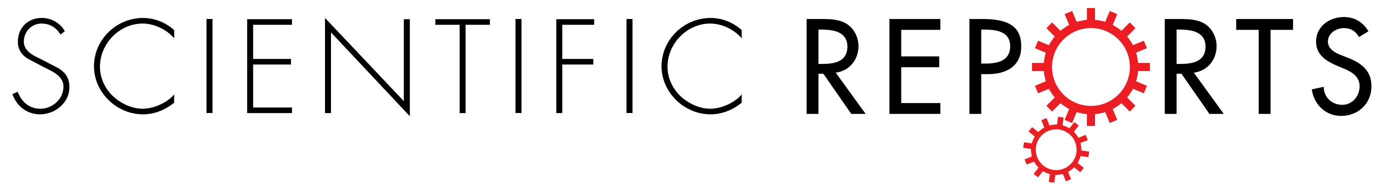

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Trans-presentation of IL-15 modulates STAT5 activation and Bcl-6 expression in T_H1 cells

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During infection, naïve CD4$^+$ T helper cells differentiate into specialized effector subsets based upon environmental signals propagated by the cytokine milieu. Recently, this paradigm has been complicated by the demonstration that alterations in the cytokine environment can result in varying degrees of plasticity between effector T helper cell populations. Therefore, elucidation of the mechanisms by which cytokines regulate T helper cell differentiation decisions is increasingly important. The gamma common cytokine IL-15 is currently undergoing clinical trials for the treatment of malignancies, due to its well-established role in the regulation of natural killer and CD8$^+$ T cell immune responses. However, the effect of IL-15 signaling on CD4$^+$ T cell activity is incompletely understood. One mechanism by which IL-15 activity is conferred is through trans-presentation via the IL-15 receptor alpha subunit. Here, we demonstrate that differentiated T_H1 cells are responsive to trans-presented IL-15. Importantly, while trans-presentation of IL-15 results in STAT5 activation and maintenance of the T_H1 gene program, IL-15 treatment alone allows for increased Bcl-6 expression and the upregulation of a T_{FH}-like profile. Collectively, these findings describe a novel role for IL-15 in the modulation of CD4$^+$ T cell responses and provide valuable insight for the use of IL-15 in immunotherapeutic approaches.

An effective immune response requires the orchestrated activity of a cohort of immune cell types. At the center of this coordinated response are CD4$^+$ T helper cells, which provide an instrumental link between the innate and adaptive arms of the immune system. These include T helper 1 (T_H1), T_H2, T_H17, and T follicular helper (T_{FH}) cell populations, all of which arise from naïve CD4$^+$ T cell progenitors[1–5]. The development of each of these effector T helper cell subpopulations is dictated by the expression of specific transcription factors, termed lineage-defining factors, the expression and activity of which are dependent upon the cytokine environment present during the activation of the naïve CD4$^+$ T cell[6–12]. Importantly, each of these populations is responsible for performing unique effector functions that range from the cell-mediated release of inflammatory cytokines during pathogenic infection to aiding in the production of humoral immunity. Despite these distinct roles, numerous studies have recently demonstrated that flexibility, or plasticity, between effector T helper cell populations can occur in response to alterations in the cytokine environment[2,13–16]. Based in part upon these insights, novel sets of immunotherapeutic tools are being developed in an effort to manipulate the immune response and capitalize on the flexible nature of T helper cell populations[17–21]. As such, it is increasingly important to understand how changes to the cytokine environment effect not only the initial differentiation of activated naïve CD4$^+$ T cells, but also the flexibility of established effector T helper cell populations.

The cytokines IL-2 and IL-15 are well-established regulators of immune cell development and function[22–24]. Both IL-2 and IL-15 signal through hetero-trimeric receptors, which are made up of the gamma common (γc) and IL-2Rβ subunits, as well as specificity-conferring IL-2Rα and IL-15Rα subunits,

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respectively[19,22]. Like IL-2, IL-15 signals through intracellular domains of the γc and IL-2Rβ subunits, which employ the janus kinase 1 (Jak1) and Jak3 kinases to phosphorylate and activate the transcription factor STAT5[19,22]. However, IL-15 is capable of signaling through a unique "trans-presentation" mechanism, which involves the expression of IL-15Rα in complex with IL-15 on the surface of a presenting cell[24–28]. This complex interacts with the remaining two thirds of the receptor on a target cell, resulting in the propagation of IL-15 signaling within that cell. Trans-presentation has been shown to increase the affinity of IL-15 for the γc and IL-2Rβ receptor subunits, leading to more robust signaling as compared to IL-15 alone[29–31].

The role of IL-15 in the stimulation of $CD8^+$ cytotoxic T and natural killer (NK) cell populations is well-characterized and has provided strong pre-clinical support for its use in cancer and vaccine immunotherapeutic approaches[24,27,32,33]. Currently, IL-15 is undergoing Phase I/II Clinical trials for the treatment of malignant melanoma and renal cell carcinoma[32–34]. However, the effects of IL-15, particularly in the context of trans-presentation, on $CD4^+$ T cell differentiation and effector function are incompletely understood. Therefore, an increased understanding of the effects of IL-15 signaling on the diverse repertoire of $CD4^+$ T cell gene programs is a critical component in not only understanding how this cytokine may affect the natural immune response, but also in helping to unravel its therapeutic potential.

In the present study, we investigated the effect of IL-15 signaling on $CD4^+$ T_H1 differentiation, using a well-established model of T_H1 cell generation[35,36]. We demonstrate that exposure of T_H1 cells to trans-presented IL-15, but not to IL-15 alone, favors the T_H1 gene program. Mechanistically, increased STAT5-activation via trans-presented IL-15 was associated with the expression of T_H1-associated genes including *Tbx21*, *Ifng*, *Prdm1*, *Il12ra* and *Il12rb*, as well as enhanced IL-12-dependent STAT4 phosphorylation. Strikingly, exposure of T_H1 cells to IL-15 in the absence IL-15Rα resulted in decreased STAT5 activation and increased expression of the T_{FH} lineage defining transcription factor Bcl-6. Elevated Bcl-6 expression levels correlated with a decrease in Blimp-1 expression and the upregulation of a T_{FH}-like gene program including *Cxcr5*, *Il6ra*, *Tnfsf8*, and *Sh2d1a*. Collectively, these results describe previously unappreciated effects of IL-15 signaling on the differential expression of T_H1 and T_{FH} gene profiles, and provide added insight into the functional properties of this immunotherapeutic cytokine.

Results

Exposure to IL-15 alone is insufficient to maintain expression of the T_H1 gene program. It has become increasingly evident that mature effector $CD4^+$ T cells are responsive to alterations in environmental stimuli in the form of cytokines[13–15,37–39]. The adaptability of effector T helper cell populations allows these cells to respond to the environment in real-time, and is particularly important in clinical applications where the use of cytokines or cytokine neutralizing antibodies is employed to promote or abrogate specific immune responses. For example, previous work has demonstrated that signaling through the gamma common (γc) cytokine IL-2 is indispensable for the promotion and maintenance of the T_H1 cell fate while, conversely, the withdrawal of IL-2 results in a loss of T_H1 cell identity and the up-regulation of a T_{FH}-like gene expression profile[36,40]. Interestingly, a second γc cytokine, IL-15, is currently undergoing Phase I/II clinical trials for the treatment of many types of cancer[33,34,41]. As the downstream signaling events of IL-15 are similar to those of IL-2, we hypothesized that IL-15 may maintain the T_H1 profile at the expense of T_{FH} gene expression. To begin to test this hypothesis, we examined whether IL-15 treatment alone would be sufficient to both promote T_H1 gene expression patterns and repress the induction of the T_{FH} gene program following IL-2 withdrawal from differentiated T_H1 cells. As with our previous study, cells exposed to high IL-2 environments repressed the T_{FH} gene program and maintained T_H1 expression patterns while, conversely, IL-2 withdrawal from T_H1 cells resulted in a significant increase in *Bcl6* expression and the induction of a T_{FH}-like profile (Fig. 1a,b)[36]. Interestingly, treatment with IL-15 similarly resulted in a significant increase in *Bcl6* expression compared to that observed in high IL-2 cells (Fig. 1a). Importantly, the increase in *Bcl6* expression was associated with an increase in the T_{FH}-associated genes *Cxcr5*, *Il6ra*, and *Tnfsf8*. Furthermore, while the T_{FH}-associated gene program was upregulated, the expression of the T_H1 genes *Ifng* and *Prdm1* was decreased (Fig. 1b). One possibility that we considered was that at day 3 (the time-point of IL-15 administration), our *ex vivo* generated T_H1 cells were not fully committed to the T_H1 phenotype and therefore maintained a higher degree of flexibility with alternative T helper cell identities. To examine this possibility, we performed a similar experiment, but added IL-15 on day 5 of the T_H1 culture (rather than day 3). Importantly, a similar induction of the T_{FH}-like profile was observed with T_H1 cells exposed to standard (3 days) or extended (5 days) T_H1 culturing conditions (Supplementary Fig. S1). Collectively, these results indicate that IL-15 treatment alone is insufficient to support T_H1 gene expression patterns and instead results in the upregulation of Bcl-6 expression and a T_{FH}-like program.

A key finding from our previous work was that the IL-2-dependent phosphorylation status of STAT5 inversely correlated with the expression of Bcl-6[36]. As IL-15 also signals via the activation of STAT5, we hypothesized that IL-15 treatment alone may not be sufficient to phosphorylate STAT5, thus resulting in increased Bcl-6 expression. To test this possibility, we performed an immunoblot analysis to assess the degree of STAT5 phosphorylation in the high IL-2-, low IL-2-, and IL-15-treated T_H1 cells. While high IL-2 cells displayed elevated levels of STAT5 phosphorylation (pSTAT5), there was very little STAT5 activation in either the low IL-2- or IL-15-treated populations (Fig. 1c). It has been demonstrated that IL-15 is capable of signaling through a unique "trans-presentation" mechanism, which involves the

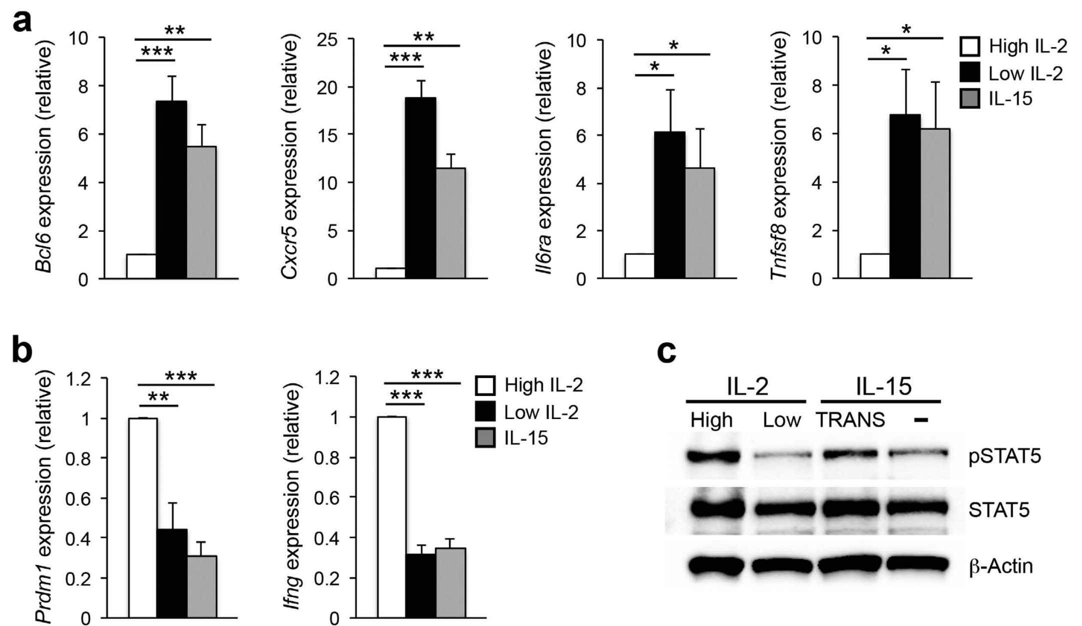

Figure 1. IL-15 treatment alone is insufficient to activate STAT5 and the T_H1 program. (**a,b**) Primary $CD4^+$ T cells were isolated from C57BL/6 mice and stimulated on plate-bound αCD3 and αCD28 for 3 days under T_H1-polarizing conditions. On day 3, cells were split and cultured with either high IL-2 (250 U/ml), low IL-2 (10 U/ml), or IL-15 (15 ng/ml). Two days following cytokine treatment, RNA was isolated to determine transcript levels for the indicated T_{FH} and T_H1-associated genes. Samples were normalized to *Rps18* as a control. Data are presented relative to the high IL-2-treated sample. Three (**a,b**) independent experiments were performed with the error bars representing SEM. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (unpaired Student's *t*-test). (**c**) Cells were treated and harvested as in "a" with the exception that an additional sample was treated with 15 ng/ml of trans-presented IL-15 (IL-15, TRANS). Following cell isolation, an immunoblot assay was performed to assess STAT5 activation levels. Total STAT5 and β-Actin are shown as controls. The image shown is representative of three independent experiments performed.

expression of IL-15Rα in complex with IL-15 on the surface of a second presenting cell. Thus, although $CD4^+$ T cells have variable expression of IL-15Rα, this suggests that IL-15Rα expression is dispensable for IL-15-responsiveness as trans-presentation provides IL-15Rα in the form of a presenting complex[27]. Therefore, we sought to determine whether T_H1 cells would display enhanced STAT5 activation in response to trans-presented IL-15. Indeed, we observed increased STAT5 phosphorylation upon exposure of T_H1 cells to IL-15 in complex with a recombinant IL-15Rα (IL-15_{Trans}) (Fig. 1c). Collectively, these data suggest that IL-15 signaling is a potential differential regulator of the opposing T_H1 and T_{FH} gene programs.

IL-15 signaling via trans-presentation represses Bcl-6 expression in T_H1 cells. The demonstration that trans-presentation of IL-15 results in increased STAT5 activation prompted us to more thoroughly explore the effect of IL-15 signaling on the expression of the T_{FH}-lineage defining transcription factor Bcl-6. We exposed T_H1 cells to two different concentrations of IL-15 (75 ng/ml and 15 ng/ml) either alone or in the trans-presented form. Interestingly, upon exposure of cells to a high environmental concentration of IL-15, *Bcl6* expression remained relatively low (Fig. 2a). Conversely, a significant increase in *Bcl6* expression was observed when T_H1 cells were exposed to a low concentration of IL-15 alone. Importantly, there was a ~6 fold decrease in *Bcl6* expression when the cells were exposed to the same low concentration of IL-15 in the trans-presented form (Fig. 2a). To determine whether Bcl-6 protein expression was consistent with the observed changes in transcript, we performed an immunoblot analysis. Indeed, while there was an increase in Bcl-6 expression at the low IL-15 concentration, we did not observe an induction of Bcl-6 expression in the trans-presented IL-15 sample (Fig. 2b). Importantly, the increase in Bcl-6 expression inversely correlated with the level of IL-15-dependent STAT5 phosphorylation. Collectively, these data indicate that differentiated $CD4^+$ T_H1 cells are capable of responding to IL-15 signaling, particularly during trans-presentation, and that increased IL-15 signaling results in the repression of Bcl-6 expression.

IL-15 signaling regulates the balance of the transcriptional repressors Bcl-6 and Blimp-1. There is a well-established antagonistic relationship between the transcriptional repressors Bcl-6 and Blimp-1 (encoded by the gene *Prdm1*) that guides a number of B and T lymphocyte developmental

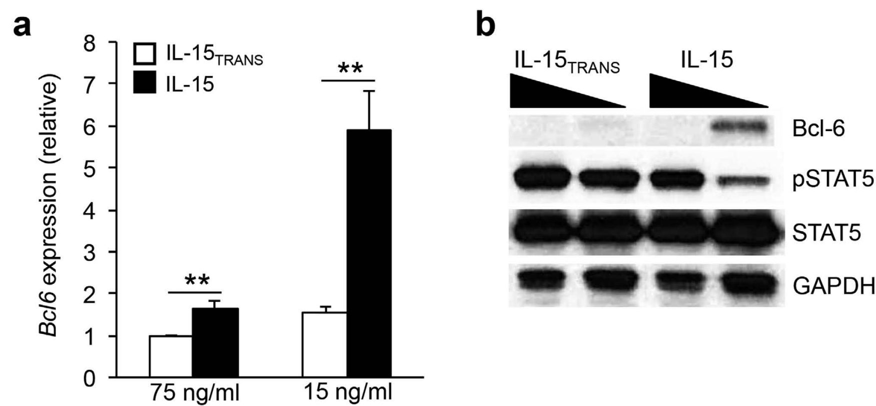

Figure 2. IL-15 signaling represses the expression of Bcl-6. (**a**) Primary CD4+ T cells were isolated from C57BL/6 mice and stimulated on plate-bound αCD3 and αCD28 for 3 days under T_H1-polarizing conditions. On day 3, cells were split and cultured with either IL-15_{TRANS} or IL-15 (75 ng/ml or 15 ng/ml). Two days following cytokine treatment, RNA was isolated to determine the transcript level for *Bcl6*. Samples were normalized to an *Rps18* control and data are represented relative to the IL-15_{TRANS} (75 ng/ml) sample. Five independent experiments were performed with the error bars representing SEM. $^{**}P < 0.01$ (unpaired Student's *t*-test). (**b**) Samples were treated and harvested as in "a". Two days following cytokine treatment, cells were harvested for each condition and an immunoblot analysis was carried out to determine the effect of IL-15 signaling on Bcl-6 expression and STAT5 activation. Total STAT5 and β-Actin are shown as controls. The displayed image is representative of three independent experiments performed.

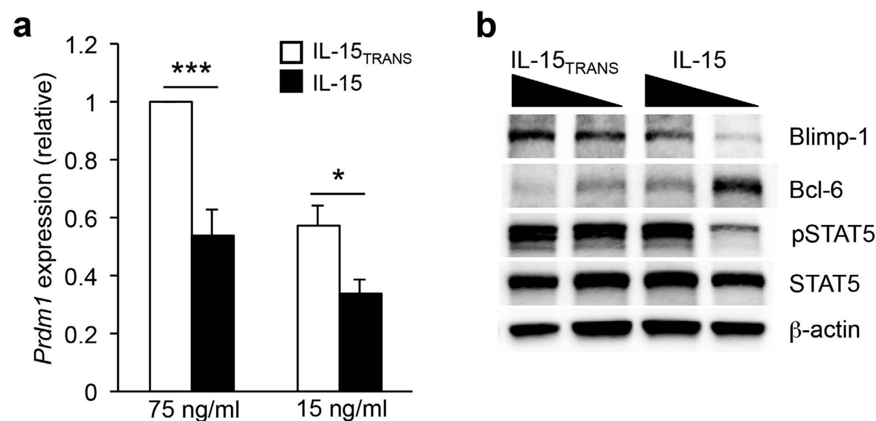

Figure 3. IL-15 trans-presentation maintains Blimp-1 expression. (**a**) Primary CD4+ T cells were cultured as described in Fig. 2. Two days following the indicated IL-15 cytokine treatment, cells were harvested for each condition and RNA was isolated to determine transcript levels for *Prdm1* under IL-15 or IL-15_{TRANS} (75 ng/mL or 15 ng/mL) conditions as indicated. Samples were normalized to *Rps18* as a control. Graphical representations are normalized to the IL-15_{TRANS} (75 ng/ml) sample. (**b**) An immunoblot analysis was performed to examine the effect of IL-15 signaling on Blimp-1, Bcl-6, p-STAT5, and STAT5 expression. β-Actin was monitored to ensure equal protein loading. For (**a**), figures are representative of five independent experiments. Error bars represent SEM. $^{*}P < 0.05$, $^{***}P < 0.001$ (unpaired Student's *t*-test). For (**b**), the image is representative of three independent experiments.

stages[42,43]. For example, previous work from our lab and that of others has demonstrated that Blimp-1 antagonizes T_{FH} cell differentiation[9,44]. In the context of T_H1 cell development, Blimp-1 inhibits the T_{FH} program by directly repressing T_{FH}-associated genes including *Cxcr5*, *Il6ra*, and *Tnfsf8*. Therefore, we hypothesized that IL-15 signaling, similar to that of IL-2, may differentially regulate the expression of these opposing transcriptional regulators and ultimately dictate the establishment of a T_H1 or T_{FH} gene program. Indeed, T_H1 cells exposed to trans-presented IL-15 maintained elevated expression of *Prdm1*, and importantly, increased Blimp-1 protein expression was also observed with trans-presented IL-15 regardless of high or low environmental concentrations of the cytokine (Fig. 3a,b). Conversely, IL-15 cytokine treatment alone resulted in a decrease in Blimp-1 expression. Importantly, the reduction in

Blimp-1 expression was coincident with a decrease in STAT5 phosphorylation, and a corresponding increase in Bcl-6 expression (Fig. 3b).

Strength of IL-15 signaling differentially regulates T_H1 and T_{FH} gene expression patterns. In our previous study, we found that the T_{FH}-associated genes *Cxcr5*, *Il6ra*, and *Tnfsf8* are all direct targets of Blimp-1-mediated repression[36]. Therefore, Bcl-6 activates the T_{FH} gene profile, at least in part, through the repression of a second transcriptional repressor (Blimp-1). Given the upregulation of Blimp-1 in response to IL-15 signaling, we hypothesized that the trans-presentation of IL-15 may also lead to the repression of the T_{FH} gene program. To investigate this possibility, we examined the expression of the T_{FH}-associated genes *Cxcr5*, *Il6ra*, *Tnfsf8,* and *Sh2d1a* under different IL-15 signaling conditions. Indeed, IL-15 signaling was associated with decreased expression of all four genes, in both dose- and trans-presentation-dependent manners (Fig. 4a). However, under low IL-15 signaling conditions, the expression of each gene was significantly induced. Once again, treating the cells with the trans-presented form of IL-15, even at low concentrations, was sufficient to maintain inhibition of *Cxcr5*, *Il6ra*, *Tnfsf8,* and *Sh2d1a* expression, most likely due to the increase in Blimp-1 expression (Fig. 3). Interestingly, not all T_{FH}-associated genes responded similarly to IL-15 signaling. For example, the expression of *Pdcd1*, *Icos*, and *Il21* did not appear to be augmented by changes to IL-15 concentration or trans-presentation (Supplementary Fig. S2). These data strongly suggest that IL-15 signaling is not a comprehensive regulator of the entire T_{FH} gene program. This is, perhaps, not surprising, as it is well-established that there are required roles for B cell interactions, as well as pro-T_{FH} cytokines such as IL-6 and IL-21, in the full commitment to the T_{FH} cell fate[17]. Thus, it is likely that the *ex vivo* setting lacks at least some of the necessary environmental factors required to promote the induction of a complete T_{FH} gene profile.

As our data suggest that enhanced IL-15 signaling is capable of maintaining T_{FH} gene repression even in the absence of IL-2, we hypothesized that trans-presented IL-15 may also be sufficient to promote the expression of T_H1-associated genes. Indeed, treatment with trans-presented IL-15 resulted in increased expression of both the T_H1 lineage-defining transcription factor *Tbx21*, and canonical cytokine *Ifng*, when compared to IL-15 treatment alone (Fig. 4b). Furthermore, cells that were exposed to trans-presented IL-15 expressed higher levels of both *Il12rb1* and *Il12rb2* compared to cells that received IL-15 alone (Fig. 4b). Consistent with the observed increase in *Il12rb1* and *Il12rb2* expression, we found that exposure of cells to trans-presented IL-15 also resulted in an increase in IL-12-dependent STAT4 activation (Fig. 4c). Taken together, these findings suggest that intracellular signaling induced by trans-presented IL-15 can promote additional cytokine-mediated responses critical for the promotion or repression of the T_H1 and T_{FH} gene programs, respectively.

Discussion

$CD4^+$ T cell differentiation from the naïve cell state into any of several effector cell types is dependent upon the cytokine environment in which the cell is located during its activation. Until recently, it was believed that $CD4^+$ T cell differentiation into an effector subset was a terminal event, but a significant body of work has established that differentiated $CD4^+$ T cells are capable of modulating their effector functions in response to changes in the cytokine environment[15,38,39,45,46]. This revelation may be particularly impactful given the use of cytokines and cytokine neutralizing antibodies as approaches to regulate the immune response in a variety of immunotherapeutic strategies.

One such example is the therapeutic use of the cytokine IL-15, which is currently under evaluation in clinical trials for the treatment of a number of types of cancer[32–34]. IL-15 may be an attractive alternative to IL-2-based therapies, as it is able to promote many of the same therapeutic effects due to similar downstream signaling pathways[47]. However, unlike IL-2, IL-15 does not support regulatory T cell populations or promote activation induced cell death (AICD), both of which may inhibit anti-cancer immune responses[48]. Although the effects of IL-15 signaling have been well characterized in $CD8^+$ T and NK cell populations, the effect of IL-15 on the $CD4^+$ T cell effector population has remained relatively unknown[49]. Thus, as the application of this promising cytokine increases, it will be crucial to understand its effects on these vital cell populations in a clinical setting.

Here, our data demonstrate that $CD4^+$ T cells are capable of responding to IL-15 treatment, particularly when IL-15 is trans-presented in complex with the IL-15Rα subunit. Subsequently, IL-15 signaling and the concurrent activation of the transcription factor STAT5 are associated with the positive regulation of T_H1 genes including *Tbx21*, *Ifng*, and *Prdm1*. Additionally, we observed increased expression of the IL-12 receptor subunits (*Il12rb1* and *Il12rb2*), as well as STAT4 activation, in response to IL-15 trans-presentation. This data is in agreement with previous work demonstrating a similar role for IL-2 in the positive regulation of the components of the IL-12R, and suggests that IL-2- and IL-15-signaling, in the context of trans-presentation, share at least some downstream effects[40].

We also found that there is an IL-15 trans-presentation-dependent inhibitory effect upon the expression of genes associated with the T_{FH} cell fate, including the expression of the transcriptional repressor Bcl-6. Our findings indicate that there is an inverse relationship between STAT5 activation and Bcl-6 expression, hinting at a potential regulatory mechanism whereby STAT5 may directly repress Bcl-6. As these data are similar to previous studies examining the repressive effect of IL-2 signaling on the T_{FH} gene program, they suggest that the combined action of STAT5 and Blimp-1 may be a conserved regulatory

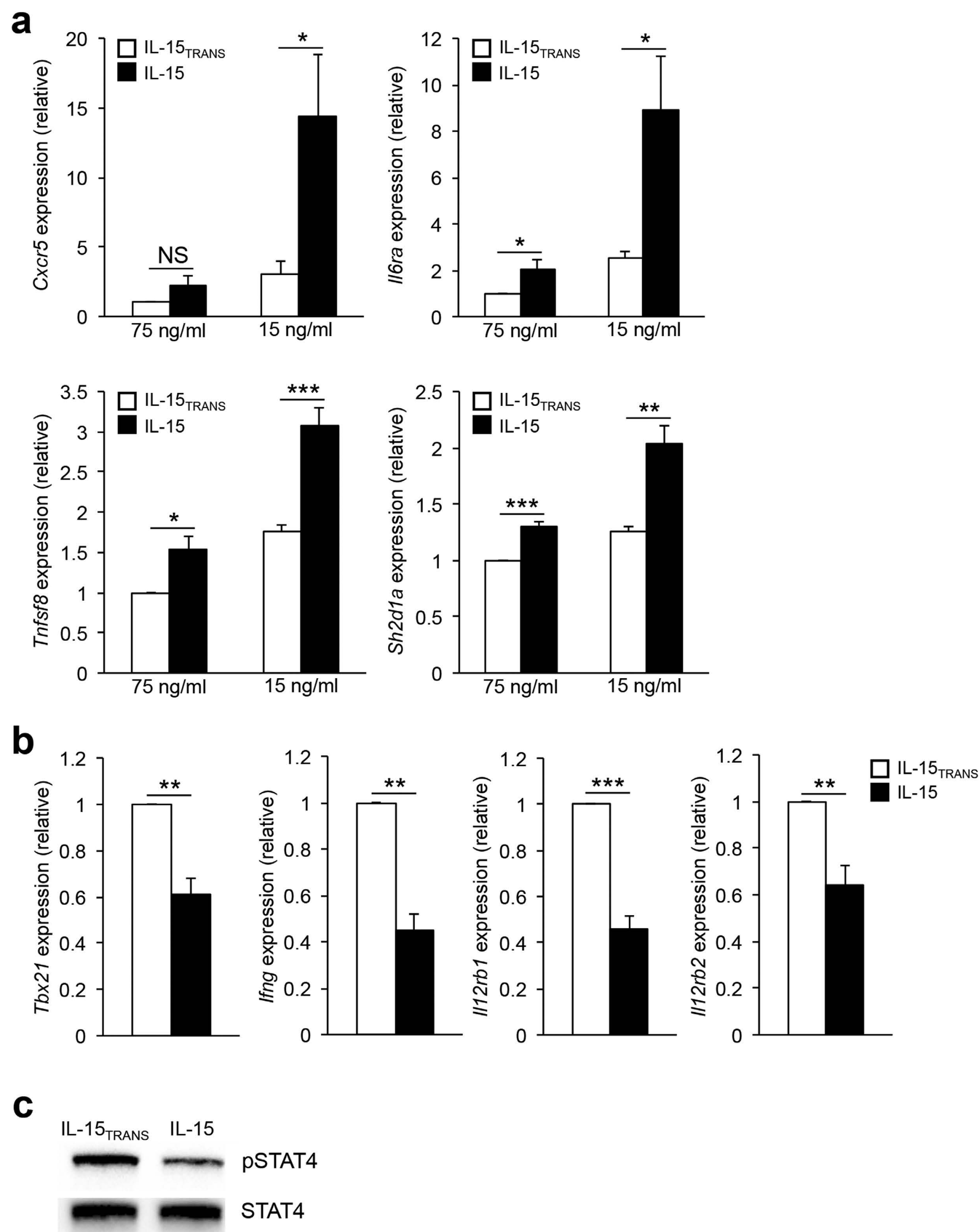

Figure 4. IL-15 signaling regulates the expression of T_{FH}-associated genes. (**a**) Primary CD4$^+$ T cells were cultured as described in Fig. 2. RNA was isolated to determine transcript levels for *Cxcr5*, *Il6ra*, *Tnfsf8,* and *Sh2d1a*. Samples were normalized to *Rps18* as a control and the data are represented relative to the IL-15_{TRANS} (75 ng/ml) sample. (**b**) Primary CD4$^+$ T cells were cultured as described as in "a". RNA was isolated to determine transcript levels for *Tbx21*, *Ifng*, *Il12rb1*, and *Il12rb2*. Samples were normalized to *Rps18* as a control. Data are presented relative to the IL-15_{TRANS} (75 ng/ml) sample. Four (**a,b**) independent experiments were performed with the error bars representing SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (unpaired Student's *t*-test). (**c**) Cells were treated and harvested as in "a". Following cell isolation, an immunoblot assay was performed to assess STAT4 activation levels. Total STAT4 is shown as a control. The image shown is representative of three independent experiments performed.

mechanism utilized by IL-2 and IL-15 signaling to promote the T_H1 cell fate[36,50,51]. Further investigation will be necessary to fully elucidate whether there are specific transcriptional mechanisms unique to IL-15 signaling that both inhibit and promote the expression of the T_{FH} and T_H1 gene programs, respectively.

Despite the apparent antagonistic nature of Bcl-6 expression and IL-15 signaling, it is interesting to note that both factors have been shown to positively influence the formation of T cell memory. Bcl-6 expression has been linked to the promotion of the effector-to-memory cell transition in both $CD4^+$ and $CD8^+$ T cells, while IL-15 is critical for the homeostatic maintenance of memory cell populations[42,52,53]. Future work will be necessary to dissect how the findings presented in this study may translate into an increased understanding of the interplay between these intrinsic and extrinsic factors that govern memory cell fate.

Collectively, this study highlights a novel role for IL-15 signaling in modulating the gene expression profiles of $CD4^+$ T helper cell populations. Consequently, these changes in gene expression dictate both the fate and function of the responding cells. As such, an increased understanding of the implications of cytokine signaling, including that of IL-15, on the plasticity of $CD4^+$ T cell populations will be critical as these environmental modulators continue to be utilized in novel immunotherapeutic strategies.

Methods

Primary cells and cell culture. Primary $CD4^+$ T cells were isolated from the spleens and lymph nodes of 5–8 week old C57BL/6 mice using the R&D Systems MagCellect $CD4^+$ T cell Isolation Kit according to the manufacturer's instructions. Cells were cultured in complete Iscove's Modified Dulbecco's medium [cIMDM; IMDM (12440053, Life Technologies), 10% FBS (26140079, Life Technologies), 1% Penicillin-Streptomycin (15140122, Life Technologies), 0.05% BME (BP176, Fisher Scientific)] and plated on αCD3 (BDB553057, Fisher Scientific) and αCD28 (BDB553294, Fisher Scientific) under T_H1 polarizing conditions (IL-12, αIL-4, and IL-2) as described previously[36,54]. After three days (or five, as in Supplementary Fig. 1), cells were split and maintained for an additional two days in IL-12 and αIL-4 and either high (75 ng/ml) or low (15 ng/ml) concentrations of IL-15, or a stable complex of IL-15 and its receptor (referred to in this manuscript as trans-presented IL-15, or IL-15_{TRANS}), as indicated. The Institutional Animal Care and Use Committee of Virginia Tech approved all experimentation involving the use of mice. All methods were performed in accordance with the approved guidelines.

Generation of the IL-15/IL15Rα complex. A stable complex of IL-15/IL-15Rα was generated by combining soluble IL-15 (447-ML, R&D Systems) with a recombinant mouse IL-15Rα Fc chimera (551-MR, R&D Systems) at a ratio of 2:1. The combination was incubated at 37 °C for 45 minutes immediately prior to use, as described previously[29,41].

RNA isolation and qRT-PCR. Two days following IL-15 cytokine treatment, cells were harvested for each condition and RNA was isolated using the Nucleospin RNA II kit from Macherey-Nagel (740955.5) per the manufacturer's protocol. cDNA was generated using the Superscript First Strand Synthesis Kit (11904018, Life Technologies). Triplicate qRT-PCR reactions using 10–20 ng of template were performed in 20μL reactions with gene-specific primers (Supplementary Table S1) and iTaq Universal SYBR Green Supermix (172-5124, Bio-Rad). All samples were normalized to the ribosomal protein S18 (*Rps18*) control. For IL-2-treated samples, expression levels were normalized to the high IL-2-treated sample. For IL-15-treated samples, expression levels were normalized to the high IL-15_{TRANS}-treated sample.

Immunoblot analysis. To examine the effect of IL-15 and IL-15_{TRANS} at the protein level, primary T cells for each condition were harvested 2 days following IL-2, IL-15, or IL-15_{TRANS} treatment. Antibodies used were as follows: pSTAT5 (611964, BD Biosciences), STAT5 (sc-482X, Santa Cruz), Bcl-6 (561520, BD Biosciences), pSTAT4 (5267S, Cell Signaling), STAT4 (sc-486X, Santa Cruz), Blimp-1 (A01647-40, Genscript), GAPDH (sc-25778, Santa Cruz), β-Actin (A00730-40, Genscript). Further details regarding the clone and/or dilution of antibodies used can be found in Supplementary Table S2).

Statistical analysis. All data represent at least three independent experiments. Error bars represent the standard error of the mean (SEM). For statistical analysis, unpaired *t* tests were performed using GraphPad Prism online software. P values < 0.05 were considered statistically significant.

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Author Contributions

I.D.C. and K.A.R. assisted with the design of the study, performed experiments, analyzed data, and wrote the manuscript. K.J.O. supervised the research, designed the study, analyzed data, and wrote the manuscript.

Additional Information

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