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TL1A Synergizes with IL-12 and IL-18 to Enhance IFN-γ Production in Human T Cells and NK Cells

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TL1A, a recently described TNF-like cytokine that interacts with DR3, costimulates T cells and augments anti-CD3 plus anti-CD28 IFN-γ production. In the current study we show that TL1A or an agonistic anti-DR3 mAb synergize with IL-12/IL-18 to augment IFN-γ production in human peripheral blood T cells and NK cells. TL1A also enhanced IFN-γ production by IL-12/IL-18 stimulated CD56+ T cells. When expressed as fold change, the synergistic effect of TL1A on cytokine-induced IFN-γ production was more pronounced on CD4+ and CD8+ T cells than on CD56+ T cells or NK cells. Intracellular cytokine staining showed that TL1A significantly enhanced both the percentage and the mean fluorescence intensity of IFN-γ-producing T cells in response to IL-12/IL-18. The combination of IL-12 and IL-18 markedly up-regulated DR3 expression in NK cells, whereas it had minimal effect in T cells. Our data suggest that TL1A/DR3 pathway plays an important role in the augmentation of cytokine-induced IFN-γ production in T cells and that DR3 expression is differentially regulated by IL-12/IL-18 in T cells and NK cells. The Journal of Immunology, 2004, 172: 7002–7007.

Interferon-γ, a hallmark cytokine produced by cells of the innate immune system and differentiated Th1 cells, plays an important role in the activation of monocytes and elimination of intracellular pathogens but it also participates in the pathogenesis of a variety of chronic autoimmune and inflammatory processes (1, 2). IFN-γ production by naive CD4+ T cells is coordinately induced by cytokines and costimulatory molecules that are expressed on APCs. Initial production of even small amounts of IFN-γ could initiate Th1 polarization in which STAT-1 activation by IFN-γ stimulates expression of T-bet (3), a Th1 “master switch” that up-regulates and stabilizes the expression of IL-12Rβ2 (4). IL-12 acting through STAT-4 powerfully reinforces Th1 polarization. IL-27, which interacts with WSX-1 (a novel class I cytokine receptor), augments IL-12-dependent IFN-γ production in naive CD4+ T cells through stimulation of T-bet and IL-12Rβ2 expression (5). IL-12 also up-regulates IL-18R on T cells and, in synergy with IL-18, further augments IFN-γ production (6–9). IL-23, a recently described cytokine, augments IFN-γ production but exerts its effect almost exclusively on memory T cells (10).

Differentiated Th1 cells produce IFN-γ upon TCR engagement, which is enhanced by costimulation with anti-CD28 Abs (11). In addition to TCR activation and CD28 costimulation, TNF and TNF-like molecules may further enhance IFN-γ production by T cells (12–16). Differentiated Th1 cells can directly produce IFN-γ in response to IL-12/IL-18 in the absence of TCR engagement in a process termed cytokine-induced IFN-γ production (17). The synergistic effect of IL-12/IL-18 on IFN-γ production is not inhibited by cyclosporin A and requires new protein synthesis (17). A similar synergistic effect of IL-12 with IL-18 for the induction of IFN-γ production has been shown for NK cells (18, 19). Several other cytokines, such as IL-15, IL-21, and IFN-α have also been shown to have a synergistic effect with IL-12 or IL-18 in inducing IFN-γ production in T and NK cells (2, 20, 21). One of the mechanisms that could partially explain the synergism of IL-12 and IL-18 for IFN-γ induction is the reciprocal up-regulation of cytokine receptors in responding cells (6, 22, 23). In addition the synergistic effect of IL-18 and IL-12 could also be mediated by simultaneous activation and cooperation of STAT-4, NF-κB, and/or AP-1 that leads to optimal activation of the IFN-γ transcription (9, 17, 24–27). Recent reports demonstrated that the combination of IL-12 and IL-18 strongly induced the expression of growth arrest and DNA damage (GADD)45β (28), an activator of mitogen-activated protein kinase (MAPK)-extracellular signal-regulated kinase kinase 4 (MEKK4), which in turn activates p38 MAPK, previously linked to IFN-γ production in CD4+ T cells (29, 30).

TL1A, a new TNF-like cytokine, was recently identified as the ligand for death domain receptor 3 (DR3), a TNF-superfamily receptor member with highest homology to TNFR1 (31). Expression of DR3 is restricted to lymphocytes and is up-regulated upon activation. TL1A interaction with DR3 leads to NF-κB activation in cells expressing endogenous receptor. TL1A increases IL-2 responsiveness and induces IFN-γ and GM-CSF release in anti-CD3 and anti-CD28 stimulated T cells (31). Recently, we have shown that TL1A or an agonistic anti-DR3 mAb enhances IFN-γ production by anti-CD3- or anti-CD2-stimulated PBL or by lamina propria lymphocytes, independently of, but in synergy with IL-12 and IL-18 (32, 33). Moreover, TL1A and DR3 expression are increased in inflamed intestinal mucosa in Crohn’s disease (33, 34). Several animal models of mucosal inflammation are characterized by an exaggerated Th1 immune response and the level of mucosal or mesenteric nodal T cell expression of IFN-γ directly correlates with the magnitude of inflammation (35).

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suggesting a potential role for TL1A/DR3 in the augmentation of Th1 responses in Crohn’s disease.

In the present report, we describe our studies of the effect of TL1A or anti-D3 mAb in the cytokine-induced IFN-γ production in PBL, T cell subsets, and NK cells. We show that the TL1A/DR3 costimulatory pathway enhances the cytokine-induced IFN-γ production in peripheral blood (PB) T cells and NK cells. Our data show differential regulation of IFN-γ production in CD4+, CD8+ T cells, and NK cells in response to TL1A and of DR3 expression in these cell subsets upon combined IL-12/IL-18 stimulation.

Materials and Methods

Cytokines and Abs

rIL-12 was from PeproTech (Rocky Hill, NJ). IL-18 was from R&D Systems (Minneapolis, MN). These cytokines were used at concentrations ten times the approximate ED50 reported by the supplier, 1 ng/ml for IL-12 and 50 ng/ml for IL-18 as indicated in each figure. Reagents were generated at Human Genome Sciences (Rockville, MD), including recombinant TL1A (aa 72–251) and anti-DR3 mAb (clone F05, IgG1) (31, 36). Isotype-or species-specific control Abs were from Jackson Immunoresearch Laboratories (West Grove, PA). Anti-CD3, anti-CD4, anti-CD8, anti-CD45RA, anti-CD45RO, anti-CD56 fluorochrome-conjugated Abs were from Caltag Laboratories (South San Francisco, CA). Anti-IFN-γ PE-conjugated Ab and isotype mouse IgG1-PE for intracellular cytokine staining were purchased from BD PharMingen (San Diego, CA).

Cell isolation and culture

Blood was obtained from normal donors after informed consent in accordance with the Human Subjects policy of the Cedars-Sinai Medical Center Institutional Review Board. PBMC were isolated on standard Ficoll/Hypaque density gradients, depleted of monocytes by culture in RPMI 1640 containing 2 mM glutamine and 25 mM HEPES buffer (Mediatech, Herndon, VA), supplemented with 10% heat inactivated FBS (Atlanta Biologicals, Norcross, GA), 0.2% amphotericin B (Gemini Bio-Products, Woodland, CA), or purified T cell subsets and NK cells (1.0 × 10^6/ml) were stimulated with IL-12, IL-18 singly or in combination with or without TL1A or anti-D3 mAb as described in the figure for 72 h. Preliminary experiments showed that the duration of TL1A incubation for optimal IFN-γ production by IL-12/IL-18-stimulated PBL or cell subsets was between 64 h and 72 h (see also Fig. 2). Supernatants were stored at −70°C for later analysis of IFN-γ content by ELISA.

Cell staining for flow cytometry

To study surface DR3 expression in T and NK cells, PBL were blocked with 5% FITC-labeled mouse IgG for 20 min on ice and incubated with anti-DR3 specific monoclonal or isotype control Ab for 30 min on ice. The cells were then washed with PBS/BSA/azide and incubated with a secondary anti-mouse Ab conjugated to PE (Caltag Laboratories) for 30 min on ice. After washing with PBS/BSA/azide and blocking with mouse IgG for 20 min, cells were stained with CD3-PE, CD56-TRIClr, and CD69-Alexa 647 for 30 min on ice and analyzed by flow cytometry (BD Biosciences, Mountain View, CA). DR3 expression was analyzed on T cells, CD56+ cells, and NK cells first by gating on lymphocytes based on their forward and side scatter and then on CD3+CD56−, CD3+CD56+, and CD3−CD56− cells, respectively. A total of 5 × 10^6 cells were acquired and both the percentage of cell subsets expressing DR3 and the mean fluorescence intensity (MFI) of DR3+ cells were analyzed. Percentage of non-specific staining with the isotype control Ab was subtracted from the percentage of specific staining for each cell subset.

Results

Synergistic effect of TL1A or anti-D3 mAb on IL-12/IL-18-induced IFN-γ production in PBL

In our previous studies, we showed that TL1A or anti-D3 mAb augments IFN-γ production by anti-CD3- or anti-CD2-stimulated PBL or lamina propria lymphocyte independently of, but in synergy with IL-12 and IL-18 (32, 33). However, TL1A activity required TCR activation and costimulatory molecule engagement for T cell IFN-γ production (31). Because T cells can produce IFN-γ in response to cytokines, notably IL-12 and IL-18 (17), we characterized in more detail the effect of the TL1A/DR3 pathway on cytokine-induced IFN-γ production in PBL. As shown in Fig. 1, addition of TL1A to PBL cultures incubated individually with IL-12 or IL-18 did not enhance IFN-γ production. However, addition of TL1A to PBL cultures with both IL-12 and IL-18 markedly enhanced IFN-γ production (Fig. 1A). There was a 4-fold increase in IFN-γ production by IL-12/IL-18-stimulated PBL with the addition of 100 ng/ml TL1A (Fig. 1A), indicating that the combination of both IL-12 and IL-18 is required for the TL1A augmentation of IFN-γ production in PBL. Although there was wide variability in the amount of IFN-γ production between donors, TL1A dose dependently enhanced IFN-γ production in PBL stimulated with the combination of IL-12/IL-18 (Fig. 1B). Incubation of PBL with an agonistic anti-D3 mAb also enhanced IL-12/IL-18-induced IFN-γ production by several fold (Fig. 1C). Similarly to TL1A, anti-D3 mAb did not enhance IFN-γ production when PBL were incubated individually with IL-12 or IL-18 (Fig. 1C).

Differential effect of TL1A on cytokine-induced IFN-γ production in T cells and NK cells

We next examined the time kinetics of IFN-γ secretion in PBL, T cells, and NK cells because IL-12/IL-18-induced IFN-γ production has been described for both T and NK cells (18, 20, 38). As shown in Fig. 2A, the total amount of IFN-γ production continues to increase over time in T cells compared with NK cells in response to IL-12/IL-18 stimulation in the presence of TL1A. However, the
fold enhancement of IFN-γ production from PBL or T and NK cell subsets was not different at 24, 48, or 72 h (Fig. 2B). Therefore, 72 h was used as the optimal time point for IFN-γ analysis in subsequent experiments. We next examined the effect of TL1A on cytokine-induced IFN-γ production on purified memory (CD45RO+) naive (CD45RA+), CD4+, CD8+, T cells, and NK cells. We also examined the effect of TL1A on the small subset of PB CD56+ T cells. They represent ~2.8% of PBL, are predominantly CD8+, and express αβ or γδ TCRs. They also express activation/memory markers and a variety of NK receptors and can lyse NK-sensitive target cell lines in vitro (37). They can be activated by TCR ligation or in response to cytokines and they can produce proinflammatory (Th1) and Th2 cytokines (37). Sorted CD45RO+ or CD45RA+ T cells along with purified CD4+, CD8+ (both depleted of CD56+ T cells), and CD56+ T cells or NK cells were incubated with IL-12/IL-18 with or without TL1A for 72 h, and IFN-γ was measured in the culture supernatants (Fig. 3A). To ascertain potential differences of PBL subset sensitivity to TL1A, we used low concentrations, ranging from 2 to 10 ng/ml, compared with the dose used in IL-12/IL-18-stimulated unfractionated PBL or T cells activated through their TCR. Fig. 3A, shows that purified PB CD4+ and CD8+ T cells exhibit a TL1A dose-dependent augmentation of IL-12/IL-18-induced IFN-γ production. TL1A enhanced IL-12/IL-18-induced IFN-γ production predominantly in memory compared with naive T cells without differential expression of DR3 in the presence of IL-12 and IL-18 between the two T cell subsets (data not shown). TL1A had a similar augmenting effect on IL-12/IL-18 induction of IFN-γ in CD56+ T cells and NK cells (Fig. 3A). Although the absolute amount of IFN-γ produced by CD56+ T cells or NK cells was significantly higher compared with that of purified CD8+ and CD4+ T cells in response to IL-12/IL-18 (Fig. 3A), the fold-induction of IFN-γ production in response to TL1A was significantly higher in purified CD4+ and CD8+ T cells than in either CD56+ T cells or NK cells (Fig. 3B). No detectable levels of IFN-γ were found in any subset culture supernatants without IL-12/IL-18 stimulation (data not shown). Our data indicate differential sensitivity of T and NK cells to TL1A stimulation in the presence of IL-12 and IL-18. Interestingly the sensitivity to TL1A stimulation was severalfold higher in CD4+ compared with CD8+ T cells (Fig. 3B).

Differential regulation of DR3 expression in response to IL-12/IL-18 in T cells and NK cells

One potential mechanism, by which IL-12/IL-18 combination may enhance TL1A-induced IFN-γ production in T and NK cells, is through its effect on DR3 receptor expression. Therefore, we studied DR3 expression in T and NK cells in response to IL-12/IL-18. To this end, PBL were incubated with IL-12, IL-18 singly or in combination and DR3 expression was examined in each cell subset by flow cytometry as described in Materials and Methods (Fig. 4). As shown in Fig. 4, B and C, either individually or combined IL-12 and IL-18 had minimal effect on DR3 expression in T cells, which is in contrast to up-regulation of DR3 expression following TCR activation (32). IL-12 alone slightly increased DR3 expression in CD56+ T and NK cells (Fig. 4B). Remarkably, IL-18 alone significantly increased the percentage of DR3+ cells in both
CD56+ T cells and more so in NK cells (Fig. 4, B and C). The combination of IL-12 and IL-18 further enhanced the expression of DR3 on CD56+ T cells and dramatically increased DR3 expression in NK cells (Fig. 4, B and C). The percentage of NK cells increased from <0.5% to 74 ± 5.5% (n = 3) after 48 h of incubation with the IL-12/IL-18 cytokine combination. Further analysis of the MFI of DR3 expression among the different PBL subsets analyzed revealed that individually IL-12 or IL-18 did not affect the MFI of DR3 expression in either T cells, CD56+ T cells, or NK cells (data not shown). However, both IL-12 and IL-18 increased the MFI of DR3 expression in CD56+ T cells and more so in NK cells, but not in T cells. In IL-12- and IL-18-treated PBL, the MFI of DR3-positive cells increased from 10 to 25 ± 9.8 (n = 3) in CD56+ T cells, and from 8 to 22 ± 6 (n = 3) in NK cells. Taken together our data suggest that the combination of IL-12 and IL-18 has differing effects on DR3 expression in T cells, CD56+ T cells and NK cells. The combination of IL-12 and IL-18 had its most dramatic effect on NK cells in terms of DR3 induction, although TL1A led to higher-fold IFN-γ production by purified T cells rather than NK cells.

**FIGURE 4.** Regulation of DR3 expression in T cells, CD56+ T, and NK cells in response to IL-12 and IL-18. PBL were incubated individually with IL-12 and IL-18 or the combination of both cytokines (IL-12/18) for 48 h, stained with CD3-FITC, CD56-tricolor, and DR3-PE, and analyzed by flow cytometry. A, The indicated gates were used to analyze expression of DR3 in CD3+CD56+ (R2), NK (R3), and CD56+ T cells (R4). B, Percentage of each cell subset that expresses DR3 (mean ± SEM of three experiments). C, A representative experiment of DR3 expression in each cell subset is shown.

**FIGURE 5.** IFN-γ production at the single cell level in PBL subsets in response to IL-12/IL-18 and TL1A stimulation. PBL were incubated with IL-12 plus IL-18 (IL-12/18) with (IL-12/18+TL1A) or without TL1A for 72 h, incubated with brefeldin A (10 μg/ml) for the last 5 h, stained with CD3-FITC and CD56-tricolor, fixed and permeabilized with saponin. The fixed and permeabilized cells were stained with anti-IFN-γ-PE or an isotype control mAb. A, The indicated gates were used to analyze IFN-γ staining in CD3+CD56+ (R2), NK (R3), and CD3+CD56+ (R4) cells. B, The percentage of IFN-γ-producing cells (top) and their MFI (bottom) were analyzed. The data represent the mean ± SEM of five experiments. C, A representative histogram of IFN-γ staining in each cell subset is shown.
Analysis of IFN-γ production at the single cell level in response to IL-12/IL-18 and TL1A

To determine whether the augmenting effect of TL1A on cytokine-induced IFN-γ production is the result of a larger number of cells producing IFN-γ or of the amount of IFN-γ produced on a per cell basis, IL-12/IL-18-stimulated T cells, CD56+ T cells, and NK cells were analyzed for IFN-γ production at the single cell level by flow cytometry (Fig. 5). As shown in Fig. 5B, a small percentage of PB T cells produce IFN-γ in response to the combination of IL-12/IL-18, and addition of TL1A enhanced the percentage of IFN-γ-producing T cells. Similarly, the percentage of IFN-γ-producing cells was increased by TL1A in CD56+ T cells and NK cells (Fig. 5B). In addition, TL1A enhanced the MFI of IFN-γ-positive cells in both T cells and CD56+ T cells (Fig. 5C), indicating that TL1A augments IL-12/IL-18-induced IFN-γ production by both increasing the percentage of responding cells and the amount of IFN-γ produced on a per cell basis. In contrast, TL1A had minimal effect on the MFI of IFN-γ staining in NK cells (Fig. 5C). Taken together our data indicate that the augmentation of IL-12/IL-18-induced IFN-γ in response to TL1A stimulation is differentially induced in T cells and NK cells.

Discussion

In this study, we showed that TL1A dose dependently enhanced IL-12/IL-18-induced IFN-γ production by unfractionated PBL. Similar results were obtained when an agonistic anti-DR3 mAb was used in conjunction with IL-12 and IL-18. Further characterization of PBL subsets revealed that TL1A directly augments IL-12/IL-18-induced IFN-γ production in purified CD4+, CD8+ T cells, NK cells, and CD56+ T cells in a dose-dependent manner. Interestingly, TL1A had the major effect in terms of the degree to which IFN-γ was induced primarily in CD4+ and CD8+ T cells. Although, CD56+ T cells and NK cells produced significantly more IFN-γ compared with purified CD4+ or CD8+ T cells in response to IL-12/IL-18, the fold-induction of IFN-γ production by the addition of TL1A was significantly higher in CD4+ and CD8+ T cells compared with NK cells or CD56+ T cells. Conversely, DR3 expression was markedly up-regulated in NK cells by the combination of IL-12/IL-18 but was minimally increased in T cells. These data indicate that the TL1A/DR3 pathway, like other ligand/receptor pairs of the TNFR family, is important in the amplification of the Th1-immune response and a critical pathway in the augmentation of cytokine-induced IFN-γ production in T cells. In addition, the data suggest that the IL-12/IL-18 cytokine combination augments IFN-γ production by T cells stimulated with TL1A by enhancing postreceptor signaling that converge to IFN-γ production, whereas in NK cells could be mostly through DR3 receptor up-regulation and recruitment of more NK cells that respond to TL1A.

The higher sensitivity of CD4+ and CD8+ T cells compared with CD56+ T cells or NK cells to TL1A stimulation suggests that TL1A/DR3 pathway plays a critical role in the augmentation of Th1 immune responses but has a less pronounced effect on NK cell IFN-γ production. However, TL1A in the presence of IL-12 and IL-18, which markedly up-regulated its receptor DR3, may have additional effects in NK cells, such as secretion of other cytokines or enhanced cytotoxicity (39). Further studies are needed to examine the effect of TL1A on functional aspects of NK cells.

Several studies have demonstrated the synergistic effect of IL-12 and IL-18 in the induction of IFN-γ in activated T and NK cells (18, 38). The synergistic effect of other cytokines, such as IL-15, IL-21, and IFN-α, when combined with IL-18 has also been reported to augment IFN-γ production in T and NK cells (2, 21). The synergism between IL-12 and IL-18 in T cell IFN-γ production could be partly mediated through reciprocal regulation of cytokine receptors in the responding cells (6, 22). Yang et al. (28) have argued that the synergistic effect of IL-12 and IL-18 on IFN-γ production in T cells involves interactions in pathways that are downstream of both cytokine receptors that directly regulate IFN-γ transcription. The synergy between IL-12 and IL-18 involves GADD45β induction (28), an activator of MEK4 and p38 MAPK that is necessary for cytokine-induced, but not TCR-induced, IFN-γ production (29).

The mechanism by which TL1A further augments IL-12/IL-18-induced IFN-γ production in T cells is currently unknown. TL1A markedly enhanced the IL-12/IL-18-induced IFN-γ in T cells by augmenting both the IFN-γ production on a per cell basis and the number of IFN-γ-producing cells as shown by intracellular cytokine staining. TL1A clearly enhanced the MFI of intracellular IFN-γ suggesting that the synergistic effect of TL1A in IL-12/IL-18-induced IFN-γ production in T cells involves pathways that are downstream of cytokine receptors. TL1A or an agonistic anti-DR3 mAb was recently shown to activate NF-κB, c-Jun N-terminal kinase/stress-activated protein kinase, p38 and extracellular signal-regulated kinase 1, and two MAPK in the human TF-1 erythroleukemic cell line (36). Several mechanisms may explain the synergism of TL1A with IL-12 and IL-18 in the induction of IFN-γ production in T cells. First, although TL1A induces transient p38 MAPK activation in TF-1 cells (36), it may be more pronounced and sustained in the presence of IL-12 and IL-18 through induction of GADD45β or other pathways. GADD45β induction could in turn be mediated through TL1A-induced NF-κB activation. All the signaling pathways may then converge to optimally activate IFN-γ gene transcription in response to TL1A, IL-12, and IL-18. However, further studies are needed to dissect the molecular mechanism by which TL1A augments the cytokine-induced IFN-γ production in T cells. Although, TL1A augments both the TCR-induced and cytokine-induced IFN-γ production, our previous studies have shown that higher levels of TL1A are required to augment TCR-induced compared with cytokine-induced IFN-γ production (32, 33) suggesting that TL1A may play a more prominent role in augmenting the cytokine-induced IFN-γ production. Interestingly, analysis of IFN-γ production at the single cell level showed that only a small percentage of PB T cells respond to IL-12/IL-18 stimulation with or without TL1A indicating that only a small number of T cells are poised to produce large amounts of IFN-γ upon combined stimulation with IL-12/IL-18 and TL1A. In addition, we showed that the mechanism of TL1A augmentation of IL-12/IL-18-induced IFN-γ production in T cells and CD56+ T cells is through both increasing the percentage of IFN-γ-producing cells and the amount of IFN-γ produced on a per cell basis, whereas the mechanism in NK cells is through increasing the percentage of IFN-γ-producing cells.

TL1A is expressed in a subset of activated T cells and is induced in HUVEC by proinflammatory cytokines such as TNF-α or IL-1, it could be involved in endothelial-T cell interactions and T cell costimulation and IFN-γ production in inflammatory diseases (40, 41). In addition the expression of cell membrane-associated TL1A by a subset of activated T cells also implicates T-T cell interactions through cell membrane-associated TL1A/DR3 in augmentation of IFN-γ production at sites of inflammation (32, 33). Other ligand/receptor pairs of the TNFR family, such as CD154-CD154 ligand (35), OX40-OX40 ligand (42), LIGHT/TNFRSF14 (43, 44), have been shown to play a dominant role in the pathogenesis of animal models of Th1-mediated inflammatory diseases. Because TL1A/DR3 plays a prominent role in augmenting cytokine-induced IFN-γ production in T cells, and elevated levels of tissue IL-18 and IL-12 have been reported for several inflammatory diseases such as Crohn’s disease (45, 46), interfering with the TL1A/DR3 pathway using specific inhibitors may, therefore, prove highly effective in the treatment of several Th1-mediated diseases.

In summary, we show that TL1A synergizes with IL-12 and IL-18 to augment IFN-γ production in PB T cells, CD56+ T cells, and NK
in addition DR3 is differentially regulated by these cytokines in T and NK cells. When expressed as fold change, the synergistic effect of TL1A with IL-12 and IL-18 on IFN-γ production was more pronounced in T cells, primarily CD4+ cells, compared with NK cells, indicating an important role of the TL1A/DR3 pathway in the augmentation of cytokine-induced IFN-γ production in T cells.

References


