

Antibody-mediated blockade of IL-15 reverses the autoimmune intestinal damage in transgenic mice that overexpress IL-15 in enterocytes

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Celiac disease (CD) is an autoimmune inflammatory disease with a relatively high prevalence especially in the western hemisphere. A strong genetic component is involved in the pathogenesis of CD with virtually all individuals that develop the disease carrying HLA-DQ alleles that encode specific HLA-DQ2 or HLA-DQ8 heterodimers. Consumption of cereals rich in gluten triggers a chronic intestinal inflammation in genetically susceptible individuals leading to the development of CD. Emerging evidence has implicated a central role for IL-15 in the orchestration and perpetuation of inflammation and tissue destruction in CD. Therefore, IL-15 represents an attractive target for development of new therapies for CD. Transgenic mice that express human IL-15 specifically in enterocytes (T3^b-hIL-15 Tg mice) develop villous atrophy and severe duodeno-jejunal inflammation with massive accumulation of NK-like CD8⁺ lymphocytes in the affected mucosa. We used these mice to demonstrate that blockade of IL-15 signaling with an antibody (TM-β1) that binds to murine IL-2/IL-15Rβ (CD122) leads to a reversal of the autoimmune intestinal damage. The present study, along with work of others, provides the rationale to explore IL-15 blockade as a test of the hypothesis that uncontrolled expression of IL-15 is critical in the pathogenesis and maintenance of refractory CD.

autoimmunity | celiac disease | immunotherapy | interleukine 15 receptor

Celiac disease (CD) is an immune-mediated enteropathy triggered by the consumption of gluten-containing cereals by genetically susceptible individuals carrying the HLA class II DQ alleles that encode DQ2 or DQ8 molecules (1, 2). Gluten peptides resulting from partial digestion of dietary cereal-derived prolamines are presented by the antigen-presenting cells in the lamina propria bearing HLA-DQ2 or HLA-DQ8 class II molecules to cognate gluten-specific CD4⁺ T lymphocytes, leading to activation and proliferation of these cells with copious IFN-γ secretion. Nonetheless, these gluten-specific CD4⁺ T cells do not appear to be directly responsible for the extensive intestinal tissue damage seen in CD (2). However, extensive intraepithelial infiltration of CD8⁺ T lymphocytes is a hallmark feature in all forms of CD-associated lesions stratified according to the Marsh classification of histologic damage (3). However, unlike the exquisite gluten specificity of proliferating lamina propria CD4⁺ T cells, infiltrating intraepithelial CD8⁺ T cells are largely devoid of any gluten specificity. It is these intraepithelial CD8⁺ T lymphocytes that show massive infiltration into the affected intestinal mucosa that cause extensive destruction of enterocytes and underlying tissues primarily via TCR-independent mechanisms that use NKG2D and other coactivating NK-cell receptors (4, 5).

Emerging evidence has implicated a pivotal role for the proinflammatory cytokine IL-15 presumably made locally by lamina propria dendritic cells, macrophages, monocytes, and intestinal epithelial cells in orchestrating immune-mediated tis-

sue destruction in CD (4–9). IL-15 is indispensable for the generation, maintenance, and homeostasis of intraepithelial lymphocytes (IEL), and consequently in IL-15 or IL-15 receptor alpha gene-deleted mice, IEL especially cells bearing CD8αα either from the TCR-α/β or γ/δ subpopulations are virtually absent in the mucosal tissues (10, 11). IL-15 also induces proliferation of CD8⁺ T lymphocytes in addition to enhancing their effector functions, including those associated with cytotoxicity and cytokine secretion. IL-15 promotes perpetuation of chronic inflammation by preventing activation-induced cell death of activated CD8⁺ T cells (12, 13). Also, in CD patients, IL-15 effectively reprograms intraepithelial CD8⁺ CTL to lymphokine-activated killer cells or natural killer-like cells capable of massive oligoclonal expansion and target cell cytotoxicity in a TCR-independent fashion, in part by coordinate induction of the NKG2D signaling pathway and other cytotoxic NK lineage receptors on these cells (5). In completing this perpetual cycle of tissue damage, IL-15 also induces surface expression of the cognate ligand of NKG2D receptor the MHC class I-related chain A (MICA) on enterocytes; thereby, establishing a sustained effector-target engagement with detrimental consequences (4). In addition to these positive modulatory effects on the activation pathways leading to persistent inflammation, the recent work of Benahmed et al. (9) indicates that IL-15 also blocks the negative regulatory pathways that are critical in maintaining immune homeostasis in the intestinal mucosa of CD patients. In the intestinal microenvironment where host immune elements are in constant contact with a plethora of commensal microorganisms, TGF-β mediates the anti-inflammatory tone of the gut mucosal immune system. IL-15 inhibits Smad-dependent signaling of TGF-β; thereby, further aggravating ongoing inflammation by disabling the operational anti-inflammatory checkpoints in the intestinal mucosa of CD patients (9). Collectively, these findings implicate a central role for IL-15 in the pathogenesis of CD, and make a compelling rationale that selective targeting of IL-15 represents a potentially valuable approach in CD treatment, where currently the only available treatment for this autoimmune disease is a life-long gluten-free diet or for a subset of CD patients who develop resistance to gluten-free diet and progress to type II refractory CD with no effective treatment.

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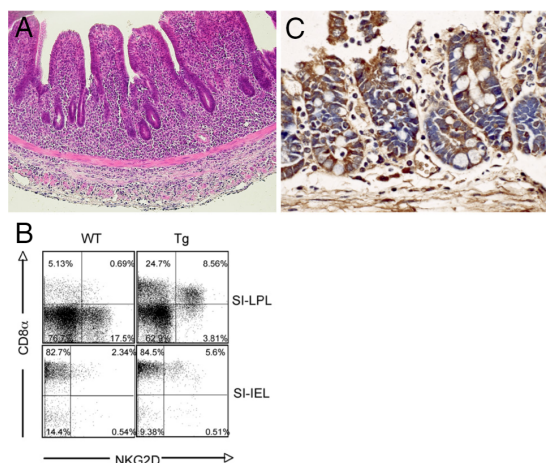


Fig. 1. T3^b-hIL-15 Tg mice manifest extensive intestinal villous atrophy and influx of lymphocytes. (A) H&E-stained section of proximal small intestine of a T3^b-hIL-15 Tg mouse showing villous atrophy and massive intraepithelial lymphocytic infiltration. Magnification, 10 $\times$. (B) Flow cytometry analysis of LPL and IEL isolated from the small intestine of T3^b-hIL-15 Tg or WT mice reveals the presence of large numbers of pathogenic CD8⁺ T cells with NKG2D expression in T3^b-hIL-15 Tg mice. Gated cells represent lymphocytes. (C) Detection of Rae-1 expression in the small intestines of T3^b-hIL-15 Tg mouse by immunohistochemistry where Rae-1 expression is indicated by the presence of reddish brown precipitants. In these immunohistochemistry experiments, an isotype control was included, but the background staining was minimal with this antibody. Each of the three untreated T3^b-hIL-15 Tg mice included in the study manifested the changes shown above.

Previously, we reported the generation of IL-15 transgenic mice that express human IL-15 in intestinal epithelial cells by using the enterocyte-specific T3^b promoter to drive the transgene, and 100% of these mice develop spontaneous inflammation in the duodeno-jejunal region (14). Although these mice do not manifest gluten sensitivity the anatomical location of the inflammatory lesions, the extensive villous atrophy with some degree of crypt hyperplasia and the massive accumulation of NK like CD8⁺ T cells in the affected mucosa of these transgenic animals closely recapitulate some of the pathological features that are observed in human CD including refractory CD. Therefore, we used these animals to test the efficacy of IL-15 blockade as a potential therapeutic modality in reversing the intestinal inflammatory pathology with a monoclonal antibody (TM- β 1) that binds to IL-2/IL-15R β (CD122) and blocks IL-15 activity (15). We show that the antibody-mediated blockade of IL-15 signaling results in effective resolution of the inflammatory lesions in the treated animals; thus, providing support for a clinical trial exploring IL-15 blockade to test the hypothesis that a similar uncontrolled expression of IL-15 is critical in the development and maintenance of refractory CD with a propensity for the development of enteropathy associated CD8 T cell lymphoma.

Results

T3^b-hIL-15 Tg Mice Display Intestinal Villous Atrophy and Extensive Infiltration of CD8⁺ T Lymphocytes, of Which Many Bear NKG2D Receptors. In T3^b-hIL-15 Tg mice, we reconfirmed our earlier observation (14) that inflammatory pathology both macroscopically and microscopically was confined to the proximal small intestine despite the demonstrable expression of IL-15 throughout the small intestines and the colon. Fig. 1A shows microscopic lesions with extensive blunting of villi in the duodeno-jejunal region. Also, when lymphocyte populations isolated from lamina

propria and intraepithelial compartments of the affected areas were assessed phenotypically by flow cytometry, there was a substantial influx of CD8⁺ T cells into these compartments with a majority of these infiltrating cells expressing NKG2D in our T3^b-hIL-15 Tg mice, as shown in Fig. 1B. Although Fig. 1B shows data from one representative T3^b-hIL-15 Tg mouse, as depicted in Fig. S1, the influx of lymphocytes into the intraepithelial compartment was consistently more pronounced in these transgenic mice than their nontransgenic litter-mates, and the increase in IEL in T3^b-hIL-15 Tg mice was statistically significant ($P < 0.05$). When we examined the extent of Rae 1 (the mouse homolog of MICA) expression in the proximal small intestinal enterocytes by immunohistochemistry, widespread expression of this NKG2D ligand was evident (Fig. 1C). Thus, our T3^b-hIL-15 Tg mice recapitulate some of the disease defining pathological features of CD, including the clear anatomic demarcation of pathological lesions to the proximal small intestines, severe blunting and atrophy of intestinal villi in the affected areas, and extensive infiltration of activated CD8⁺ T lymphocytes, of which many bear NKG2D receptors in addition to the overexpression of IL-15 locally in the affected mucosa. Also, consistent with the hyperexpression of NKG2D ligands MICA/B, ULBP, and HLA-E on intestinal enterocytes that provide a causal link to CD8⁺ T cell mediated tissue damage in CD, the abundance of Rae1 in affected intestinal mucosa of T3^b-hIL-15 Tg mice collectively suggest that these mice could serve as a reasonable model for assessing the efficacy of agents that block IL-15 action on these intestinal pathologic disorders.

Effective Blockade of IL-15 Signaling by TM- β 1 Antibody That Binds to Murine CD122.

IL-15 is made by various cell types, including monocytes and dendritic cells, and has potent effects on both innate and adaptive immune systems (12, 16). IL-15 mediated effects are transduced via a tripartite receptor complex consisting of IL-15R α , which is the high affinity private receptor of IL-15 along with two signaling components CD122 (IL-2/IL-15R β) and CD132 (γ_c). In most situations, the three-receptor subunits are not coexpressed by the same cell. Rather IL-15R α , the private receptor of IL-15 is expressed by activated antigen-presenting dendritic cells, monocytes, and by some nonhematopoietic cells of the lung and gastrointestinal tract (17). These IL-15R α bearing cells present IL-15 *in trans* to CD122 and CD132 expressing NK and CD8⁺ T cells as part of an immunological synapse.

To achieve durable *in vivo* blockade of IL-15 activity in T3^b-hIL-15 Tg mice, a rat monoclonal antibody TM- β 1 that reacts with the murine CD122 was evaluated (15). The TM- β 1 antibody blocks the interaction of *trans*-presented IL-15 by IL-15R α with the CD122/CD132 signaling receptor complex on responsive NK, and CD8⁺ T cell subsets. As shown in Fig. S2, the TM- β 1 antibody was very effective in inhibiting IL-15-induced proliferation of murine splenocytes. Although both IL-2 and IL-15 use CD122 for their signal transduction events, it has been demonstrated previously (18) that IL-2 induced proliferation of mouse splenocytes that express the high-affinity IL-2R α (CD25) is not affected by TM- β 1 antibody. One possibility for this discordant effect is that an antibody directed toward CD122 can effectively block IL-15 signaling by cells that only express the CD122/CD132 complex, but not IL-2 when cells express the trimeric receptor complex formed by most activated cells in the presence of IL-2, which induces CD25, the private receptor of IL-2 on these cells.

Having confirmed that the TM- β 1 antibody is effective in the efficient blockade of IL-15 activity, we administered the TM- β 1 antibody as a bolus of 200 μ g i.p. twice a week, 3–4 days apart for a period of 8 weeks to a group of three T3^b-hIL-15 Tg mice that were 6 months of age at the beginning of the experiment. Two other control groups (each having three animals) were

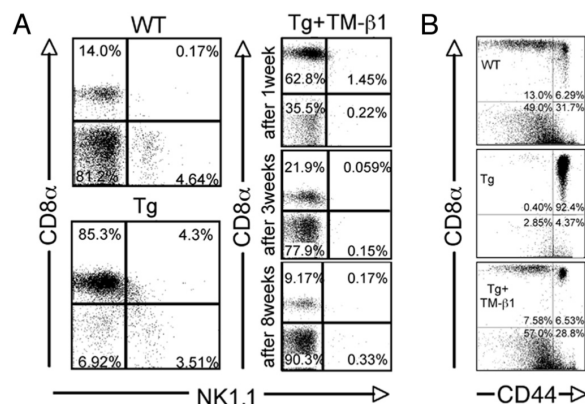


Fig. 2. TM-β1 antibody-mediated blockade of IL-15 activity reversed the abnormal peripheral blood lymphocytosis seen in T3^b-hIL-15 Tg mice in a dose- and a time-dependent manner. PBMC were isolated from WT, T3^b-hIL-15 Tg mice while undergoing antibody treatment longitudinally. The modulation of CD8⁺ T cell subsets with NK and activation markers were assessed by flow cytometry. (A) CD8α and NK1.1, (B) CD8α and CD44 expression. Gated cells represent lymphocytes. Data shown were at the termination of the experiment (6 months plus 8 weeks of therapy) from one representative mouse from each group of three animals and the other mice displayed similar profiles.

included to provide base line cellular, gross pathological, and histopathological data for comparison with the antibody treated group, and comprised of age matched nontransgenic litter-mates and T3^b-hIL-15 Tg mice with no antibody therapy.

TM-β1 Antibody Administration Reverses Abnormal Peripheral Blood Cell Profiles. In T3^b-hIL-15 Tg mice, the expression of human IL-15 mRNA is exclusively limited to the intestines of these animals (14). However, the sera of these animals contain measurable levels of circulating human IL-15 in the range of 50–100 pg/mL as measured by ELISA, which probably reflects the seepage of enterocyte-synthesized IL-15 into the circulation of these animals. When a complete blood count (CBC) was performed on peripheral blood samples of these T3^b-hIL-15 Tg mice (Table S1), there was consistent evidence of leukocytosis with total white blood cell counts being ≈3- to 5-fold higher than those seen in the nontransgenic litter-mates. We attribute the expansion of peripheral blood leukocytes to increased levels of circulating IL-15 in these animals in a manner similar to what one would see in mice with repeated exogenous IL-15 administrations that leads to the expansion of peripheral blood lymphocytes. With repeated TM-β1 administration, we noted a decline in the total white blood cell counts in blood samples collected longitudinally from animals during the course of antibody treatment (Table S1); thus, providing a way to monitor the effectiveness of TM-β1 antibody treatment. Having confirmed a measurable impact on total white blood cell counts of peripheral blood with *in vivo* infusions of TM-β1, we decided to examine the dynamics of peripheral blood cell profiles in detail primarily focusing on the two cell populations that are most sensitive to IL-15, namely, CD8⁺ T lymphocytes and NK cells, by flow cytometry with continued TM-β1 administration. As shown in quadrant percentages in Fig. 2, there was considerable expansion of CD8⁺ T as well as CD8⁺ NK1.1⁺ T cell subsets in T3^b-hIL-15 Tg mice (Fig. 2A). It should be noted that the increase in the actual number of cells in each of these subsets, including the NK cell subset, is even more dramatic when one takes into consideration that the absolute numbers of CD8⁺ and NK cells in the blood of these T3^b-hIL-15 Tg mice are significantly greater than their nontransgenic litter mates as shown in Fig. S3. More

importantly, the blockade of IL-15 had an immediate and striking impact on the cells bearing the NK1.1 phenotypic marker resulting in the disappearance of these cells from the blood within the very first week after initiation of TM-β1 treatment. However, the impact on the massively expanded CD8⁺ T cells was more gradual, although equally profound, requiring a longer period of therapy for their elimination from the peripheral circulation. Intriguingly, even after 8 weeks of TM-β1 infusions, the CD8⁺ T cell subset was reduced only to a level that was only slightly lower than those seen in the nontransgenic litter-mates (compare 9.17 versus 14%). This observation is consistent with previous studies where administration of TM-β1 antibody, although it eliminated NK cells, had no meaningful impact on the number of CD8⁺ T lymphocytes in normal WT mice (19, 20). This limited effect of TM-β1 antibody on total circulating CD8⁺ T cell numbers is also in accord with the observations in IL-15 knockout mice where there is only a modest reduction in the total CD8⁺ T cell population. To evaluate the possibility that within the CD8⁺ T cell population perhaps only a minor subset is exquisitely IL-15 dependent, we examined the modulation of CD44^{high} subpopulation of CD8⁺ T lymphocytes with TM-β1 treatment. As shown in Fig. 2B, in T3^b-hIL-15 Tg mice, the expansion of peripheral blood lymphocytes was almost exclusively limited to the CD8⁺CD44^{high} subset, the same population that was ablated by TM-β1 therapy. A parallel observation has been made in IL-15 knockout mice where CD8⁺CD44^{high} cells were absent, but CD8⁺CD44^{low} populations were retained (21). Nonetheless, there appears to be a potential difference in naturally occurring CD8⁺CD44^{high} cells versus IL-15-induced CD8⁺CD44^{high} cells in response to TM-β1 treatment, because in treated animals, even after 8 weeks of antibody infusions, a treatment refractory population of CD8⁺CD44^{high} lymphocytes persisted. Although the mechanisms that account for these differences if any in IL-15 dependency in the two cell populations remain unknown, it is important to note that the blockade of IL-15 signaling with TM-β1 eliminated only the fraction of CD8⁺ T cells that expanded under IL-15 influence, and after antibody treatment, animals were still able to maintain normal numbers of CD8⁺ T lymphocytes. Of note, NK cells were also eliminated with IL-15 blockade. From the above observation, a key clinical implication of CD122 directed blockade of IL-15 is that only T lymphocytes that are vitally dependent on IL-15 are eliminated by this approach, and the treatment is not likely to result in the profound immunosuppression that would occur with total elimination of CD8⁺ T lymphocytes.

Macroscopic and Microscopic Pathological Changes in the Intestines of T3^b-hIL-15 Tg Mice Are Reversed by TM-β1 Therapy. After 8 weeks of TM-β1 infusions, animals in all three groups were killed for macroscopic and microscopic evaluation. At the time of necropsy, all animals were 8 months of age. As previously reported (14), in T3^b-hIL-15 Tg mice, the macroscopic evidence of intestinal inflammation usually become apparent at 3 months of age. As shown in Fig. 3A, all T3^b-hIL-15 Tg mice that were in the untreated control group showed extensive macroscopic evidence of inflammation that was strikingly limited to the duodenojejunal region. There was extensive swelling and distention of the affected region with serosal hemorrhage and prominent distended blood vessels on the serosal surface. The serosal surface itself was dull and somewhat granular in appearance compared with the uniformly smooth and shiny serosal surface seen in the nontransgenic litter-mates. However, no ulcerations or granulomatous lesions were noted in the intestines. Also, as shown in Fig. 3B, the mesenteric lymph nodes and spleens were greatly enlarged in these T3^b-hIL-15 Tg mice and displayed expanded periarteriolar lymphoid sheaths (PALS) consistent with extensive lymphoid proliferation, but no other architectural changes

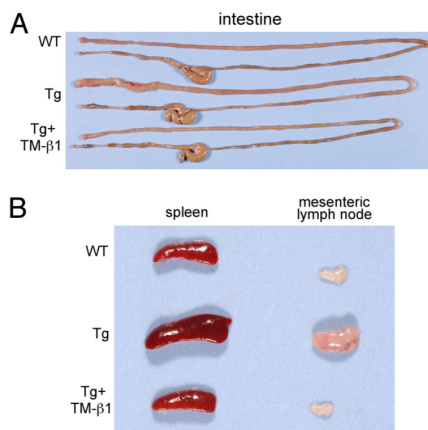


Fig. 3. TM-β1 antibody-mediated blockade of IL-15 signaling reverses the macroscopic inflammatory pathologic lesions in T3^b-hIL-15 Tg mice. (A) Small intestine, and (B) spleen and mesenteric lymph node. Data shown are from one representative mouse from each group of three animals and the other mice displayed similar profiles.

were evident. However, after 16 infusions of TM-β1 antibody over a period of 8 weeks, all observed small intestinal macroscopic changes were reversed in all of the treated animals shown in Fig. 3A. Also, TM-β1 treatment was associated with the involution of greatly enlarged spleen and mesenteric lymph nodes to their normal size in the treated animals (Fig. 3B).

Having confirmed the reversal of gross pathologic changes of the intestines with TM-β1 treatment in T3^b-hIL-15 Tg mice, tissue sections were made from representative areas of the intestines for histologic evaluation as well. The microscopic abnormalities in these T3^b-hIL-15 Tg mice were strictly confined to the duodeno-jejunal region and included massive lymphocytic infiltration into the lamina propria even extending to some areas below the smooth muscle layer. Also there was significant IEL infiltration as well, along with vacuolar degeneration of enterocytes, especially at the tips of villi, which were extensively blunted resulting in the markedly reduced ratio of villus to crypt height, as shown in Fig. 4. However, in all T3^b-hIL-15 Tg mice that were treated with TM-β1 antibody, there was a reversal of the entire spectrum of histologic changes, including the reestablishment of normal villus heights that resembled those of nontransgenic litter-mates, as shown in Fig. 4.

TM-β1 Antibody Treatment Reestablishes Normal Lymphocytic Cell Profiles in the Small Intestinal Mucosa. It was remarkable that the TM-β1 antibody that is not directed to IL-15 per se but blocks its signaling reversed both gross pathological as well as histologic changes within a span of 8 weeks without leaving any of the florid persistent proximal small intestinal inflammation that was

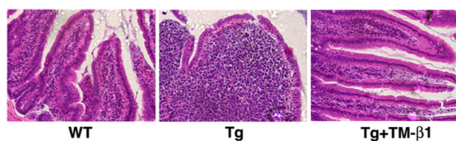


Fig. 4. TM-β1 antibody mediated blockade of IL-15 signaling reverses the microscopic inflammatory pathologic lesions in T3^b-hIL-15 Tg mice. H&E-stained sections of small intestine of WT, T3^b-hIL-15 Tg, and TM-β1-treated T3^b-hIL-15 Tg mice with original magnification at 20×. Data shown are from one representative mouse from each group of three animals at the end of the therapeutic study period and the other mice displayed similar profiles.

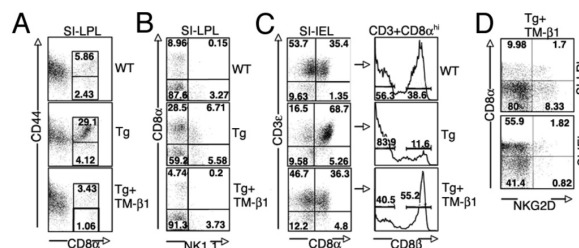


Fig. 5. TM-β1-mediated blockade of IL-15 signaling virtually eliminated pathogenic and abnormal CD8⁺ T cell populations from small intestinal IEL and LPL of T3^b-hIL-15 Tg mice. LPL and IEL were isolated from the small intestines of WT, T3^b-hIL-15 Tg, and TM-β1-treated Tg mice, and subjected to surface phenotypic analysis by flow cytometry. (A) Cell surface expression of CD44 and CD8α on LPL. (B) Cell surface expression of CD8α and NK1.1 on LPL. (C) Cell surface expression of CD3ε and CD8α on IEL were analyzed (Left), CD8α^{high} CD3ε⁺ cells were gated and analyzed for CD8β coexpression (Right). (D) Cell surface expression of CD8α and NKG2D on LPL and IEL from small intestines of TM-β1 treated T3^b-hIL-15 Tg mice were analyzed by flow cytometry. Data shown are from one representative mouse from each group of three animals and the other mice displayed similar profiles.

present in these T3^b-hIL-15 Tg mice. This dramatic resolution of the inflammation may likely have been facilitated at least in part by the high degree of tissue remodeling and turn-over that is inherent to intestinal mucosa. Thus, it is the influx of lymphocytes in response to locally synthesized IL-15 that appears to cause extensive tissue damage. Therefore, we decided to examine in more detail how TM-β1 treatment had impacted different subsets of T lymphocytes that accumulated in the proximal small intestines of T3^b-hIL-15 Tg mice by flow cytometry after collecting IEL and LPL fractions from euthanized animals after 8 weeks of TM-β1 antibody treatment. In T3^b-hIL-15 Tg mice, CD8⁺ T lymphocytes were increased in both the lamina propria and intraepithelial compartments, and a majority of these cells, particularly the lamina propria CD8⁺ T cells, were of memory phenotype with high expression levels of CD44 (Fig. 5A). Also, as seen in CD, there was a meaningful increase in CD8⁺ T cells that coexpressed NK cell markers (CD8⁺NK1.1⁺), a subset of T cells that was virtually absent in the lamina propria of nontransgenic animals (Fig. 5B). However, in animals treated with TM-β1, these expanded CD8⁺CD44^{high} as well as CD8⁺NK1.1⁺ cell populations were virtually eliminated from the lamina propria. Nonetheless, again as seen in peripheral blood, there appeared to be a small subpopulation of CD8⁺CD44^{high} lymphocytes (~3%) in the lamina propria of TM-β1 treated mice just as in nontransgenic animals that was refractory to TM-β1 treatment. However, the factors that account for their refractory nature to TM-β1 treatment remain to be fully defined. In previous studies (22), we noted that long term memory CD8⁺ T cells coexpress IL-15Rα along with CD122 and CD132 subunits. Such cells that express the heterotrimeric receptors *in cis* are likely to be resistant to antibodies such as TM-β1 directed against CD122 in blocking IL-15 action. In the intraepithelial compartment of T3^b-hIL-15 Tg mice, the expanded cell population was primarily of TCR positive CD8⁺ cells with α/α homodimers, and these cells were markedly reduced by TM-β1 treatment, as shown in Fig. 5C. The induction of killer cell receptor NKG2D in intestinal CD8⁺ T lymphocytes by locally synthesized IL-15 is pivotal in their tissue destructive activity in CD (4, 5). As depicted in Fig. 1B, in T3^b-hIL-15 Tg mice, a greater proportion of LPL and IEL CD8⁺ T cells displayed NKG2D receptors on their cell surface. The expression of NKG2D and other NK cell-associated receptors on CD8⁺ T cells confers TCR-independent NK-like killing activity, cytokine secretion, and proliferative activity in response to stress signals

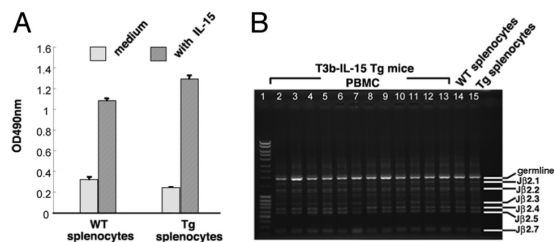


Fig. 6. Proliferation of lymphocytes from T3^b-hIL-15 Tg mice depended on IL-15, and showed polyclonality. (A) Splenocytes were isolated from WT and T3^b-hIL-15 Tg mice at the termination of the experiment (6 months plus 8 weeks of treatment), and these splenocytes were cultured in vitro with 50 ng/mL of human IL-15 for 72 h. Cell proliferation was analyzed by a colorimetric assay. (B) DNA PCR gel shows normal Jβ2 chain gene rearrangement on lymphocytes of T3^b-hIL-15 Tg mice. Lane 1, DNA marker; lanes 2–13, PBMC isolated from 12 different T3^b-hIL-15 Tg mice; lane 14, splenocytes from WT mouse; lane 15, splenocytes from T3^b-hIL-15 Tg mouse.

from epithelial enterocytes to such cells, and these pathogenic T cells with NKG2D expression cause extensive tissue damage in a number of autoimmune diseases, including rheumatoid arthritis, type 1 diabetes, and CD (2). Importantly, with the TM-β1 treatment, these pathogenic CD8⁺ T cells in both intraepithelial and lamina propria compartments were largely eliminated (compare Fig. 1B with Fig. 5D). Thus, from the phenotypic analyses of both peripheral blood and small intestinal mucosal lymphocytes, the TM-β1 treatment resulted in the reestablishment of normal lymphocytic composition in the intestinal mucosa and the peripheral blood without causing any abnormal lymphocytopenia and attendant immunodeficiency.

Because of the possibility that the infiltrating CD8⁺ T cells no longer require IL-15 for their survival or proliferation, we assessed whether splenocytes from our T3^b-hIL-15 Tg mice were capable of spontaneous proliferation *ex vivo* when cultured in medium without any added T cell growth factor cytokines. As shown in Fig. 6A, no meaningful proliferative differences were noted between splenocytes from nontransgenic litter-mates and T3^b-hIL-15 Tg mice. Also, when the culture medium was supplemented with IL-15, splenocytes from both groups displayed robust proliferation in response to IL-15 with a slightly higher level in T3^b-hIL-15 Tg mice that may likely result from having a higher surface density of CD122 on those cells (16). Fehniger et al. (23) demonstrated that transgenic mice with an MHC-Class I promoter driven modified murine IL-15 developed fatal lymphocytic leukemia between 3 to 12 months of age depending on the mouse strain (FVB versus C57BL/6). Therefore, we determined whether the expanded cell pool in the 8-month-old T3^b-hIL-15 Tg mice represented an aberrant clonal outgrowth of CD8⁺ lymphocytes or a global expansion of the natural CD8⁺ T cell repertoire of these animals. When we evaluated the TCR gene rearrangement profiles of these cells by PCR amplification of the TCR Jβ2 region (23), the amplified fragment profile of CD8⁺ lymphocytes from T3^b-hIL-15 Tg mice was similar to those of nontransgenic litter-mates as depicted in Fig. 6B; thus, revealing that the expansion of CD8⁺ T cells in T3^b-hIL-15 Tg mice at 8 months of age was in fact global and is not restricted to a particular aberrant clone. The differences in the promoters and their tissue expression, the levels of IL-15 produced or the shorter duration of our study period may explain the differences observed in development of lymphocytic leukemia when compared with the MHC-Class I promoter driven murine IL-15 transgenic mice.

Discussion

The T3^b-hIL-15 Tg mice we generated in the year 2002 develop spontaneous autoimmune inflammation in the proximal small

intestine as we reported previously (14) as a model that might contribute to the understanding of the role of CD8⁺ T cells in Crohn's disease. However, there are greater parallels between the pathological changes in CD and the changes in these mice that include intestinal villous atrophy and extensive accumulation of NK like CD8⁺ T cells. Also, the disorders of IL-15 that underlie the mouse model parallel those that have been suggested to have a pathogenic role in CD especially refractory CD. In particular, since 2002, emerging evidence from several studies has implicated a central role for locally produced IL-15 in orchestrating continued autoimmune inflammation leading to extensive intestinal tissue damage in CD (4–9); thus, prompting us to reevaluate our T3^b-hIL-15 Tg mice as a potential model for IL-15 mediated intestinal immune pathology. In these mice, IEL infiltration is evident very early in life microscopically, but by approximately the third month after birth, there is florid lymphocytic infiltration with partial or complete villous atrophy.

In the pathophysiology of CD, the emerging consensus is that the extensive indiscriminate enterocyte destruction is caused by activated intraepithelial CD8⁺ T lymphocytes. The destruction caused by these T cells is independent of their TCR-specificity, but is very much dependent on the induction of NKG2D and other NK cell associated receptors on these cells, as well as the induction and expression of stress-induced NKG2D ligands such as MICA/B, ULBPs, and HLA-E on epithelial enterocytes caused by locally synthesized IL-15 (4–6). As was demonstrated here in Fig. 1B, in T3^b-hIL-15 Tg mice, the infiltrating lymphocytes expressed NKG2D and these NKG2D⁺CD8⁺ cells accounted for a significant proportion of IEL and lamina propria lymphocyte (LPL) pools seen in T3^b-hIL-15 Tg mice compared with WT litter-mate control mice (IEL, 2.3 to 5.6%; LPL, 0.7 to 8.6%). Also, the expression of Rae1, the mouse homolog of MICA in abundance, on the intestinal enterocytes of T3^b-hIL-15 Tg mice was evident (Fig. 1C), providing a crucial link to the smoldering inflammation seen in the proximal small intestine. Thus, the duodeno-jejunal anatomic location of the inflammatory lesions, the display of extensive intraepithelial lymphocytic infiltration and villous atrophy that are not seen in inflammatory bowel diseases other than CD, along with the demonstrable expression of NKG2D in the infiltrating CD8⁺ cells and the induction of Rae1 in epithelial enteric cells that are considered as pivotal elements in the perpetuation of inflammation with attendant tissue damage in CD highlight the pathologic similarities seen in T3^b-hIL-15 Tg mice with those of active CD. It is of interest that the intestinal overexpression of IL-15 alone in these mice is able to cause extensive villous atrophy and lymphocytic infiltration further strengthening the notion that IL-15 could be a principal causal element in the pathophysiology of CD.

With the use of 6-month-old T3^b-hIL-15 Tg mice that manifest established autoimmune intestinal pathology in all animals (orchestrated by locally synthesized IL-15), we have provided evidence that supports the view that an antibody directed against the CD122 that blocks IL-15 signaling, administered starting at 6 months can effectively reverse the established immunopathologic lesions in the proximal small intestines of these animals, without causing any profound lymphocytopenia or immunodeficiency. This approach ablates the offending “pathogenic” CD8⁺ T lymphocytes at the apex of the pathological cascade rather than affecting downstream effector-mediators. A monoclonal antibody, MiK-(β)1 directed against the human CD122 that blocks IL-15 activity is already in Phase I trials for evaluation in the treatment of T cell large granular lymphocyte leukemia (24). The extensive evidence published by others that support a pivotal role for disordered IL-15 expression in the pathogenesis of refractory CD, together with the findings from the present study demonstrating that administration of an antibody directed to CD122 can reverse established IL-15 mediated intestinal damage, provide a rationale to explore whether Hu-MiK-Beta-1

[humanized version of MiK-(β 1)] is of value in the treatment of patients with refractory CD, including those developing enteropathy associated CD8 T cell leukemia (EATL). Hu-Mik-Beta-1 has been produced under current good manufacturing practices, and has been shown to be well tolerated without hematological or chemical toxicities, evidence of immunogenicity or induction of infectious or autoimmune complications in two toxicology studies in cynomolgus monkeys. Also, in two Phase I trials involving patients with T cell large granular lymphocytic leukemia and those with HAM/TSP, no generalized immunosuppression or autoimmunity has been observed in the 14 patients studied to date. Such a trial in patients with CD would provide an *in vivo* test of the prevailing hypothesis that uncontrolled expression of IL-15 is not merely a correlate of the disease, but is critical in the pathogenesis of refractory CD and EATL. Also, such therapy might prevent the transition of patients from a nonmalignant clonal CD8⁺ proliferation that characterizes the refractory type II CD (RCDII) to EATL, a disorder for which no effective treatment exists.

Materials and Methods

Mice. The generation of T3^b-human IL-15 Tg mice has been reported previously (14). All of the animal experiment protocols were approved by the Tokyo Metropolitan Institute of Medical Science animal care and use committee.

Reagents. Purified anti-mouse CD122 Ab (clone TM- β 1) was either purchased from Serotec or obtained as a gift from UCB. Recombinant human IL-15 was purchased from PeproTech.

Cell Preparation. Peripheral blood mononuclear cell (PBMC) and splenocytes were prepared as previously described (14). Isolation of IEL and LPL by Percoll gradient centrifugation has been described previously (14).

Study Protocol. Three 6-month old T3^b-human IL-15 Tg mice were injected i.p. with 200 μ g of TM- β 1 antibody in 0.3 mL of sterile PBS twice a week (3 days apart) for 2 months, and three control T3^b-human IL-15 Tg mice received PBS only in an identical manner. All mice were killed at 8 months of age for pathological examinations.

Flow Cytometry Analysis. Lymphocytes were analyzed by flow cytometry by using standard protocols. Briefly, cells were washed in FACS buffer containing 10% FBS and 2 mM EDTA. Next Fc receptors were blocked with anti-CD16/32 antibody for 20 min at 4 °C, and lymphocytes were stained with combinations of antibodies to: CD3 ϵ -FITC, CD8 α -FITC, NK1.1-APC, NKG2D-PE, and NKG2A/C-E-FITC (BD-PharMingen) for 20 min at 4 °C. Samples were then analyzed with a FACSCalibur flow cytometer (Becton Dickinson) using FLOWJO software (Tree Star).

Tissue Staining and Histology. For histology, tissues from small intestines were fixed in 4% *para*-formaldehyde and embedded in paraffin. Four-micrometer sections were affixed to slides, deparaffinized, and stained with hematoxylin and eosin. Morphological changes in the stained sections were examined by light microscopy.

Cell Proliferation Assay. Cellular proliferation was determined by using Cell Titer 96 Aqueous One Solution Cell Proliferation Assay (Promega). Briefly, 1×10^5 cells were cultured in RPMI with 10% FBS, 2ME, and 50 ng/mL of recombinant human IL-15 (Peprotec). After 4 days in culture, cell proliferation assay reagent was added into wells and incubated for 4 h, and the absorbance at 490 nm was measured using a microplate reader (Molecular Devices).

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