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Intrinsic Requirement for the Vitamin D Receptor in the Development of CD8αα-Expressing T Cells

Danny Bruce and Margherita T. Cantorna

Vitamin D and vitamin D receptor (VDR) deficiency results in severe symptoms of experimental inflammatory bowel disease in several different models. The intraepithelial lymphocytes of the small intestine contain large numbers of CD8αα+ T cells that have been shown to suppress the immune response to Ags found there. In this study, we determined the role of the VDR in the development of CD8αα+ T cells. There are fewer total numbers of TCRββ+ T cells in the gut of VDR knockout (KO) mice, and that reduction was largely in the CD8αα+ TCRββ+ cells. Conversely TCRγδ+ T cells were normal in the VDR KO mice. The thymic precursors of CD8αα+ TCRββ+ cells (triple-positive for CD4, CD8αα, and CD8αβ) were reduced and less mature in VDR KO mice. In addition, VDR KO mice had a higher frequency of the CD8αα+ TCRββ+ precursors (double-negative [DN] TCRββ+ T cells) in the gut. The proliferation rates of the DN TCRββ+ gut T cells were less in the VDR KO compared with those in wild type. Low proliferation of DN TCRββ+ T cells was a result of the very low expression of the IL-15R in this population of cells in the absence of the VDR. Bone marrow transplantation showed that the defect in VDR KO CD8αα+ TCRββ+ cells was cell intrinsic. Decreased maturation and proliferation of CD8αα+ TCRββ+ cells in VDR KO mice results in fewer functional CD8αα+ TCRββ+ T cells, which likely explains the increased inflammation in the gastrointestinal tract of VDR KO and vitamin D-deficient mice. The Journal of Immunology, 2011, 186: 000–000.

The human body is composed of ~100 trillion cells, and 10 times that many bacteria reside in the lumen of the intestine (1). The intestinal epithelial layer not only forms a physical barrier to protect against invading pathogens but also contains a highly specialized immune system. The GALT has evolved to have effector responses to invading pathogens while maintaining tolerance to harmless commensal flora (2). When the balance between effector and tolerogenic response is lost, intestinal inflammation can occur like that seen in inflammatory bowel disease (IBD) (2). The intestinal epithelial layer contains a highly specialized immune system. The GALT has a physical barrier to protect against invading pathogens but also contains a highly specialized immune system. The GALT has evolved to have effector responses to invading pathogens while maintaining tolerance to harmless commensal flora (2). When the balance between effector and tolerogenic response is lost, intestinal inflammation can occur like that seen in inflammatory bowel disease (IBD) (2).

The IEL contains several unique cell types including CD8αα+ T cells. Unlike the TCR coreceptor CD8ββ, CD8αα does not act as a coreceptor, and T cells that express CD8αα are not MHC I class restricted (3, 4). CD8αα has been shown to bind to the nonclassical MHC molecule thymic leukemia (TL) Ag with a higher affinity than that for MHC class I (5). CD8αα+ TCRββ+ IELs are self-reactive but not self-destructive and are believed to be regulatory T cells that help to maintain tolerance in the gut (6). In addition, CD8αα+ TCRββ+ IELs have been shown to suppress intestinal inflammation in the T cell transfer model of IBD (7). The homodimeric form of CD8 can be expressed on both αβ and γδ T cells in the gut, and expression of CD8αα is IL-15 dependent (8, 9). In addition, IL-15 has been shown to induce maturation and enhance survival and proliferation of both CD8αα+ TCRββ+ and CD8αα+ TCRγδ+ IELs (9).

The intestine can support lymphopoiesis as is evident by the presence of CD8αα+ IELs in athymic nude mice and in irradiated neonatal thymectomized mice reconstituted with bone marrow (BM) (4). However, the CD8αα+ IELs in athymic mice are largely of the TCRγδ variety (4, 10). More recent data suggest that the thymus is required for the CD8αα+ TCRββ+ (8). TCRγδ+ cells diverge from the TCRββ+ cells at an early double-negative (DN) stage in the thymus. Like conventional TCRββ+ T cells, CD8αα+ TCRββ+ IEL progenitors develop from double-positive (DP) thymocytes (8). The DP thymocytes that become CD8αα+ TCRββ+ IEL precursors become triple-positive (TP) expressing CD4, CD8αα, and CD8αβ (8). The development of these self-reactive T cells requires exposure to self-antigen peptides for selection in the thymus like other regulatory T cell populations (4). After surviving agonist selection, CD8αα+ TCRββ+ IEL precursors downregulate expression of CD4 and CD8 to become CD4-CD8- TCRββ+ thymocytes that express CD5 (8). Unlike conventional T cells, DN TCRββ+ thymocytes egress the thymus and migrate directly to the intestine (11). Upon entering the IL-15–rich environment of the intestine, DN TCRββ+ cells downregulate CD5 and become mature CD8αα+ TCRββ+ IELs (8). Even though the gut contains both CD8αα+ TCRββ+ and TCRγδ+ T cells and there may be some overlap in function, the two cell types are developmentally distinct.

The vitamin D receptor (VDR) is a member of the steroid hormone family of nuclear receptors (12). The VDR contains a DNA binding domain that is accountable for the high-affinity binding of the active form of vitamin D (1,25-dihydroxyvitamin D3), for dimerization with retinoid X receptor, and for binding...
other transcription factors (12). The hetrodimeric complex of VDR and retinoid X receptor binds to vitamin D response elements and regulates transcription of the target genes (12).

Vitamin D is an important modulator of the immune system. Signaling through the VDR has been shown to suppress multiple models of Th1- and Th17-driven autoimmune diseases including IBD (13). Vitamin D can affect T cell function as well as the development of specific T cell populations. In vitro, supplementation with 1,25-dihydroxyvitamin D3 limits secretion of IFN-γ by CD4 T cells and promotes IL-5 and IL-10, which favors Th2 responses over Th1 responses (14, 15). In addition, VDR knockout (KO) TCRγδ+ T cells show an impaired ability to migrate to the intestine when adoptively transferred to Rag KO mice (16). VDR-deficient mice have normal numbers of conventional CD4 and CD8 T cells in the peripheral lymphoid organs. VDR KO mice have increased proportions of Th1 cells, reduced Th2 responses, and fewer iNKT cells and CD8α+ TCRγδ+ T cells compared with those of wild-type (WT) mice (16–18). CD4 T cells from VDR KO mice overproduce IFN-γ and proliferate twice as much in MLRs (13). VDR KO and vitamin D-deficient WT mice have a significant reduction in the number of CD4+ IELs that coexpress CD8αα (16).

We show here that intrinsic defects occur during the development of VDR KO CD8αα+ TCRδδ+ T cells that result in the impaired development of these cells in the IEL. VDR KO mice have normal numbers of CD8αα+ TCRγδ+ IELs. There is a significant difference in the percentages and total numbers of T cells expressing TCRδδ in the VDR KO IELs compared with those of WT IELs. WT BM can reconstitute VDR KO IELs to normal levels, but VDR KO CD8αα+ TCRδδ+ IELs fail to develop normally in a WT host. The number of TP thymocytes and the frequency of maturing TCRβ+ TP thymocytes are significantly reduced in neonatal VDR KO mice. The less mature VDR KO DN TCRδδ+ IELs are more prevalent in the IELs, fail to become IL-15+, and do not mature and proliferate in response to IL-15. Our results suggest that the VDR is an important factor in the development and maturation of CD8αα+ TCRδδ+ IELs.

Materials and Methods

Mice

Age- and sex-matched VDR KO and WT C57BL/6 mice were produced at the Pennsylvania State University (University Park, PA). VDR KO and WT mice were housed in the same room and under the exact same housing conditions. For embryonic thymocytes, timed breedings were performed. Breeding pairs were caged together in the evening, and the following morning females were inspected for seminal plugs. Females with plugs were separated to establish embryonic day 0 and monitored for weight gain as an indicator of pregnancy. Experimental procedures received approval from the Office of Research Protection Institutional Animal Care and Use Committee at the Pennsylvania State University.

T cell isolation and cell culture

For IELs, the small intestine was removed and flushed with HBSS (Sigma-Aldrich, St. Louis, MO) containing 5% FBS, and the Peyer’s patches were suspended at 1.0 × 10⁶ cells/ml in RPMI 1640 supplemented with 10% FCS (Thermo-Fisher Scientific), 2 mM l-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin, and 5 mM 2-mercaptoethanol (Invitrogen, Carlsbad, CA) and stimulated with 0.5 µg/ml recombinant IL-15 (R&D Systems, Minneapolis, MN). Reciprocal BM chimeras were produced using VDR KO and WT mice. Reconstitution of WT→WT (donor→recipient), VDR KO→WT, and WT→VDR KO mice was >80% complete in the blood of

Immunofluorescence

Cells were stained according to standard procedures and analyzed on a FC500 benchtop cytometer (Beckman Coulter, Brea, CA). The following Abs were used: ECD anti-CD4 (Southern Biotech, Birmingham, AL), PE anti-CD8β, FITC anti-CD122, PE anti-CD25, FITC anti-TCRγδ, PE-Cy5 anti-TCRδ, FITC anti-CD45, FITC CD45.1 or CD45.2, and PE-Cy7 anti-CD8α and appropriate isotype controls including FITC IgG1, λ (A110-1), PE-Cy7 IgG2α, κ (R5-95), PE IgG2b, κ (A55-1), FITC IgG2a, κ (G155-178), or PE-Cy5 IgG2, λ (Ha 4/8) (BD Biosciences). Isotype controls were used to set appropriate gating. CD8αα was detected with PE-labeled TL-tetramer (T3b) (8). The tetramers were a gift from Dr. Hilde Cheroutre (La Jolla Institute for Allergy and Immunology, La Jolla, CA) and the National Institutes of Health Tetramer Core Facility (Atlanta, GA). Data were evaluated with WinMDI 2.9 software (Scripps Institute, La Jolla, CA).

BM transplantation

Donor bone marrow cells were isolated and transferred into sublethally irradiated CD45 allele matched recipients. Mice were allowed 6 wk to recover, and reconstitution of BM was evaluated in the blood by flow cytometry and staining for donor CD45 expression.

BrdU incorporation assay

Two-week-old mice were injected i.p. with 50 µl 25 mg/ml BrdU dissolved in PBS at days 0, 2, and 4 of the experiment. The mice were sacrificed on day 7, and the IELs were analyzed for BrdU incorporation using a BrdU flow kit (BD Pharmingen) according to the manufacturer’s instructions. PE-IgG1, κ (MOPC-21) was used as isotype control (BD Pharmingen).

Statistics

Bar graphs are represented as mean ± SEM. Data was analyzed by two-tailed unpaired t test using Prism 5.0 statistical software (GraphPad Software, La Jolla, CA). The p values ≤0.05 were considered statistically significant.

Results

Gastrointestinal TCRδδ+ T cells are reduced in the absence of vitamin D signaling

Equal total numbers of cells were isolated from WT and VDR KO IELs (Supplemental Fig. 1A). The frequency of innate immune cells including dendritic cells (WT, 42 ± 4%; VDR KO, 30 ± 4%), macrophage (WT, 6 ± 2%; VDR KO, 11 ± 2%), and NK cells (WT, 2.3 ± 0.3%; VDR KO, 3.2 ± 0.6%) were the same in the IELs of WT and VDR KO mice. The frequency of both CD4+ and CD8αβ+ T cells were not different in VDR KO and WT mice (16). Staining for the TCRγδ-chain showed that WT and VDR KO mice had similar frequencies of γδ T cells in the IELs (Fig 1A). Staining for TCRδδ showed significantly fewer TCRδδ+ T cells in VDR KO mice (Fig 1A). More than 40% of the IELs in WT mice expressed the TCRδδ compared with only 25% of the VDR KO IELs (Fig 1A). CD8αα can be expressed on many different cell types but primarily on γδ and αβ T cells (6). The majority of the CD8αα+ IELs are γδ T cells in WT mice (Fig 1B). In the VDR KO IELs, the frequencies of CD8αα+ TCRγδ (VDR KO, 67%; WT, 54%) and of CD8αα non-T cells (VDR KO, 12%; WT, 5%) were higher than those in the WT IELs (Fig 1B). However, the total numbers of CD8αα+ TCRγδ+ and CD8αα+ non-T cells in WT and VDR KO IELs were the same (Supplemental Fig. 1B, 1D). Conversely, the frequency and total cell number of CD8αα+ TCRδδ+ T cells in WT IELs were higher than the VDR KO values (WT, 39%; VDR KO, 17%; Fig 1B, Supplemental Fig. 1C). In the absence of the VDR, fewer TCRδδ+ and fewer CD8αα-expressing TCRδδ+ T cells were present in the gut.

Intrinsic defect in VDR KO cell CD8αα+ TCRδδ+ T cells

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all mice tested (Fig. 1C). Importantly, VDR KO mice were reconstituted with the same efficiency as WT mice (Fig. 1C). The IELs of WT mice reconstituted with WT BM (WT–WT) had 43% of the CD8α+ TCRβ+ T cells of donor origin (Fig. 1D, 1E). VDR KO recipients of WT BM (VDR KO–WT) had slightly lower reconstitution with WT donor cells, but the values were not significantly different from those of WT–WT (Fig. 1D, 1E). Fewer CD8α+ TCRβ+ T cells of VDR KO origin were recovered from WT recipients (VDR KO–WT), and the difference was significantly different from both the WT–WT and WT–VDR KO chimeras (Fig. 1D, 1E). The data suggest that VDR KO BM has a cell intrinsic defect in the generation of CD8α+ TCRβ+ T cells that reside in the IELs.

**Early CD8α+ TCRβ+ thymic precursors in the VDR KO mice**

CD8α+ T cell precursors are first detectable in the thymus at embryonic day 16 of fetal development (8). Expression of CD8α along with CD8β and CD4 results in a TP cell type (8). The TP thymocytes in WT mice make up >40% of the thymus at embryonic day 16, peak at embryonic day 17 (58%), and are only 10% by birth (day 1, Fig. 2A). Similar frequencies of TP thymocytes with the same kinetics were found in the VDR KO thymus (Fig. 2A). Frequencies of DP thymocytes were also not different in the fetal thymus of VDR KO and WT mice.

From birth to 3 wk of age, WT mice maintained the percentage of TP thymocytes (~10%), whereas the percentage of VDR KO TP thymocytes significantly decreased (Fig. 2B). The frequency of TP cells in the thymus then declined to that found in the adult thymus by 6 wk (~5%), and at 6 wk there were equal numbers of TP cells in WT and VDR KO thymi (Fig. 2B). The frequencies of DP thymocytes are not different in WT and VDR KO mice regardless of age (Fig. 2C). Expression of the TCR is a step in the maturation of the CD8α+ T cell precursors (8). There were more WT TP cells that express the TCRβ receptor than VDR KO TP cells that express the TCRβ receptor at 2 and 3 wk of age. The kinetics for the appearance of TCRβ+ TP thymocytes was the same in VDR KO and WT mice, but the WT mice had a higher frequency of TCRβ+ CD8α precursors (Fig. 2C). By 6 wk when the frequency of TP cells was low and not different between WT and VDR KO mice, the expression of TCRβ on TP cells was also not different (Fig. 2D).

The first 3 wk of life are a critical time for the appearance of CD8α+ TCRβ+ T cells in the IELs (4). WT IELs contained 20% TCRβ+ T cells by 1 wk of age, and that percentage gradually increased as the mouse matured to the adult levels of 30% (Fig. 2E). CD8α+ TCRβ+ IELs can be found in low frequencies at 1 and 2 wk in WT mice, and a significant increase occurs between 2 and 3 wk of age to when >40% of the TCRβ+ T cells express CD8α (Fig. 2F). VDR KO TCRβ+ IELs fail to appear in the intestine at the same rate as WT T cells, and this defect can...
be seen as early as the first week of life (Fig. 2E). The frequency of VDR KO CD8α+ TCRβ+ T cells in the gut is the same as that of WT at 1 and 2 wk of age (Fig. 2F). By 3 wk, there are lower frequencies of VDR KO CD8α+ TCRβ+ IELs than WT CD8α+ TCRβ+ IELs, and the numbers of the CD8α+ TCRβ+ fail to increase further as the mice age (Fig. 2F).

Reduced proliferation of TCRβ+ IELs in the absence of the VDR

At the time of CD8α+ TCRβ+ appearance in the IELs (between 2 and 3 wk of age in WT mice; Fig. 2F), mice were treated with BrdU to measure the in vivo proliferation of the cells. Total BrdU incorporation of the IELs of WT mice showed that 34% of the cells had proliferated (Fig. 3A). Only 22% of the VDR KO IELs incorporated BrdU over the same time period, which was significantly less than that of WT IELs (Fig. 3A). To identify what population of IELs was proliferating at a slower rate in the VDR KO mice, the cells were stained for TCRβ, CD8α, and BrdU. Incorporation of BrdU by the TCRβ− IELs (CD8 T cells, NK cells, etc.) was similar in the VDR KO and WT mice (Fig. 3B). Incorporation of BrdU in the CD8α+ TCRβ− IELs (largely the CD8α+ TCRγδ+ cells; Fig. 1B) was higher but not significantly higher in VDR KO (32%) than that in WT (24%) mice (Fig. 3C). The frequency of proliferating TCRβ+ T cells in WT IELs was twice as high as that of VDR KO IELs (Fig. 3D). Different TCRβ+ subsets proliferated at different rates. In WT mice, the frequency of proliferating DN TCRβ+ T cells was significantly greater compared with that of CD8α+ TCRβ+ T cells (Fig. 3D). The frequency of proliferating DN TCRβ+ IELs from VDR KO mice was not different than that of CD8α+ TCRβ+ T cells (Fig. 3D). There was a significant decrease in the frequency of proliferating DN TCRβ+ and CD8α+ TCRβ+ IELs in VDR KO mice compared with that in WT mice (Fig. 3D). Only the VDR KO CD8α+ TCRβ+ IELs and their precursors had impaired homeostatic proliferation.

Increased frequency but decreased numbers of CD8α+ TCRβ+ IEL precursors in VDR KO mice

CD8α+ TCRβ+ IEL precursors mature from DN TCRβ+ IELs (8). In the WT IELs, there was a minor population of less mature DN TCRβ+ T cells that had not upregulated CD8α+ in the IELs (Fig. 4A). VDR KO mice had a higher frequency of DN TCRβ+ IELs than that in WT mice (Fig. 4A). Because VDR KO mice have fewer TCRβ+ cells, the total number of immature T cells was less in the VDR KO IELs than that in the WT IELs (Fig. 4B, 4C). The increased frequency of DN TCRβ+ IELs in the VDR KO IELs was not due to an increase in NKT cells because the percentage of TCRββ/NK1.1+ expressing cells was also less in the VDR KO mice than that in the WT mice (Fig. 4D). Immature DN TCRβ+ IELs first express CD5 that is later downregulated as the
cell matures and expresses CD8α (8). Of the DN cells in the IELs of WT mice, 25% express CD5 (Fig. 4E). More than 42% of the DN IELs in the VDR KO expressed CD5, which was significantly more than that of the WT DN cells (Fig. 4E). As a result of the reduced numbers of TCRβ+ cells, the total number of CD5+ DN TCRβ+ IELs in VDR KO mice was significantly less than that in the WT mice (Fig. 4F). VDR KO mice had a higher frequency of immature DN TCRβ+ IELs than that of WT mice.

**IL-15 unresponsiveness of VDR KO CD8αα precursors in the VDR KO mice**

IL-15 is required to induce the maturation of CD8αα+ TCRβ+ IELs (8, 19). In addition, IL-15 is required for CD8αα+ TCRγδ+ IELs, CD44highCD8+ memory T cells, and NK cells (20). CD8αα+ TCRγδ+ IEL numbers were not different in WT and VDR KO mice (Fig. 1, Supplemental Fig. 1B). Of the CD8+ T cells in the spleen, 49 ± 3% WT and 45 ± 6% VDR KO were of the memory phenotype or CD44high. Similarly, the frequencies of NK cells (NK1.1+ and CD3−) were similar in the spleens of VDR KO and WT mice (4–5%). Of the four cell types that are IL-15 dependent, only the CD8αα+ TCRβ+ IEL is affected by VDR deficiency.

Expression of the IL-15R was measured on T cells in the thymus and IELs by measuring CD122 on the CD25− T cells. The mean fluorescence intensity (MFI) of the IL-15R on DN T cells in the thymus of WT mice was low (Fig. 5A). The level of IL-15R expressed on WT and VDR KO DN T cells in the thymus was similar (Fig. 5A). Expression of the IL-15R in all IELs of WT and VDR KO mice was also low and similar (Fig. 5B). The MFI of IL-15R was slightly higher on VDR KO (MFI 6.9) CD8αα+ TCRβ+ IELs than on WT CD8αα+ TCRβ+ IELs (MFI 5.9), but the difference did not reach significance (Fig. 5C; \( p = 0.07 \)). The MFI of IL-15R in the CD8αα+ TCRβ− cells (largely γδ T cells; Fig. 1B) was ~4.0 in both the WT and VDR KO IELs (Fig. 5D). The level of expression of IL-15R on WT and VDR KO TCRβ+ IELs was significantly higher (MFI 7.0) than that on VDR KO DN TCRβ+ IELs (MFI 4.1, \( p = 0.01 \)). The frequency of IL-15R expression was low on all CD8αα+ cells in the IELs of either VDR KO or WT mice: CD8αα+ TCRβ− (2–3%; Fig. 5E), CD8αα+ TCRβ+ (1.8–2.3%), and CD8αα+ TCRγδ+ (0.9–1.2%). Only 2% of WT and 3% of VDR KO CD8αα+ TCRβ+ IELs express high levels of the IL-15R (Fig. 5E). Conversely, 37% of DN TCRβ− cells expressed the IL-15R in the WT IELs (Fig. 5F). VDR KO DN TCRβ+ IELs failed to express the IL-15R (0.6%; Fig. 5F). Exogenously delivered IL-15 induces the expression of CD8αα in activated thymocytes (8). Twenty percent of the cells expressed CD8αα when WT thymocytes were activated and cultured with IL-15 (Fig. 5G). Ten percent or half as many of the similarly treated VDR KO thymocytes upregulated CD8αα in response to IL-15 (Fig. 5G). The data point to defects in DN cell expression of the IL-15R and intrinsic defects in the response to exogenous IL-15 in the absence of the VDR.

**Discussion**

We show here that there is a T cell intrinsic defect in VDR KO CD8αα T cell development that is TCRβ T cell specific. In VDR KO mice, TP thymocyte development was normal during fetal development and in the adult thymus, but there are fewer TP thymocytes during the first 3 wk of life. VDR KO DN precursors failed to mature and proliferate under the influence of IL-15 due to the low expression of IL-15R. These results identify the VDR as an important regulator of homeostasis, development, maintenance, and proliferation of CD8αα+ TCRβ+ cells in the IEL.

The GALT can support extrathymic development of CD8αα T cells as evident by the discovery that T cells can be found in the IEL compartment of athymic nude mice (10). The majority of the T cells found in athymic mice are CD8αα+ TCRγδ+ T cells, but the numbers were ~4-fold less than those of normal euthymic mice, and very few CD8αα+ TCRβ+ IELs existed in athymic mice (10). These discoveries indicated that the thymus is more important for the development of CD8αα+ TCRβ+ than CD8αα+ TCRγδ+ IELs. Under normal physiological conditions, TCRγδ+ T cell development occurs both thymically and extrathymically during fetal development and continues through adulthood (21). The discovery that TP cells are thymic precursors of CD8αα+ TCRβ+ IELs supports a thymic requirement for these cells (8). The ability of CD8αα+ TCRγδ+ T cells to develop normally in VDR KO mice suggests that signals specific for CD8αα+ TCRβ+ T cells are provided by the GALT.
T cell development such as thymic selection may be VDR dependent. The development of both CD8αα⁺ TCRαβ⁺ and CD8αα⁺ TCRγδ⁺ IELs is severely impaired in the absence of IL-15, IL-15Ra, or IL-15/IL-2RB (9). In addition, NK cells and memory CD8⁺ T cells fail to develop in the absence of IL-15 and/or the IL-15 receptors (9). IL-15 is produced and trans-presentation via the IL-15Ra by the epithelial cells of the intestine (9). Because VDR KO mice had normal numbers of CD8αα⁺ TCRγδ⁺ IELs and WT CD8αα⁺ TCRαβ⁺ IELs developed normally in a VDR KO host, expression and presentation of IL-15 by intestinal epithelial cells must be adequate for induction of these IL-15–dependent cell types. Furthermore, the selective defect in only the CD8αα⁺ TCRαβ⁺ IEL suggests that there are not global VDR-mediated effects on the IL-15 pathway. We found that VDR KO DN TCRαβ⁺ T cells failed to become IL-15R⁺ and proliferated less than WT DN T cells in the IELs. The severe reduction in the level of proliferation in VDR KO DN TCRαβ⁺ reflects the loss of IL-15 signaling in only this cell type. In addition, thymocytes from VDR KO mice expressed normal amounts of the IL-15R but failed to respond to exogenous IL-15 as well as the WT thymocytes to upregulate CD8αα expression. These data indicate that in the VDR KO mice, CD8αααα precursors also have a defect in IL-15R signaling. The maturation of CD5⁺ DN TCRαβ⁺ T cells requires IL-15, and VDR KO IELs contain a higher frequency of CD5⁺ DN TCRαβ⁺ T cells (8). The increased percentage of VDR KO DN TCRαβ⁺ T cells that remain in an immature CD5⁺ state also reflects a lack of IL-15 signaling and likely contributed to the overall reduction of the mature CD8αα⁺ TCRαβ⁺ T cells in the IELs of VDR KO mice. The data suggest that expression of the VDR and other transcription factors found only in the precursor of the CD8αα⁺ TCRαβ⁺ are required for IL-15R signaling and to upregulate IL-15R expression that results in survival, maturation, and CD8αα⁺ expression in the IELs. Other IL-15–dependent cells do not use vitamin D to regulate IL-15R expression.

iNKT cell development is also impaired in VDR KO mice (18). CD8αα⁺ TCRαβ⁺ T cells have been called “gut NKT cells” because of their expression of NK receptors (6). Like NKT cells, CD8αα⁺ TCRαβ⁺ T cells express NK receptors including members of the Ly49 family, CD94 and NKR-IL, and use the invariant signaling component FcRγI as a part of their CD3 complex (6, 22). Both cells have a memory phenotype and rapidly produce cytokines upon stimulation (6, 22). In addition, both cells can develop in the absence of MHC class I or class II but do require β2-microglobulin (6, 11, 22). An interesting similarity between CD8αα⁺ TCRαβ⁺ T cells and NKT cells is their developmental dependence on expression of the NF-κB family transcription factor RelB (6). RelB deficiency is associated with autoimmunity that is believed to be linked to dysfunctional T cell selection in the thymus, which allows the survival of conventional T cells that express high-affinity receptors that would normally be deleted during the selection process (23, 24). The reduced numbers of CD8αα⁺ TCRαβ⁺ and NKT cells and the survival of conventional T cells with self-reactive TCR suggests that RelB plays a role in thymic function during agonist selection (6). Notably, RelB is transcriptionally regulated by vitamin D and has been shown to have VDR response elements in its promoter in both mice and humans (25). It would be interesting to determine what role of RelB is in the VDR-mediated development of CD8αα⁺ TCRαβ⁺ cells.

The process of agonist selection allows T cells carrying “forbidden repertoires” to survive the selection process but reprograms the cell to be regulatory in nature, carrying a self-reactive TCR while maintaining a nondestructive function (6, 10). Both CD8αα⁺ TCRαβ⁺ and NKT cells undergo agonist selection in the thymus as DP cells (6, 22). Each cell type then diverges along a separate developmental pathway. NKT cells that survive selection downregulate CD8 or CD4 and CD8, rapidly expand, and upregulate expression of CD44 and NK1.1 (22). At the DP stage, CD8αα⁺ TCRαβ⁺ precursors transition to a TP stage in a pre-TCR signal dependent manner (8). The TP thymocytes complete TCR rearrangement and undergo agonist selection (8). After surviving selection, TP cells downregulate all CD4 and CD8 becoming DN TCRαβ⁺ T cells that egress the thymus and continue their maturation in the gut under the influence of IL-15 (8). The similarities in the selective requirement for the VDR in CD8αα⁺ TCRαβ⁺ and NKT cells suggest a common mechanism for regulation. Further investigation is needed to determine the role of vitamin D signaling in the thymus during agonist selection.

Vitamin D and signaling through the VDR are important for regulating intestinal health. VDR deficiency increases the severity of inflammation in several mouse models of intestinal disease (13). The prevalence of Crohn’s disease and ulcerative colitis is higher in northern versus southern climates and urban versus rural areas (26, 27). These are also factors that correlate with vitamin D deficiency. Several studies have reported deficiencies in adult and pediatric patients with IBD, and vitamin D deficiency is common even when the patient is in remission (28, 29). VDR KO and vitamin D-deficient mice are highly susceptible to multiple different models of experimental IBD (13, 30, 31). VDR KO mice do not develop overt inflammation, but there is microscopic evidence of increased TNF-α and IFN-γ in the gastrointestinal tract of the VDR KO mouse, and as it ages the amounts and variety of cytokines increased [IL-12 was detected (32)]. The data suggest that IBD is not a vitamin D-deficiency disease but rather vitamin D may be an environmental factor that regulates inflammation in the gut. Genome-wide association studies have shown that polymorphisms within the VDR gene increase susceptibility to IBD (33, 34). It seems likely that reduced signaling through the VDR would limit the development of CD8αα⁺ TCRαβ⁺ T cells in the human gut.

We have shown that there is an intrinsic and specific defect in the development of VDR KO CD8αα⁺ TCRαβ⁺ T cells. TP precursors in the thymus are reduced in neonatal VDR KO mice. Immature DN TCRαβ⁺ T cells fail to upregulate the IL-15R, proliferate less, and maintain a more immature phenotype. CD8αα⁺ TCRαβ⁺ T cells are important regulators of intestinal immune responses, and their failure to mature in VDR KO mice likely contributes to the increased susceptibility of these mice to intestinal inflammation.

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Disclosures
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References


