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VI. 研究成果の刊行物・別刷

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Efficient induction of oral tolerance by fusing cholera toxin B subunit with allergen-specific T-cell epitopes accumulated in rice seed

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ABSTRACT

Cholera toxin B (CTB) subunit is an efficient mucosal carrier molecule for induction of oral tolerance to antigens and allergens. Here, T-cell epitopes of Cry j 1 and Cry j 2, major allergens in Japanese cedar pollen, were expressed in rice seed as a fusion protein with either CTB or rice glutelin as a control. Feeding mice with rice seed containing CTB-fused T-cell epitopes suppressed allergen-specific IgE responses and pollen-induced clinical symptoms at 50-fold lower doses of T-cell epitopes than required when using control seed. Our findings present a novel potential strategy for immunotherapy of type-I allergy.

1. Introduction

Oral peptide immunotherapy using dominant T-cell epitopes provides a safe, easy, and effective strategy against allergic and autoimmune diseases [1,2]. We have recently reported that feeding mice with transgenic rice seed containing T-cell epitopes derived from major Japanese cedar pollen allergens Cry j 1 and Cry j 2 suppressed pollen-induced allergic responses and clinical symptoms [3]. These results demonstrate the clinical potential of T-cell epitopes accumulated in rice seed for immunotherapy of allergic disorders.

Cholera toxin B (CTB), a GM1 ganglioside-binding subunit of cholera toxin, has been used as an efficient mucosal carrier for induction of oral tolerance [4–6]. Compared to native antigens/allergens, 15–800-fold lower amounts of CTB-fused antigens/allergens are sufficient for induction of tolerance, indicating promising applications of CTB for immunotherapy of allergic and autoimmune diseases [4–6]. Thus, in the present study, we expressed T-cell epitopes of Cry j 1 and Cry j 2 as a fusion protein with CTB in rice seed, and examined the efficacy of CTB-fused T-cell epitopes in our experimental mouse model of allergy.

In BALB/c mice, two dominant T-cell epitopes (P1-277–290 of Cry j 1 and P2-246–259 of Cry j 2) and one subdominant T-cell epitope (P2-70–83 of Cry j 2) have been identified [7]. These three T-cell epitopes were linked together into one peptide [7], designated 3Crp, and were expressed in rice seed as a fusion protein with either CTB (CTB-3Crp) or rice glutelin acidic subunit (GLU-3Crp) as a control. In feeding experiments, a relatively high dose of GLU-3Crp control seed (equivalent to 15 μg of 3Crp) was required for suppression of allergen-specific IgE responses, whereas a much lower dose of CTB-3Crp seed (equivalent to 0.3 μg of 3Crp) was sufficient. These results indicate that the dose of 3Crp required for oral tolerance induction was decreased approximately 50-fold by fusing with CTB.

2. Materials and methods

2.1. Plasmid construction and rice transformation

A DNA coding for 3Crp was designed using optimized codons frequently used by rice seed storage protein genes [8–10]. First, two pairs of complementary DNAs coding for N- and C-terminal half regions of 3Crp were cloned into pUC19, forming a complete DNA sequence of 3Crp. This 3Crp gene was amplified by PCR, and fused to the 3′ end of the CTB gene [10], generating the expression

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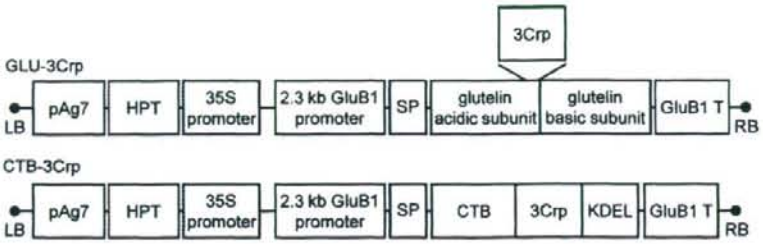

Fig. 1. Constructs for expression of GLU-3Crp and CTB-3Crp in rice seed. DNA fragments coding for GLU-3Crp and CTB-3Crp were linked to the rice seed storage protein glutelin promoter. The *hpt* gene was used for the selection of transgenic rice plants. *GluB-1*, rice glutelin *GluB-1*; 35S, cauliflower mosaic virus 35S promoter; *hpt*, hygromycin phosphotransferase gene; pAg7, agropine synthase polyadenylation signal sequence; RB, right border; LB, left border.

plasmid CTB-3Crp (Fig. 1). A KDEL endoplasmic reticulum retention signal was attached to the C-terminus of CTB-3Crp to increase accumulation levels [9]. In the control, the PCR-amplified 3Crp gene was inserted into the variable region of the C-terminal region of the glutelin GluA-2 (GLU) acidic subunit, as described [11], producing the expression plasmid GLU-3Crp (Fig. 1). These genes were ligated downstream of the 2.3-kb *GluB-1* promoter with or without a signal peptide sequence. After these two expression cassettes were separately inserted into the binary vector pGPTV-35S-HPT, they were introduced into the rice genome (*Oryza sativa* L. cv Kitaake) by *Agrobacterium tumefacience*-mediated transformation [12].

2.2. Detection of CTB-3Crp and GLU-3Crp

More than 30 independent transgenic lines were evaluated for accumulation of 3Crp in mature seed grain by Western blot analysis. Seed protein was analyzed as previously described [3]. Briefly, rice seed was ground to a fine powder, and total seed protein was extracted with extraction buffer containing 4% (w/v) SDS, 8 M urea, and 5% (w/v) β-mercaptoethanol [3]. Total seed protein was separated by electrophoresis on 15% SDS-PAGE, and transferred to Hybond-P PVDF membranes (Amersham Biosciences) for Western blot analysis. Each membrane was probed with rabbit polyclonal antibodies raised against either CTB [10] or a synthetic peptide (CKTSSSHFTFKVD: residues 23–35 of 3Crp) (Scrum, Japan), and incubated with a goat anti-rabbit IgG secondary antibody conjugated to horseradish peroxidase (Promega). For quantification of the products, GLU-3Crp and CTB-3Crp proteins were expressed in *E. coli* as 6× His-tagged proteins, purified by affinity chromatography, and used as standards. Accumulation levels of 3Crp in seed were extrapolated against standard curves generated by densitometric scanning of Western blots of purified standards.

2.3. Oral tolerance induction and allergen challenge

BALB/c male mice at 6 weeks of age were purchased from CLEA Japan. Mice in groups of four to five were given 200 mg fine powder of rice seed, containing different doses of 3Crp as described in Fig. 3, orally in 1.0 ml PBS once a day for 10 days. Each dose of 3Crp in 200 mg seed powder was prepared by mixing wild-type rice seed with GLU-3Crp or CTB-3Crp seed. Mice were then intraperitoneally challenged twice on days 10 and 17 with 0.1 mg of total protein extract of Japanese cedar pollen (Cosmo Bio, Japan) adsorbed on 5 mg aluminum hydroxide (Cosmo Bio). At the first challenge, recombinant mouse IL-4 (R&D Systems) was mixed with the allergen solution at 0.1 μg per mouse to maximize the induction of allergen-specific IgE responses [3].

2.4. ELISA

On day 31, mice were bled to measure allergen-specific IgE titers by ELISA as described [3]. Briefly, ELISA plates were coated with 2 μg/ml of anti-mouse IgE (Southern Biotechnology), and serial dilutions of serum were applied to the plates. After washing, biotinylated pollen extract was used as the detection reagent. Streptavidin–horseradish peroxidase conjugate (Pierce) was then added to the plates, and the reaction was developed with peroxidase substrate solution (Moss). The last serum dilution yielding an OD 450 value of 0.1 over the background was recorded as the endpoint titer.

2.5. Histamine release and nasal symptoms

Histamine release and nasal symptoms were evaluated as described [3]. Briefly, mice in groups of 10–11 were fed wild-type rice seed, GLU-3Crp seed containing 30 μg 3Crp/grain, or CTB-3Crp seed containing 0.5 μg 3Crp/grain once a day for 10 days. These mice were intraperitoneally sensitized with total pollen extract, and then intranasally challenged with 20 μl of 1 μg/ml total pollen extract once a day for 1 week. Nasal symptoms were evaluated by counting the number of sneezes for 5 min after the last intranasal challenge. Statistical significance was determined by Student's *t*-test.

3. Results

3.1. Expression of GLU-3Crp and CTB-3Crp in rice seed

GLU-3Crp and CTB-3Crp were specifically expressed in endosperm of rice seed under the control of the 2.3-kb *GluB-1* promoter (Fig. 1). As a control for CTB-fused 3Crp, we first attempted to express 3Crp directly, but its accumulation was undetectable in the transgenic rice seed (data not shown). As an alternative, 3Crp was expressed as a fusion protein with rice glutelin, because it was known that several peptides fused with glutelin accumulated in rice seed [8,11,13]. In rice endosperm, endogenous glutelin is first synthesized as a precursor, and then post-translationally processed into two mature acidic and basic subunits [11]. As shown in Fig. 2, a GLU-3Crp signal was detected as a part of the acidic subunit with the expected molecular mass of the mature form of GLU-3Crp (34 kDa) at 35 μg/grain, whereas the precursor form of GLU-3Crp (56 kDa) was not detected. On the other hand, CTB-3Crp was detected as a band with the expected molecular mass of 17 kDa at 0.4 μg/grain (Fig. 2).

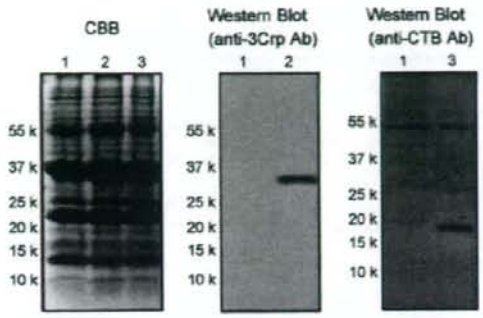

Fig. 2. Detection of GLU-3Crp and CTB-3Crp. Total protein of rice seed was separated by electrophoresis on 15% SDS-PAGE, transferred to PVDF membranes for Western blot analysis, and probed with anti-3Crp or anti-CTB antibody. Lane 1, wild-type seed; lane 2, GLU-3Crp seed; lane 3, CTB-3Crp seed.

3.2. Suppression of allergen-specific IgE responses by feeding of CTB-3Crp seed

Next, we examined the effect of orally administered rice seed containing GLU-3Crp (GLU-3Crp seed) or CTB-3Crp (CTB-3Crp seed) on pollen allergen-specific IgE responses. In our experimental mouse model, oral administration of a relatively high dose of GLU-3Crp seed (equivalent to more than 15 μg of 3Crp) were required for suppression of allergen-specific IgE, whereas lower amounts of GLU-3Crp seed (equivalent to 0–6 μg of 3Crp) did not affect IgE levels (Fig. 3). In the groups of mice orally administered with CTB-3Crp seed containing 0.3, 0.5, and 5 μg of 3Crp, the development of allergen-specific IgE was inhibited (Fig. 3). These results indicate that the dose of 3Crp required for suppression of allergen-specific IgE responses was decreased approximately 50-fold by fusing 3Crp with CTB.

3.3. Suppression of pollen-induced histamine release and clinical symptoms by feeding of CTB-3Crp seed

Levels of histamine release were 52.4 ± 12.7 ng/ml in the group of mice fed wild-type rice seed [3], whereas oral feeding of GLU-3Crp seed (containing 30 μg 3Crp) and CTB-3Crp seed (containing 0.5 μg 3Crp) suppressed levels of histamine release to 20.5 ± 10.0 and 7.5 ± 3.1 ng/ml, respectively ($P = 0.076$). In the control group of mice fed wild-type rice seed, significant nasal symptoms of sneezing developed (Fig. 4). In contrast, the number of sneezes was reduced in the group of mice fed GLU-3Crp seed (containing 30 μg 3Crp) and CTB-3Crp seed (containing 0.5 μg 3Crp) (Fig. 4). These results indicate that feeding of CTB-3Crp seed was also 60-fold more effective than GLU-3Crp seed at inhibiting allergic symptoms in the nasal tract.

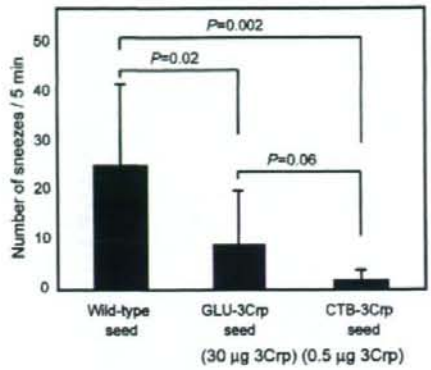

Fig. 4. Suppression of pollen-induced clinical symptoms. Mice in groups of 10–11 were fed rice seed, intraperitoneally sensitized with total pollen extract, and intranasally challenged with total pollen extract once a day for 1 week. Nasal symptoms were evaluated by counting the number of sneezes for 5 min after the last intranasal challenge.

4. Discussion

Allergen-specific IgE antibodies play a major biological role in the induction of inflammatory responses; therefore suppression of allergen-specific IgE is expected to be a key strategy for modulating allergic disorders [14]. In the present study, we showed that threshold amounts of 3Crp required for suppression of allergen-specific IgE responses were dramatically decreased by feeding of CTB-3Crp seed. Further, we demonstrated that allergic symptoms were suppressed in the group of mice fed CTB-3Crp seed. Our findings indicate that the clinical efficacy of 3Crp was significantly enhanced by fusing 3Crp with CTB.

The efficacy of CTB-fused antigens for modulating Th1-mediated autoimmune responses is well established [5]. Concerning Th2-driven responses, effects of CTB fusion were recently examined using OVA as a model antigen, showing that CTB-fused

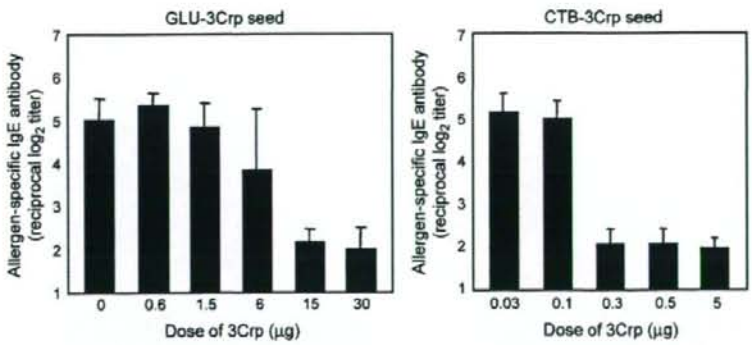

Fig. 3. Suppression of allergen-specific IgE responses. Mice in groups of four to five were orally administered once a day for 10 days with finely powdered rice seed containing different doses of 3Crp. Mice were then intraperitoneally challenged with total protein extract of Japanese cedar pollen, and allergen-specific serum IgE titers were determined by ELISA.

OVA induces oral tolerance at a 100-fold lower dose than control OVA [6,15]. Our results using pollen allergen-derived T-cell epitopes are consistent with these reports, providing additional evidence supporting the potential of CTB fusion to modulate Th2-mediated immune responses.

Based on high-affinity binding to GM1 ganglioside, CTB is considered to act as an efficient mucosal carrier molecule for induction of oral tolerance. However, CTB has recently been shown to have immunomodulatory properties, which are associated with induction of oral tolerance. A recent study showed that oral administration of CTB-fused OVA induces regulatory T-cells with strong suppressive activities [15]. Another study reported that intranasal administration of CTB-free Bet 1 v allergen suppressed allergen-specific IgE, IgG2a, and IgA responses, whereas CTB-fused Bet v 1 enhanced allergen-specific IgG2a and IgA levels, suggesting that CTB modifies the immunological properties of Bet 1 v [16]. Further, orally administered CTB has adjuvant activities such as induction of cellular activation, surface molecule expression, and cytokine production [17]. Although the mechanism is not clear, these immunomodulatory properties of CTB might be a clue to explain the reduction of histamine release and sneezes in CTB-3Crp-fed mice compared to GLU-3Crp-fed mice, while no significant differences in allergen-specific IgE levels were observed between the two groups (Fig. 3). Further studies on the effect of CTB are required prior to future clinical applications of CTB-fused T-cell epitopes.

Production platforms using rice seed offer several benefits such as low-cost production and storage, and safety without contamination by mammalian pathogens. Rice seed also provides convenient and effective mucosal delivery of antigens/allergens for immunotherapy [3]. Our study here showed that the efficacy of 3Crp was significantly improved by genetic fusion with CTB, further supporting the potential of rice seed containing T-cell epitopes for clinical applications in immunotherapy of type-I allergy.

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Antibody-mediated blockade of IL-15 signaling reverses autoimmune intestinal damage in a transgenic mouse model of celiac disease

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Running title: IL-15 blockade as a therapeutic modality for celiac disease

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Abstract

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 mediated by both innate and T cell-mediated mechanisms 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 for which the only available treatment is a life-long gluten-free diet and even then in some patients the disease continues to exacerbate and ultimately results in refractory CD with increased incidence of lymphomas. Transgenic mice that express human IL-15 specifically in enterocytes with an enterocyte-specific promoter ($T3^b$-hIL-15 Tg mice) develop severe duodeno-jejunal inflammation that closely recapitulates the pathognomonic features of active CD. We used these mice as a model in a proof-of-concept study to evaluate the efficacy of IL-15 blockade as a potential therapeutic modality for CD with a MAb (TM-β1) that binds to murine CD122 and abrogates IL-15 signaling. We show that TM-β1-mediated blockade of IL-15 signaling reverses the autoimmune intestinal pathology in these mice thus providing a compelling rationale to explore IL-15 blockade as a potential therapeutic modality especially for refractory CD.

Introduction

Celiac disease (CD) also known as celiac sprue or gluten sensitive enteropathy is an immune-mediated enteropathy triggered by the consumption of gluten-containing cereals such as wheat, barley or rye (reviewed in ref 1, 2). The prevalence of CD in North American and European populations is estimated to be in the range of 1 in 150-300 although it may be less prevalent in certain Asian populations such as those in Japan (reviewed in ref 3). The genetic susceptibility to CD shows a tight linkage with the presence of human leukocyte antigen (HLA) class II DQ alleles either in *cis* or *trans*, coding for HLA DQ2 or DQ8 class II molecules and possibly with other less obvious genetic components conferring some degree of susceptibility as well. The spectrum of clinical manifestations of CD ranges from inapparent latent disease to overt disease with intestinal symptoms including diarrhea often leading to extraintestinal manifestations of weight loss, stunted growth in childhood disease, and iron deficiency anemia resulting from malabsorption of nutrients from the affected gut mucosa (4). In addition although not very common, celiac disease could lead to adenocarcinoma or enteropathy associated T cell lymphomas as well, especially in individuals who develop refractory disease that is not amenable to the standard therapy of dietary gluten withdrawal.

Unlike many other autoimmune diseases, the principal elements of the pathogenesis of celiac disease have been elucidated (reviewed in ref 1, 5). Gluten peptides resulting from partial digestion of dietary cereal–derived prolamins are presented by the antigen presenting cells (APC) including dendritic 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 interferon gamma (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. On the other hand, extensive intra-

epithelial 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 (6). However, unlike the exquisite gluten specificity of proliferating lamina proprial $CD4^+$ T cells, infiltrating intra-epithelial $CD8^+$ T cells are largely devoid of any gluten specificity. It is these intra-epithelial $CD8^+$ 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 utilize NKG2D and other co-activating NK-cell receptors (7, 8).

Emerging evidence has implicated a pivotal role for the proinflammatory cytokine interleukin 15 (IL-15) presumably made locally by lamina proprial dendritic cells, macrophages, and monocytes as well as intestinal epithelial cells in orchestrating immune-mediated tissue destruction seen in CD (7-12). IL-15 is indispensable for the generation, maintenance and homeostasis of intra-epithelial lymphocytes and consequently in IL-15 or IL-15 receptor alpha gene-deleted mice, intraepithelial lymphocytes are virtually absent in the mucosal tissues (13, 14). IL-15 also induces proliferation of $CD8^+$ T lymphocytes in addition to enhancing their effector functions including those associated with cytolysis and cytokine secretion. IL-15 promotes perpetuation of chronic inflammation by preventing activation-induced cell death of activated $CD8^+$ T cells (reviewed in ref 15). Furthermore, in CD patients IL-15 effectively reprograms intra-epithelial $CD8^+$ CTL to lymphokine-activated killer cells or natural killer-like cells capable of massive oligoclonal expansion and target cell cytolysis in a TCR-independent fashion, in part by coordinate induction of the NKG2D signaling pathway and other cytolytic NK lineage receptors on these cells (7). In completing this perpetual cycle of tissue damage in CD, 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 (8). In addition to these positive modulatory effects on the activation pathways leading to persistent

inflammation, the recent work of Benahamed et al. (12) 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 micro-environment where host immune elements are in constant contact with a plethora of commensal organisms, TGF-β mediates the anti-inflammatory tone of the gut mucosal immune system while promoting oral tolerance and mucosal IgA responses. 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 (12). 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 therapeutic approach in CD, an autoimmune disease for which the only available treatment currently is a life long gluten-free diet or in the case of refractory CD where there is no effective treatment.

Previously, we reported the generation of IL-15 transgenic mice that express human IL-15 in intestinal epithelial cells using the enterocyte-specfic $T3^b$ promoter to drive the transgene. These mice develop florid spontaneous inflammation in the duodeno-jejunal region (16). The anatomical location of the inflammatory lesions, extensive villous atrophy and the massive accumulation of NK like $CD8^+$ T cells in the affected mucosa of these transgenic animals closely recapitulate the pathognomonic lesions of human 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-β1) that binds to IL-2/IL-15Rβ (CD122) and blocks IL-15 activity (17). We show that the antibody-mediated blockade of IL-15 signaling results in effective resolution of the inflammatory lesions in the treated animals and thus attests to the value of exploring IL-15 blockade as a rational therapeutic modality in treating CD as well as refractory CD with a high propensity for developing into T cell lymphomas.

Material and Methods

Mice. The generation of $T3^b$-human IL-15 Tg mice has been reported previously (16). 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-β1) was either purchased from Serotec or obtained as a gift from UCB Inc, UK. Recombinant human IL-15 was purchased from PeproTech.

Cell preparation. PBMC and splenocytes were prepared as previously described. (16), Isolation of IEL and LPL by Percoll gradient centrifugation has been described previously (16).

Antibody Infusion Protocol. Mice were injected intraperitoneally with 200 micrograms of TM-β1 antibody in 0.3ml of sterile phosphate buffered saline (PBS) twice a week (3 days apart) for two months.

Flow-Cytometry Analysis. Lymphocytes were analyzed by flow cytometry by using standard protocols. Briefly, cells were washed in FACS buffer containing 10% Fetal Bovine Serum (FBS) and 2mM EDTA, Fc receptors were blocked with anti CD16/32(BD-Pharmingen) for 20min at 4°C. After Fc receptor blocking, lymphocytes were stained with combinations of antibodies to: CD3ε-FITC, CD8α-FITC, NK1.1-APC, NKG2D-PE, NKG2A/C/E-FITC (BD-Pharmingen) for 20min at 4°C. Samples were then analyzed with a FACSCalibur flowcytometer (Becton Dickinson). Data were analyzed using FLOWJO software(Tree Star Inc.,).

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 under light microscopy.

Cell proliferation assay. Cellular proliferation was determined using Cell Titer96 AQueous One

Solution Cell Proliferation Assay (Promega). Briefly, $1x10^5$ cells were cultured in 10%FBS RPMI with 2ME for 4days with 50ng/ml of recombinant human IL-15 (Peprotec). After 4 days in culture, Cell Titer96 AQueous One Solution Cell Proliferation Assay reagent was added into culture wells and incubated for 4 hours and the absorbance at 490 nm was measured using Emax precision microplate reader (Molecular Devices).

Results and Discussion

$T3^b$-hIL-15 Tg mice display salient pathophysiological features of Celiac disease. The lack of a suitable animal model has been a serious impediment in developing effective therapeutic modalities for celiac disease for which the only available treatment at present is life-long gluten free diet for affected individuals. Also some individuals fail to respond to dietary withdrawal of gulten, and in these individuals the condition exacerbates despite being on a gluten-free diet leading to life-threatening neoplasia thus underscoring the necessity for more effective therapies (18). 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 (16) and shown again in Fig.1 A. Since then, 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 (7-12) thus prompting us to re-evaluate our $T3^b$-hIL-15 Tg mice as a potential model for IL-15 mediated immune pathology that occurs in CD. In these mice, intraepithelial lymphocytic infiltration is evident very early in life microscopically but by around the third month after birth, the extent of lymphocytic infiltration mimics Marsh (6) stage 3/4 active CD with partial or complete villous atrophy as shown in Fig 1, panel B. Macroscopic nodular mosaic pattern of the mucosa as well as the prominence of submucosal blood vessels in these mice (Fig. 1,panel A) bear striking resemblance to the pathology seen in active CD.

In the pathophysiology of CD, the emerging consensus is that the extensive indiscriminate enterocyte destruction is driven 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 of the expression of stress-induced NKG2D ligands such as MICA/B, ULBPs, HLA-E on epithelial enterocytes as a result of

locally synthesized IL-15 (7, 8, 9). As shown in Fig1, panel D, in $T3^b$-hIL-15 Tg mice, the infiltrating lymphocytes expressed NKG2D as determined by flow cytometry and these $NKG2D^+CD8^+$ cells accounted for a significant proportion of IEL and LPL pools seen in $T3^b$-hIL-15 Tg mice in comparison to WT litter-mate control mice (2.3% to 5.6%: IEL, 0.7% to 8.6% LPL). The expression of Rae1, the mouse homolog of MICA on the intestinal enterocytes of $T3^b$-hIL-15 Tg mice was also examined by immunochemistry and the expression of Rae1 was readily demonstrable as shown in Fig 1, panel C. Thus, the duodeno-jejunal anatomic location of the inflammatory lesions, the display of two cardinal CD pathognomonic features of massive intraepithelial lymphocytic infiltration and villous atrophy that is 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 which are considered as pivotal elements in the perpetuation of inflammation with attendant tissue damage in CD, in our view collectively qualify the $T3^b$-hIL-15 Tg mice as a *bona fide* model to study the pathophysiology of CD and to evaluate potential therapeutic approaches for active CD in humans. Indeed, it is remarkable that the intestinal over-expression of IL-15 alone in these mice is sufficient singularly to recapitulate the entire spectrum of immune pathologic lesions seen in CD, further strengthening the notion that IL-15 is a principal causal element in the pathophysiology of CD and hence a rational target for therapeutic intervention in the treatment of CD.

Effective blockade of IL-15 signaling by TM-b1 antibody that binds to murine CD122. IL-15 is a 14-kDa, 114-amino-acid polypeptide that belongs to the four-α-helix bundle family of cytokines and is made by a variety of cell types including monocytes and dendritic cells and has potent effects on both innate and adaptive immune systems (reviewed in refs 15, 19). IL-15 mediated effects are transduced via a tripartite receptor complex consisting of IL-15Rα which is a high affinity private receptor of IL-15

along with two signaling components CD122 (IL-2/IL-15Rβ) and CD132 (γ_c). In most situations, the three-receptor subunits are not co-expressed by the same cell. Rather IL-15Rα, the private receptor of IL-15 is expressed by activated antigen presenting dendritic cells, momocytes and by some non-hematopoeitic cells of the lung and gastrointestinal tract (20, 21). These IL-15Rα bearing cells present IL-15 in *trans* to IL-2/IL-15Rβ (CD122) and γ_c (CD132) expressing NK and CD8+ T cells as part of an immunological synapse. The *trans*-presented IL-15 stimulates the proliferation of activated $CD4^-$ $CD8^-$, $CD4^+$ $CD8^+$, $CD4^+$, and $CD8^+$ T cells and promotes the maintenance of $CD8^+$ $CD44^{hi}$ memory phenotype T cells. It is critical for the generation, proliferation, and activation of NK and NKT cells and plays an essential role in the homeostasis of intraepithelial T cells bearing CD8αα either from the TCR α/β or γ/δ subpopulations of T lymphocytes in mucosal surfaces (13, 14). IL-15 also prevents apoptosis of activated T cells via induction of anti-apoptotic proteins Bcl-2, Mcl-1 and Bcl-X_L while limiting the expression of the pro-apoptotic Bim protein (22).

In designing a strategy to achieve durable in vivo blockade of IL-15 activity in $T3^b$-hIL-15 Tg mice, we opted to evaluate a rat monoclonal antibody TM-β1 that reacts with the murine CD122 (17). The TM-β1 antibody blocks the interaction of *trans*-presented IL-15 by IL-15Rα with the CD122/CD132 signaling receptor complex on responsive NK, and CD8+T cell subsets. As shown in Fig 2, the TM-β1 antibody was very effective in inhibiting IL-15-induced proliferation of murine splenocytes. Despite the fact that both IL-2 and IL-15 utilize CD122 for their signal transduction events, it has been demonstrated previously (23) that IL-2 induced proliferation of mouse splenocytes is not affected by TM-β1 antibody. One possibility for this discordant effect is that an antibody directed toward CD122 can effectively block cells that express only CD122/CD132 complex as is true for IL-15 but not when responsive cells express the trimeric receptor complex as is true for most activated cells in the presence of IL-2 which induces CD25, the private receptor of IL-2 on these cells.