, spilling into the marginal zone and red pulp. GCs were large and very active but usually with normal cytology. At higher power (Fig. 2A), the centroblasts, with small nuclei adjacent to the nuclear membrane, and lymphoblast-like cells with central nuclei were also infiltrated by varying numbers of histiocytes having large open nuclei and plentiful cytoplasm.

As the disease progressed, both the spleen (data not shown) and lymph nodes (Fig. 2B) exhibited enlarged follicles that seemed to...
expand progressively from GC-like structures in the nodes, trapping the few residual normal small lymphocytes in the cords, finally resulting in the nearly complete obliteration of normal lymph node architecture (Fig. 2A4). In the spleen, follicular expansion led to marked compression of the red pulp (data not shown). In both the spleen and nodes, histiocytic infiltrates ranged from modest to quite intense.

Mice with enlarged spleens and MLNs frequently exhibited features of overt lymphoma with centroblasts, immunoblasts, or plasma cells. These lymphoid neoplasms were frequently interspersed

Table 1. Changes in gene expression in Col13a1Δdel mice compared with littermate controls

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<thead>
<tr>
<th>Gene name</th>
<th>Gene symbol</th>
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<tr>
<td>Immuneологic function</td>
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<tr>
<td>Cryptdin 4</td>
<td>Defcr4</td>
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<td>Peptidoglycan recognition protein 1</td>
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<td>Dectin-2 (C-type lectin domain family 4, member n)</td>
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<td>Small proline-rich protein 2</td>
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<td>C-reactive protein</td>
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<td>Metabolic enzymes</td>
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<td>Butyrobetaine (γ), 2-oxoglutarate dioxygenase 1</td>
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<td>Cytochrome P450</td>
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<td>Transmembrane protease, serine 8 (intestinal)</td>
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<tr>
<td>Signaling</td>
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<tr>
<td>Apoptosis</td>
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<tr>
<td>Caspase-12</td>
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<tr>
<td>Others</td>
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<tr>
<td>Tripartite motif-containing 59</td>
<td>Trim59</td>
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<tr>
<td>Ribosomal protein L14</td>
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<td>Laminin, α3</td>
<td>Lama3</td>
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<tr>
<td>McLeod syndrome gene homologue</td>
<td>Xkh</td>
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<td>β-Carotene 9, 10'-dioxygenase 2</td>
<td>Bdeo2</td>
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<td>α-Fetoprotein</td>
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<td>Transthyretin</td>
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<td>Melanoma cell adhesion molecule</td>
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<td>Otopetin 3</td>
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NOTE: Small intestine tissue from four mutant mice and two age-matched controls were subjected to microarray analysis, and genes significantly altered in expression in at least three of four mutant mice compared with controls are listed. The fold change represents the mean of the changes in jejunum samples of four transgenic mice studied compared with control.
These hematopoietic neoplasms included one histiocytic sarcoma lymphomas in 399 mice), 10 were characterized histologically. An immunoblastic lymphoma with plasmacytoid differentiation among prominent populations of histiocytes, leading to the diagnosis of histiocyte-associated lymphomas. An example of an immunoblastic lymphoma with plasmacytoid differentiation (Fig. 2B1) exhibited classic immunoblasts with large nuclei, reticular chromatin, and large, magenta, sometimes bar-shaped nucleoli, attached on one side to the nuclear membrane. Plasmablasts had nuclei and chromatin compacting around the nuclear membrane and smaller, centrally placed nucleoli. These cells were laid out against a pink frothy background of cytoplasm belonging to histiocytes with large nuclei and varying patterns of small nucleoli and usually very open chromatin. The dominance of the histiocyte population was readily seen at lower power (Fig. 2B2). In a very unusual case, a striking accumulation of histiocytes arrayed in a pseudo-rosette pattern almost totally obscured small populations of malignant immunoblasts and centroblasts (Fig. 2B3). Histiocytic accumulations consistent with the diagnosis of histiocytic sarcoma but without an associated lymphoma were seen in several instances (Fig. 2B4).

Of 47 lymphomas observed in the Col13a1del mice (47 lymphomas in 399 mice), 10 were characterized histologically. These hematopoietic neoplasms included one histiocytic sarcoma and nine B-cell lineage tumors. The B-cell lineage tumors were primarily of GC or post-GC origin with three follicular lymphomas, three centroblastic lymphomas (one histiocyte associated), and one immunoblastic lymphoma. There were also two cases of lymphoblastic lymphoma in mice without thymic enlargement, which in other series are almost uniformly of mature B-cell origin (21, 22). Of the 4 lymphomas found in the control mice (4 lymphomas in 264 mice), 1 was used for histopathologic analysis and diagnosed as a follicular B-cell lymphoma. It should be noted that there was no histologic evidence of leukemia in tissues of Col13a1del mice lacking macroscopic lymphomas and that they had normal white cell, red cell, and platelet counts and normal hemoglobin levels, indicative of normal bone marrow function.

Expression and localization of collagen XIII. We characterized expression patterns for collagen XIII protein in the lymphomas using an antibody that would recognize both the endogenous and mutant forms of the protein. As shown in Fig. 2C, lymphomas that stained intensely for CD45R(B220) (Fig. 2C1) characteristically expressed very little or no collagen XIII (Fig. 2C2). This result was supported by in situ hybridization studies that revealed little if any signal from the lymphomas (data not shown).

RT-PCR was carried out to study the expression of the Col13a1del transgene and endogenous collagen XIII in the lymphomas of the transgenic mice. Despite lack of signals in tissue staining, all the lymphomas in the mesenteric nodes expressed both the transgene and the endogenous collagen XIII, with a higher expression level for the transgene (Fig. 3A, lanes 1–5). Similarly to lymphomas, MLNs of control mice expressed collagen XIII mRNA at a low level (Fig. 3A, lane 6), and macroscopically normal MLNs of Col13a1del mice expressed both endogenous and transgene mRNAs (Fig. 3A, lane 7). Nevertheless, collagen XIII protein was virtually absent in control and Col13a1del MLNs studied by immunostaining (data not shown).

Thus, the virtual lack of the corresponding protein in the macroscopically normal MLNs did not favor the possibility that the endogenous gene and transgene might be expressed by normal B cells but that expression was lost on transformation.

The likelihood that the B-cell lymphomas seen in the MLNs originated from the intestine prompted us to study the dense B-cell populations of normal Peyer’s patches from wt and Col13a1del mice. Peyer’s patch B cells defined by expression of CD45R(B220) and CD19-positive B cells of these cells. Southern blot analysis of the lymphomas revealed clonal immunoglobulin gene rearrangement (lanes 1–3, three individual lymphoma cases). Arrow. HpaI-digested, 16.4-kb germ-line arrangement; arrowheads, rearranged forms.

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Collagen XIII in Lymphoma Development

Figure 1. Analysis of the lymphomas of the intestine. A, macroscopic appearance of a mesenteric lymphoma. B and C, according to the fluorescence-activated cell sorting analysis, varying proportions of the cells were found to express the T-cell antigen CD3 (B) or the B-cell marker CD19 (C). The histograms are gated on total viable lymphocytes and the numbers in B and C are the percentages of CD3-positive T-cells and CD19-positive B-cells of these cells. D, Southern blot analysis of the lymphomas revealed clonal immunoglobulin gene rearrangement (lanes 1–3, three individual lymphoma cases). Arrow. HpaI-digested, 16.4-kb germ-line arrangement; arrowheads, rearranged forms.

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Figure 1. Analysis of the lymphomas of the intestine. A, macroscopic appearance of a mesenteric lymphoma. B and C, according to the fluorescence-activated cell sorting analysis, varying proportions of the cells were found to express the T-cell antigen CD3 (B) or the B-cell marker CD19 (C). The histograms are gated on total viable lymphocytes and the numbers in B and C are the percentages of CD3-positive T-cells and CD19-positive B-cells of these cells. D, Southern blot analysis of the lymphomas revealed clonal immunoglobulin gene rearrangement (lanes 1–3, three individual lymphoma cases). Arrow. HpaI-digested, 16.4-kb germ-line arrangement; arrowheads, rearranged forms.

Among the 47 lymphomas in the Col13a1del mice (47 lymphomas in 399 mice), 10 were characterized histologically. These hematopoietic neoplasms included one histiocytic sarcoma lymphomas, three centroblastic lymphomas (one histiocyte associated), and one immunoblastic lymphoma. There were also two cases of lymphoblastic lymphoma in mice without thymic enlargement, which in other series are almost uniformly of mature B-cell origin (21, 22). Of the 4 lymphomas found in the control mice (4 lymphomas in 264 mice), 1 was used for histopathologic analysis and diagnosed as a follicular B-cell lymphoma. It should be noted that there was no histologic evidence of leukemia in tissues of Col13a1del mice lacking macroscopic lymphomas and that they had normal white cell, red cell, and platelet counts and normal hemoglobin levels, indicative of normal bone marrow function.
Additional studies of lymph nodes and the thymus showed that they did not express collagen XIII (data not shown). Of the lymphoid tissues analyzed, only the spleen showed strong collagen XIII staining, where it seemed to colocalize with the endothelial cell marker PECAM in small vessels (Fig. 4C), the larger vessels being collagen XIII negative (Fig. 4C1 and C3, asterisk). Two-color studies of sections from normal spleen using antibodies to collagen XIII, desmin, and PECAM showed at higher magnification that collagen XIII (Fig. 4D1) did not fully colocalize with PECAM (Fig. 4D2) but exhibited a similar distribution to desmin (Fig. 4D3).

**Electron microscopy of the small intestine.** The demonstration that collagen XIII is expressed at high levels in the intestines prompted us to examine the small intestine in greater detail by electron microscopy. The epithelial cells of the small intestines of control mice (Fig. 5A) showed a well-defined BM, whereas an abnormal appearance of the BM was observed in the jejunum of the 12- to 18-month-old *Col13a1del* mice (Fig. 5B) in that the normal lamina lucida and lamina densa layers of the BM could not be detected. This abnormal BM was less obvious in the *Col13a1del* mice 2 months of age (data not shown), indicating that the structural abnormalities of the BM progressed with age. It is noteworthy that cytoplasmic changes were not seen in the intestinal epithelial cells of young or old *Col13a1del* mice.

**Gene expression profiles differ for small intestinal tissues from wt and *Col13a1del* mice.** To determine if the basis for enhanced lymphoma susceptibility of the *Col13a1del* mice might correlate with altered gene expression profiles, we studied RNAs prepared from small intestinal tissue using a gene chip representing 45,000 transcripts. The mice chosen for study appeared to be healthy and did not have any macroscopic lymphomas or enlarged MLNs. The genes significantly altered in expression in mutant mice compared with controls are listed in Table 1.

Strikingly, the preliminary gene ontology classification of the data using the dChip Gene Function Enrichment analysis revealed that in at least three of four *Col13a1del* mice, a significantly overrepresented number of genes with altered levels of expression were only observed in functional groups defined by the annotation terms immune response, defense response, and antigen binding (Supplementary Table S2). Further analysis with the Affymetrix GeneChip Operating System indicated that 11 of the 23 genes expressed at substantially higher levels in the small intestines of mutant mice encoded proteins involved in various aspects of innate and acquired immunity. *Defcr4* and *Defcr-rs7* encode defensins, mediators of innate immunity with bactericidal activity produced by Paneth cells. *Dggrp1* specifies a member of a family of proteins expressed by intestinal and immune cells that recognize peptidoglycans, ubiquitous components of bacterial cell walls. The product of *Clec4n* is Dectin-2, a C-type lectin with mannose/fucose-like specificity with expression under the dome of Peyer's patches and on dendritic cell and monocytes enhanced by inflammatory stimuli. *H2-DMb1*, *H2-DMb2*, and *H-2DMa* encode heteromeric molecules differentially expressed by subsets of antigen-presenting cells. The protein encoded by *Mst4a4b* enhances...
Collagen XIII in Lymphoma Development

Figure 3. Expression of the Col13a1del transgene and endogenous collagen XIII in the lymphomas and intestine. A, RT-PCR analysis of the expression of the transgene (tg; 297 bp) and the endogenous collagen XIII gene (567 bp) in several lymphomas (lanes 1–5) from two transgenic lines, in normal MLN in control (lane 6) and transgenic mouse (lane 7), and in the large intestine of the transgenic mouse (lane 8) studied by Southern blotting. Lane 9, negative control for RT reaction; lane 10, negative control for PCR reaction. The selected primers allowed simultaneous amplification of both the endogenous and transgenic mRNAs. Transgene expression is equal to or higher than endogenous collagen XIII expression in all tumors studied. B, RT-PCR analysis showed that endogenous collagen XIII mRNA was highly expressed both in large (lane 3) and small (lane 4) intestine of control mice. Both the transgene and endogenous collagen XIII were clearly expressed in large (lane 1) and small (lane 2) intestine of Col13a1del mice at comparable levels. Lane 5, negative control for RT reaction; lane 6, negative control for PCR reaction. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control.

the production of Th1 cytokines, including IFN-γ and interleukin-6, by CD4+ T cells responding to peptides presented by MHC class II–expressing cells. Finally, enhanced expression of the metallothionein encoded by Mt2 would provide an antioxidant that could provide some protection from a vigorous inflammatory response and activation of Casp12 could inhibit the mucosal responses to bacteria engendered by intracellular NOD proteins. Taken together, the results of the transcription profiling suggest that the small intestinal tissues of Col13a1del mice might be involved in a heightened response to intestinal bacteria, possibly both commensal and pathogenic. The only gene with altered expression encoding a BM protein was Lama3, a component of laminin 5. Expression of Itgα8 gene, encoding one of the matrix receptors, was also increased.

Discussion

The results of this study show that collagen XIII is a critical determinant of BM structure in the intestines. They also show that alterations in protein structure induced by one copy of a mutant transgene that affects trimerization result in activated expression of genes encoding the innate and acquired arms of the immune response in the intestinal tissue associated with the accelerated development of B-cell lineage lymphomas, and that the incidence of these tumors may be affected by the nature of the intestinal flora. An alternative possibility is that mutant collagen XIII is temporarily expressed in immune cells during their maturation or activation eventually leading to abnormal proliferation and lymphoma development. However, we do not favor the latter possibility because we were not able to detect expression of collagen XIII in the immune cells.

Mature B-cell lymphomas occur with varying frequencies in old mice of different strains (23). The B6D2F1 mice used to create the transgenic mouse line had a ∼2% incidence of lymphoma when housed under conventional conditions but an incidence of only 0.8% when raised in SPF conditions. In contrast, the incidences of lymphomas in the Col13a1del mice were 15.8% under conventional and 7.4% under SPF conditions, respectively. The decrease in the incidence and size of the lymphomas under SPF conditions, and lack of pathogens found in the conventionally reared mice, suggests that environmental influences contributed to the initiation and/or progression of the lymphomagenic process.

Almost all the lymphomas appearing in the Col13a1del mice originated from GC or after GC B cells, comprising follicular, DLBCL, and plasma cell neoplasms. Most were associated with significant populations of activated, nonclonal T cells and macrophages. Importantly, histologic and molecular studies of human B-cell lineage tumors have revealed subsets with similar features. For example, a subset of human follicular B-cell lymphomas is characterized by a large number of genes encoding T-cell markers and genes expressed at high levels in activated macrophages (9). In addition, molecular profiling of DLBCL identified a subset, designated “host response,” defined by inflammatory/immune cell infiltrates (8). The host response subset of DLBCL bears many similarities to the category of T-cell–rich/histiocyte-rich lymphomas as defined in the WHO classification of hematologic malignancies (24). Mouse B-cell lineage tumors were previously recognized as including a histiocyte-associated subset of DLBCL (25), whereas more recent studies have characterized a subset of plasmablastic/anaplastic plasmacytomas with an inflammatory signature (26).

The mechanisms responsible for the association of B-cell tumors and inflammatory populations are not known, although a series of studies has shown that changes in the intestinal flora and inflammatory stimuli can profoundly influence the development of different types of B-cell lineage neoplasms in mice. First, conventionally housed BALB/c mice that are highly sensitive to plasmacytoma induction by i.p. injection of pristane were completely tumor resistant when raised under SPF conditions (27). Furthermore, there was a dramatic inhibition of plasmacytoma development when pristane-injected mice were treated with the nonsteroidal anti-inflammatory drug indomethacin (28). Second, mice deficient in granulocyte-macrophage colony-stimulating factor and IFN-γ, which exhibit inflammatory lesions in many tissues, had a high incidence of B-cell neoplasms that was completely suppressed when they were treated with antibiotics (29). Finally, studies of NFS.V+ mice, which develop a high incidence of B-cell lymphomas when raised under SPF conditions (29), showed that the latency of the disease was prolonged and tumor incidence was reduced when the mice were raised under SPF conditions. The reduced incidence of lymphomas in the present study of SPF Col13a1del mice provides a striking parallel to these earlier findings.

8 J.W. Hartley et al., unpublished data.
The contributions, if any, of the activated CD4+ T-cell and histiocyte (macrophage) populations to the processes of lymphoma initiation, progression, or maintenance are not known. Previous studies have shown that CD4+ T cells specific to antigens expressed on the surface of B cells can drive lymphoma development in mice (30), whereas in gastric mucosa-associated lymphoid tissue (MALT) lymphomas of humans, CD4+ T cells that engage *H. pylori* antigens displayed by antigen-presenting cells stimulate chronic proliferation of autoreactive B cells and their eventual transformation (31). The T-cell dependence of this disease is evidenced by complete regression of the lymphoma during the early stages of the disease following successful antibiotic treatment of *H. pylori*. Of particular relevance to the current study is the observation that immunoproliferative small intestinal disease, recognized as one of the prototypes of MALT lymphoma but with features of atypical plasma cells, is driven by T-cell recognition of *Campylobacter jejuni* antigenic peptides (32) and can respond to broad-spectrum antibiotics.

Unexpectedly, the expression of collagen XIII protein was below the limits of detection in lymphomas from the transgenic mice, although transcripts for both the endogenous and mutant genes were present. In addition, the spleen was found to be the only lymphoid tissue to express collagen XIII, where it was found in the small blood vessels, possibly in association with pericytes. As expected from previous studies (16), collagen XIII was expressed at high levels in both the villi and the crypts of the basal aspect of the epithelium in the intestines. Studies of transcripts suggest that the endogenous and mutant proteins were expressed at comparable levels.

Ultrastructural studies of the small intestine showed that the attachment of epithelial cells to the BM was abnormal, with the BM in older mice lacking the normal lamina lucida and lamina densa layers. Previous studies had shown that collagen XIII is involved in the attachment of muscle fibers to the extracellular matrix (33). In addition, these studies of the gene-targeted mouse line Col13a1<sup>N/N</sup>, which expresses an N-truncated collagen XIII, revealed a disorganized fuzzy structure to the BMs of the skeletal muscle. The BM abnormalities seen in the Col13a1<sup>del</sup> transgenic mice were more severe, however. This difference may be due to the fact that the endogenous and mutant proteins are expressed at comparable levels in the transgenics, whereas only the mutant protein is expressed by the knockin mice. Coexpression could result in the formation of greatly perturbed supermolecular complexes of collagen XIII and its extracellular partners, which may include fibronectin, heparin, and two BM components, nidogen-2 and perlecan (34). The marked alteration in the structure of the BM underlying intestinal epithelial cells could undermine their normal barrier function. The persistent activation of antigen-responsive T cells and B cells could be a component of the initiating phase of B lymphoma development.

An alternative or perhaps supplementary explanation for the role played by mutant collagen XIII in lymphomagenesis could relate to the BMs of the skeletal muscle. The BM expressed by the knockin mice. Coexpression could result in the formation of greatly perturbed supermolecular complexes of collagen XIII and its extracellular partners, which may include fibronectin, heparin, and two BM components, nidogen-2 and perlecan (34). The marked alteration in the structure of the BM underlying intestinal epithelial cells could undermine their normal barrier function. The persistent activation of antigen-responsive T cells and B cells could be a component of the initiating phase of B lymphoma development.

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Collagen XIII expression in the Peyer’s patches, small intestine, and spleen. A, collagen XIII is not expressed by the lymphocytes (arrowheads) within the Peyer’s patches in either the wt (A2) or Col13a1<sup>del</sup> (A4) mouse intestine as identified with the B-cell marker CD45R (A1 and A2) and type IV collagen staining (A5 and A6). Only nonspecific staining (marked with an asterisk) is detected. B, strong collagen XIII staining is localized underneath the epithelial cell layer in the villus area of the small intestine both in control (B1) and in Col13a1<sup>del</sup> mice (B2) and also adjacent to the Peyer’s patch (A3 and A4). In addition to the epithelial BM, collagen IV is localized in lamina propria around the blood vessels (B3 and B4). C, in both wt and Col13a1<sup>del</sup> mice, collagen XIII (C1 and C2) is colocalized with PECAM (C3 and C4) in the small vessels of the spleen, whereas the large vessels (marked with an asterisk) are negative for collagen XIII. D, collagen XIII (D1), PECAM (D2), and desmin (D3) staining in serial sections of the wt spleen suggests a pericyte localization of collagen XIII. Scale bars, 100 μm (A and B, as indicated in A1), 50 μm (C, as indicated in C1), and 20 μm (D, as indicated in D1).
the demonstration that collagen XIII is one of several collagen family ligands with a high affinity for the inhibitory leukocyte-associated Ig-like receptor LAIR-1 (12). Cross-linking of the receptor in vitro induces a potent inhibitory signal that affects the function of natural killer (NK) cells, T cells, monocytes, and dendritic cell precursors (35–38) and serves as a negative regulator of BCR-mediated signaling (36). The heterotrimer formed by mutant and wt proteins in the Col13a1 Ddel mice may result in disruption of the normal triple helical conformation that characterizes most of the collagen XIII ectodomain and reduce its affinity for LAIR-1. It is well established that the loss of other inhibitory immune receptors or down-regulation of their ligands can result in chronic inflammation or autoimmune disease (39–41). Interestingly, collagen XIII is targeted by autoantibodies in patients with the congestive form of Graves’ ophthalmopathy, an immune-mediated inflammation of the periorbital connective tissues in the absence of eye muscle dysfunction (42). As suggested for other autoimmune conditions in which collagens have been identified as autoantigens, antibodies directed to the collagen ligand may inhibit interactions with LAIR-1 as a contributory factor to the disease (12).

These considerations reinforce the suggestions made above that the altered structure of the epithelial BM of the intestine caused by structurally abnormal collagen XIII heterotrimers in Col13a1 Ddel mice is associated with enhanced exposure to bacterial products. These engage pattern recognition receptors on T cells, NK cells, and macrophages, inducing the expression of inflammatory cytokines and chemokines. The reduced thresholds for cell activation occasioned by reduced signaling through LAIR-1 could contribute to this overactive immune response. The exposure of activated B cells to mutagenic agents would set the stage for B-cell transformation.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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A Mutant Collagen XIII Alters Intestinal Expression of Immune Response Genes and Predisposes Transgenic Mice to Develop B-Cell Lymphomas

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