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IL-21 promotes CD4 T cell responses by PI3-kinase dependent upregulation of CD86 on B cells

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Abstract

The cytokine interleukin-21 (IL-21) is a potent immune modulator with diverse mechanisms of action on multiple cell types. IL-21 is in clinical use to promote tumour rejection and is an emerging target for neutralisation in the setting of autoimmunity. Despite its clinical potential, the biological actions of IL-21 are not yet fully understood and the full range of effects of this pleiotropic cytokine are still being uncovered. Here we identify a novel role for IL-21 as an inducer of the costimulatory ligand CD86 on B lymphocytes. CD86 provides critical signals through T cell-expressed CD28 that promote T cell activation in response to antigen engagement. Expression levels of CD86 are tightly regulated *in vivo*, being actively decreased by regulatory T cells and increased in response to pathogen-derived signals. Here we demonstrate that IL-21 can trigger potent and sustained CD86 upregulation through a STAT3 and PI3-kinase dependent mechanism. We show that elevated CD86 expression has functional consequences for the magnitude of CD4 T cell responses both *in vitro* and *in vivo*. These data pinpoint CD86 upregulation as an additional mechanism by which IL-21 can elicit immunomodulatory effects.

Introduction

IL-21 is known to influence multiple parameters of the immune response. The clinical importance of this pathway was first appreciated nearly a decade ago with the demonstration that IL-21 could augment anti-tumour immunity(1, 2), and this has since become an active area of research(3-5). In addition to augmenting immunity against tumours, IL-21 signalling can directly induce apoptotic pathways in chronic lymphocytic leukaemia (CLL) B cells(6, 7) and diffuse large B cell lymphoma(8).

The role of IL-21 in T cell-dependent B cell responses has been extensively documented. IL-21 critically regulates antibody production, partly in cooperation with IL-4(9), and promotes plasma cell differentiation in both mice(10) and humans(11). The intimate interaction between follicular helper T cells (T_{FH}) and germinal centre B cells is also shaped

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by provision of IL-21; T_{FH}-derived IL-21 directly targets germinal centre B cells, reinforcing their fate decision by sustaining bcl6 expression(12, 13).

Alongside effects on B cells, several studies have also reported that IL-21 promotes T cell activation. Pre-exposure to IL-21 has been shown to increase the antigen responsiveness of CD8 T cells(14) and permit triggering by weak TCR agonists(15). CD4 T cell responses can also be augmented by IL-21, in part due to its ability to counteract Treg suppression(16, 17). The mechanisms by which IL-21 directly or indirectly promotes T cell responses are not yet fully defined. In this study we identify a novel role for IL-21 in upregulating the expression of the costimulatory ligand CD86 on B cells. We show that this requires activation of the PI3-kinase pathway and is dependent on the PI3-kinase subunit p110 δ , a molecule currently being targeted in the setting of several B cell malignancies (CLL, NHL)(18). The increased expression of CD86 on B cells is shown to have functional consequences for T cell expansion both *in vitro* and *in vivo*. Collectively these data suggest an additional mechanism by which IL-21 may augment adaptive immune responses and reveal a further level of T cell / B cell interaction directed by this cytokine.

Materials and Methods

Mice

DO11.10 TCR transgenic and BALB/c mice were purchased from The Jackson Laboratory. IL-21R^{-/-} mice were provided by Manfred Kopf (ETH Zurich) and were bred with DO11.10 TCR transgenic mice to generate IL-21R^{-/-} DO11.10 TCR transgenic progeny. p110 δ ^{D910A} mice were provided by K.O. Mice were housed at the University of Birmingham Biomedical Services Unit or at University College London and used according to Home Office and institutional guidelines.

Flow cytometry

Cells were stained with mAb against CD25 (PC61.5; eBioscience), CD4 (LT34; eBioscience), CD19 (1D3), CD86 (GL1; eBioscience), CD80 (16-10A1), pSTAT1 (14/P-STAT1), pSTAT3 (49/P-STAT3), pSTAT5 (47) and DO11.10 TCR (KJ1.26; eBioscience). All antibodies were purchased from BD Biosciences unless otherwise indicated. For pSTAT staining, cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized with 100% ice-cold methanol for 30 minutes. Statistics were performed using an unpaired 2-tailed t test with a 95% confidence interval.

Short-term splenocyte cultures

1 \times 10⁵ BALB/c splenocytes were cultured for 15-16 hours alone, with IL-21 at 25, 50, 100 or 200ng/ml (Peprotech), or with 1 μ g/ml LPS (Sigma). For time-course experiments, cells were harvested at 2, 4, 6, 8 or 15 hours.

Short-term B cell cultures

Magnetic separation (Miltenyi Biotec) was used to purify CD19⁺ B cells from BALB/c or p110 δ ^{D910A} spleen. 1 \times 10⁶ cells were cultured for 16 hours alone or in the presence of 200ng/ml IL-21 (Peprotech) or 10ng/ml IL-4 (Peprotech). For STAT3 inhibition experiments cultures were supplemented with 10, 50 or 100 μ M S3I-201 (Calbiochem) as indicated. For PI3K inhibition experiments cultures were supplemented with 10 μ M LY-294002 (Invitrogen) as indicated. For assessment of activated STAT proteins cells were cultured for 2 hours alone or in the presence of 200ng/ml IL-21 (Peprotech). For experiments to determine the target of IL-21 2.5 \times 10⁴ BALB/c or IL-21R^{-/-} B cells were cultured with 2.5 \times 10⁴ magnetically separated (Miltenyi Biotec) CD4⁺CD25⁻ T cells from

BALB/c or IL-21R^{-/-} lymph node for 16 hours alone or in the presence of 200ng/ml IL-21 (Peprotech).

Confocal microscopy

Magnetic separation (Miltenyi Biotec) was used to purify CD19⁺ B cells from BALB/c spleen. 1×10^6 cells were cultured for 6 hours alone, with 200ng/ml IL-21 (Peprotech), with 10 μ g/ml cycloheximide (Sigma) or both. Cells were stained with mAb against CD86 (GL1; eBioscience) and imaged using glass bottom culture dishes (MatTek). Imaging was carried out using a 100x oil immersion objective.

RT-PCR

Magnetic separation (Miltenyi Biotec) was used to purify CD19⁺ B cells from BALB/c spleen. mRNA was isolated at this point or after culture of 1×10^6 cells for 16 hours alone or in the presence of 200ng/ml IL-21 (Peprotech). Quantitative PCR was performed to assess the expression of β_2 microglobulin (Eurofins MWG Operon) or CD86 (Taqman gene expression assay; Applied Biosystems).

In vitro proliferation assays

Magnetic separation (Miltenyi Biotec) was used to purify CD4⁺CD25⁻ T cells from IL-21R^{-/-} lymph node. 2.5×10^4 cells were cultured with 2.5×10^4 CD19⁺ B cells from BALB/c or IL-21R^{-/-} spleen, with 0.8 μ g/ml anti-CD3 (BD Biosciences) alone or in the presence of 10 μ g/ml anti-CD86 (BioXcell). Triplicate wells were pooled and harvested at day 1 to assess CD86 expression or day 3 to determine cell counts by flow cytometry.

Adoptive transfers

CD19⁺ B cells from BALB/c or IL-21R^{-/-} spleen were isolated by magnetic separation (Miltenyi Biotec) and cultured for 16 hours with 1 μ g/ml OVA peptide. 3×10^6 peptide pulsed cells were injected intravenously into IL-21R^{-/-} recipients. Magnetic separation (Miltenyi Biotec) was used to purify CD4⁺ cells from IL-21R^{-/-} DO11.10 TCR⁺ lymph node. 24 hours after B cell transfer a total of 2×10^6 CellTrace Violet labelled IL-21R^{-/-} DO11.10 TCR⁺ T cells were injected intravenously and 1 μ g IL-21 (Peprotech) or PBS intraperitoneally. Where indicated recipients were also given 100 μ g anti-CD86 (BioXcell) or PBS intraperitoneally immediately after T cell transfer. Mice were culled at day 7 for analysis of inguinal lymph node and splenic cells.

Results

IL-21 upregulates CD86 expression on B cells

To explore the influence of IL-21 on costimulatory ligand expression, murine splenocytes were cultured in the presence or absence of IL-21 for 16h and the expression of CD86 and CD80 assessed by flow cytometry. We noted a marked elevation of CD86, but not CD80, on CD19⁺ B cells in the timeframe examined (Fig. 1A). Titration (Fig. 1B) and timecourse (Fig. 1C) data established that IL-21-mediated CD86 upregulation was dose-dependent and appeared maximal around 8h following stimulation. The magnitude of CD86 upregulation observed in response to IL-21 was comparable, if not greater, than that seen with a 1 μ g/ml dose of LPS, a TLR ligand known to drive CD86 upregulation (Fig. 1D). In contrast, splenic dendritic cells showed some CD86 expression at baseline and this was not further elevated by IL-21 under the conditions assessed (Supplemental Fig. 1).

CD86 upregulation requires direct signalling to B cells and protein neosynthesis

Since the above assays did not use purified B cells it remained possible that IL-21 was altering CD86 expression indirectly by acting on a different cell type. In this regard we have previously shown that conventional CD4 T cells express high levels of IL-21 receptor and can respond to IL-21 by acquiring resistance to Treg suppression(19). To dissect whether regulation of CD86 expression involved direct signalling to B cells or was an indirect consequence of IL-21 signalling to T cells, we took advantage of mice genetically deficient for the IL-21 receptor(20). Co-cultures of T cells and B cells were established in which the cells derived from either wildtype or IL-21 receptor deficient mice and these were incubated for 16h with or without IL-21. As expected, when both T and B cells expressed IL-21R, IL-21 upregulated CD86 expression (Fig. 2A, far left panels) and when neither cell type expressed IL-21R, CD86 was unchanged (Fig. 2A, second panels). Crucially, in situations where B cells were IL-21R^{+/+} but T cells were IL-21R^{-/-}, addition of IL-21 retained the capacity to upregulate CD86 (Fig. 2A, third panels). Furthermore, preventing B cells alone from receiving IL-21 signals completely abrogated the ability of IL-21 to upregulate CD86 (Fig. 2A, far right panels). Collectively these data indicate that direct signalling of IL-21 to B cells is responsible for mediating CD86 upregulation.

The upregulation of CD86 by the cytokine IFN γ has been reported to be independent of protein neosynthesis(21). To examine whether upregulation of CD86 by IL-21 required protein neosynthesis we performed experiments using purified B cells in the presence or absence of cycloheximide. The ability of IL-21 to upregulate B cell CD86 expression was completely abrogated in the presence of cycloheximide, indicating a requirement for protein neosynthesis (Fig. 2B, 2C). We next assessed whether upregulation of CD86 by IL-21 was transcriptionally regulated; this revealed that mRNA for CD86 was strongly upregulated by exposure to IL-21 (Fig. 2D). Together these data indicate that de novo transcription and translation of CD86 mRNA is required for IL-21-mediated CD86 upregulation.

CD86 upregulation requires STAT3 and Pi3-kinase

IL-21 signaling is known to involve STAT3 activation in both mouse(22) and human(23, 24) B cells, and the ability of STAT3 to mediate IL-21 effects has been demonstrated using B cells isolated from STAT3-deficient patients(25). We confirmed the ability of IL-21 to activate the STAT3 pathway in B cells (Fig. 3A) then sought to determine whether this pathway was involved in IL-21-mediated CD86 upregulation. In the presence of the STAT3 inhibitor S3I-201, IL-21 was no longer able to induce significant CD86 upregulation (Fig. 3B, 3C) suggesting a critical role for STAT3 in this process.

A second major pathway triggered by IL-21 is the activation of the enzyme phosphoinositide-3-kinase (PI3-kinase)(22, 26), and the ability of BCR engagement to upregulate CD86 is known to be dependent on PI3-kinase signalling(27). To determine whether IL-21 upregulates CD86 via a PI3-kinase-dependent pathway, we first employed the PI3-kinase inhibitor LY-294002. Strikingly, IL-21-mediated CD86 upregulation was completely abrogated in the presence of this inhibitor (Fig. 4A). The related cytokine IL-4 retained the ability to upregulate CD86 in the presence of the PI3-kinase inhibitor, consistent with previous studies(27). We next took advantage of mice expressing a catalytically inactive form of the PI3-kinase p110 δ subunit (D910A mice)(28), since this subunit is known to be important in the B cell lineage(29, 30). Purified B cells from D910A animals failed to upregulate CD86 in response to IL-21, while their ability to upregulate CD86 in response to IL-4 remained intact (Fig. 4B). Collectively these data indicate that IL-21 uses STAT3 and PI3-kinase to drive CD86 upregulation and pinpoint p110 δ as the critical PI3-kinase subunit responsible.

IL-21 mediated CD86 upregulation has functional consequences *in vitro*

To test whether the upregulation of CD86 in response to IL-21 had functional consequences, *in vitro* T cell proliferation assays were performed. CD4⁺CD25⁻ T cells from IL-21R^{-/-} mice were used to preclude direct effects of IL-21 on the T cells themselves. IL-21R^{-/-} T cells were activated by anti-CD3 in the presence of B cells that were either wildtype or IL-21R deficient; in this setting any IL-21 made by the T cells would potentially be able to upregulate CD86 on the wildtype B cells but not the IL-21R^{-/-} ones. In line with this, CD86 was upregulated to a greater extent on the IL-21R^{+/+} B cells than the IL-21R^{-/-} ones (Fig. 5A). IL-4 did not appear to contribute to CD86 upregulation in this setting as assessed by inclusion of blocking anti-IL-4 Ab (data not shown). The increased CD86 expression on IL-21R^{+/+} B cells was associated with significantly greater T cell proliferation (Fig. 5B) and activation marker expression (Fig. 5C and data not shown). T cell proliferation and activation marker expression was dependent on CD86 as demonstrated by antibody blockade of this pathway (Fig. 5B, 5C).

IL-21 mediated CD86 upregulation has functional consequences *in vivo*

To extend the above observations to an *in vivo* setting, we established a system in which peptide-pulsed B cells were used to stimulate the proliferation of antigen-specific CD4 T cells. Wildtype or IL-21R^{-/-} B cells were pulsed with OVA₃₂₃₋₃₃₉ peptide and adoptively transferred into IL-21R^{-/-} hosts. OVA-specific T cells (DO11) that were also IL-21R^{-/-} were CellTrace labelled and injected into the same hosts, so that their response to the OVA-pulsed B cells could be tracked. To maximise the effects of IL-21 on B cell phenotype, recombinant IL-21 was injected i.p. where indicated. Ensuring that the host animals and the adoptively transferred T cells were IL-21R^{-/-} allowed us to restrict the IL-21 effects to the B cell compartment. At d7, single cell suspensions from inguinal LN and spleen were stained by flow cytometry to identify the adoptively transferred antigen-specific T cells and assess their proliferation status (Fig. 6A). CellTrace profiles for gated antigen-specific T cells revealed greater proliferation in mice that had received IL-21R^{+/+} B cells compared with those that had received IL-21R^{-/-} B cells. Proliferation was particularly evident in the spleen consistent with the likely trafficking of the adoptively transferred peptide-pulsed B cells to this site. The enhanced T cell response to IL-21R^{+/+} B cells was also reflected in higher absolute numbers of antigen-specific T cells (Fig. 6B). The ability of IL-21 signalling to B cells to augment the T cell response was dependent on CD86 as assessed by *in vivo* antibody blockade (Fig. 6C). Recovery of injected B cells at day 1 confirmed increased expression of CD86 on IL-21R^{+/+} B cells compared with IL-21R^{-/-} B cells (Supplemental Fig. 2). Collectively these data demonstrate that IL-21 increases expression of CD86 on B cells and that this increased availability of costimulatory ligand has functional consequences for the magnitude of T cell responses *in vitro* and *in vivo*.

Discussion

IL-21 is recognised to exert multiple effects on the differentiation and function of B cells. While first recognised for its ability to promote human B cell proliferation(31), it can also trigger B cell apoptosis(32) with the outcome of IL-21 signalling most likely dictated by its context(10, 33). IL-21 has a well established role in the induction of plasma cell differentiation(10, 11) and this involves the cooperative binding of STAT3 and IRF4 to the IL-21 response element in the *Prdm1* gene encoding Blimp-1(34). Intriguingly, IL-21 also promotes the expression of Bcl6 a key transcriptional regulator of germinal centre B cell differentiation(12, 13), despite the fact that Blimp-1 and Bcl6 have mutually antagonistic effects. Thus IL-21 acts at multiple stages to modulate B cell activation, differentiation and death. Our recent findings provide additional insight into the effects of IL-21 on B cells,

demonstrating an upregulation of CD86 that has consequences for T cell dependent immune responses.

The role of IL-21 in DCs is less well studied and our side by side comparison of B cells and DCs suggested CD86 was not obviously upregulated by IL-21 in the latter population. While it has been shown that IL-21 can inhibit DC maturation(35, 36), other studies suggested IL-21 can enhance DC function(37), and recent analysis has identified a role for IL-21 in triggering DC migration in a virus-induced diabetes model(38). Interestingly, in the latter study, reduced migration of IL-21R^{-/-} DC was accompanied by decreased CD86 expression. Thus, further work is required to fully elucidate the biological function of IL-21 in DCs.

The provision of CD28 costimulation is known to be of critical importance in thresholding T cell responses. As a consequence, stimuli that alter expression of the costimulatory ligands CD86 and CD80 are likely to have a significant impact on T cell immunity. This is particularly true of CD86, as it is thought to be the dominant ligand for driving T cell responses(39). Expression of CD86 and CD80 is tightly regulated, and the CD28 homologue CTLA-4 serves to limit their availability by competitive inhibition and ligand downregulation(40-42). Unregulated availability of ligands, in mice lacking CTLA-4, results in unfettered T cell responses that culminate in lethal autoimmunity(43, 44). Thus, restricting CD86 and CD80 expression represents a major control point for adaptive immunity. Counteracting the downregulation of ligand by CTLA-4, various inflammatory stimuli serve to enhance ligand expression. Accordingly TLR ligands and cytokines such as IFN γ have been shown to upregulate CD86 expression. Here we identify IL-21 as a potent upregulator of CD86 expression on B cells.

IL-21 is one of several cytokines associated with systemic autoimmunity(45) and has been linked with lupus pathogenesis in mice(10, 46) and humans(47). The capacity of IL-21 to upregulate CD86, and promote T cell costimulation, could conceivably contribute to its pathogenic effects. Consistent with this, B cell expression of the costimulatory ligands CD86 and CD80 has been shown to be essential for autoreactive T cell activation and development of joint pathology in the proteoglycan-induced arthritis model in mice(48).

A major cellular source of IL-21 is the follicular helper T cell (T_{FH}) subset that provides crucial help to B cells during the germinal center (GC) reaction(49-51). Interestingly, IL-4 is also produced by T_{FH} cells and is known to positively regulate the expression of CD86 during productive interactions with B cells. Since B cell-derived CD86 provides critical signals for T_{FH} cell maintenance(52), the production of IL-21 and IL-4 provides an elegant mechanism for T_{FH} to solicit their own survival signals.

We show that IL-21-dependent CD86 upregulation is strictly reliant on STAT3 phosphorylation and PI3-kinase, revealing unappreciated roles for these pathways in IL-21-mediated B cell responses. Using B cells expressing a catalytically inactive isoform of p110 δ , we further identify this PI3-kinase subunit as essential for the promotion of CD86 by IL-21. In contrast, IL-4-driven CD86 upregulation is independent of PI3-kinase, consistent with previous studies that instead identified a role for STAT6(53). The precise relationship between PI3-kinase and STAT3 in orchestrating IL-21-dependent CD86 upregulation is unclear, however preliminary studies suggest that STAT3 activation is independent of the PI-3kinase pathway (Supplemental Fig. 3). The PI3-kinase pathway is an important mediator of lymphocyte survival(54) and inhibitors of the p110 δ isoform are in clinical use for the treatment of CLL(55). Our data suggest that PI3-kinase inhibition may have a greater impact on the nature of the immune response than previously appreciated; in addition to blocking antigen-dependent proliferation and survival signals in B cells, abrogating PI3-kinase

signalling may also influence the costimulatory profile of B cells and their ability to drive CD4 T cell responses.

IL-4 and IL-21 exhibit redundancy in their ability to support germinal centre formation, with deficiency in each pathway alone having only a minor impact(9). Likewise either IL-4 or IL-21 appears sufficient to support IgG secretion from mouse(56) or human(57) B cells *in vitro* with synergistic activity being seen in the presence of both. Redundant roles for IL-4 and IL-21 in B cell CD86 upregulation might explain why mice lacking the PI3-Kinase p110 δ subunit in B cells retained the capacity to form germinal centers(58), since IL-4-mediated CD86 upregulation would be predicted to be intact in this setting. Although IL-21 and IL-4 are frequently co-expressed in germinal centre resident T_{FH} cells(59, 60), the observation that Th17 cells can also provide cognate help to B cells(61, 62) suggests at least one scenario in which IL-21 provision may be uncoupled from that of IL-4.

Similar to IL-21, the cytokine IL-10 is well known as a plasma cell differentiation factor. Despite their largely overlapping roles in promoting plasma cell differentiation these cytokines have notably distinct effects on CD86 expression, with IL-10 being generally accepted to downregulate CD86. Intriguingly, the receptors for these cytokines appear to be subject to differential kinetic regulation during B cell differentiation, with IL-21 playing an earlier role than IL-10(63). This might imply a set window during which CD86 expression is amenable to IL-21-directed up-regulation. If late IL-10 signals downregulate CD86 this could suggest a requirement for appropriate curtailment of T cell costimulatory signals for optimal T cell / B cell collaboration. In keeping with the idea of active downregulation (as well as upregulation) of costimulatory ligands, Bcl6 is known to downregulate CD80 in germinal centre B cells (64). The fine tuning of CD86 and CD80 expression in germinal centre B cells, and the consequences for T_{FH} homeostasis(52, 65) are incompletely understood and represent key areas for further investigation.

In summary we have shown that IL-21 upregulates CD86 expression on B cells in a manner depends on the PI3-Kinase p110 δ subunit. This finding is likely to have important implications for T cell / B cell collaboration within the germinal center, and for T cell responses in the wider context of anti-tumor responses and autoimmunity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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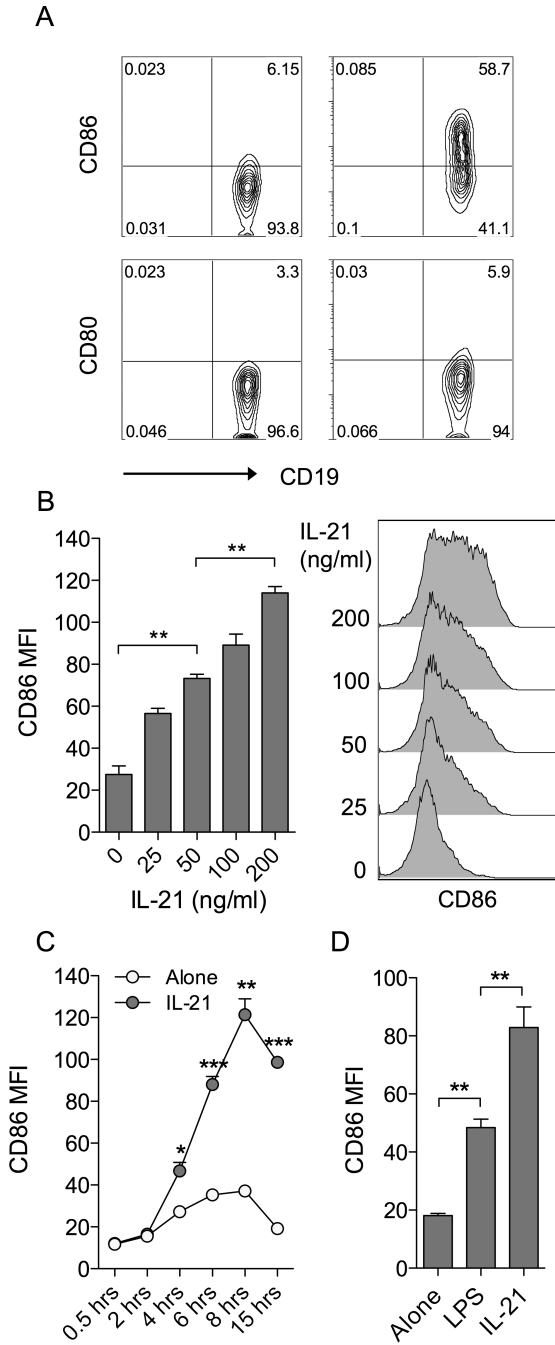


Figure 1. IL-21 upregulates CD86 expression on B cells

(A) 1×10^5 BALB/c splenocytes were cultured for 16 hours alone or in the presence of 200ng/ml IL-21. Plots show representative CD80 and CD86 expression for gated CD19+ B cells. (B) 1×10^5 BALB/c splenocytes were cultured for 16 hours alone or in the presence of the indicated doses of IL-21. Histograms show CD86 expression for gated CD19+ B cells and graph shows collated CD86 MFI data. (C) 1×10^5 BALB/c splenocytes were cultured alone or in the presence of 200ng/ml IL-21 and harvested at the indicated time points. Graph shows collated CD86 MFI data for gated CD19+ B cells. (D) 1×10^5 BALB/c splenocytes were cultured for 16 hours alone or in the presence of either 200ng/ml IL-21 or $1 \mu\text{g/ml}$ LPS.

Graph shows collated CD86 MFI data for gated CD19+ B cells. Data are representative of at least 3 independent experiments. * $P < .05$. ** $P < .01$. *** $P < .001$.

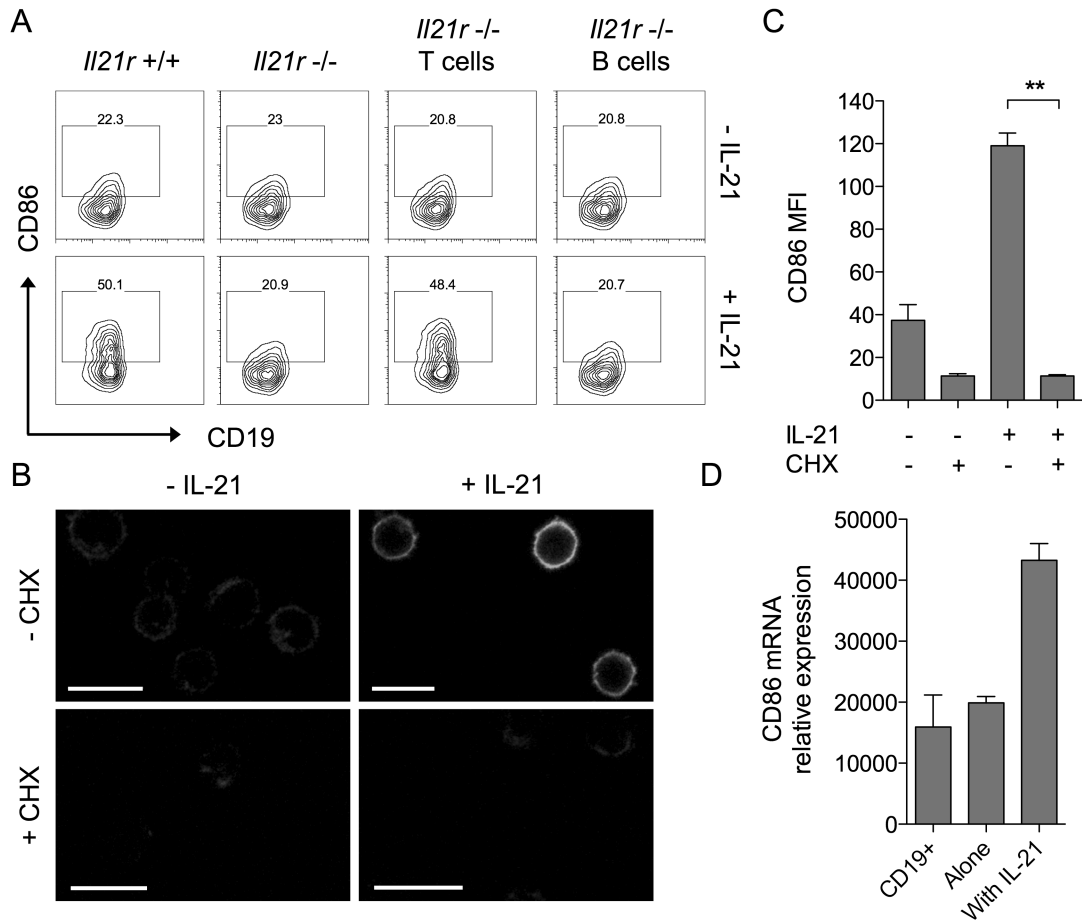


Figure 2. Induction of CD86 by IL-21 requires direct signalling to B cells and is dependent on protein neosynthesis

(A) 2.5×10^4 BALB/c CD4+CD25⁻ T conv were cultured with 2.5×10^4 CD19⁺ B cells for 16 hours alone or in the presence of 200ng/ml IL-21. Cell populations were deficient for the IL-21R as indicated. Plots show CD86 expression for gated CD19⁺ B cells. (B) 1×10^6 BALB/c CD19⁺ B cells were cultured for 6 hours alone, with 200ng/ml IL-21, 10 μ g/ml cycloheximide or both. Representative confocal microscopy images show CD86 staining. Scale bars = 10 μ m. (C) Graph shows collated CD86 MFI for cells from B analysed by flow cytometry. Data are representative of 3 independent experiments. (D) 1×10^6 BALB/c CD19⁺ B cells were cultured for 16 hours alone or in the presence of 200ng/ml IL-21. Graph shows relative CD86 mRNA expression for cultured cells and freshly isolated CD19⁺ B cells. Data are representative of 3 independent experiments. ** P < .01.

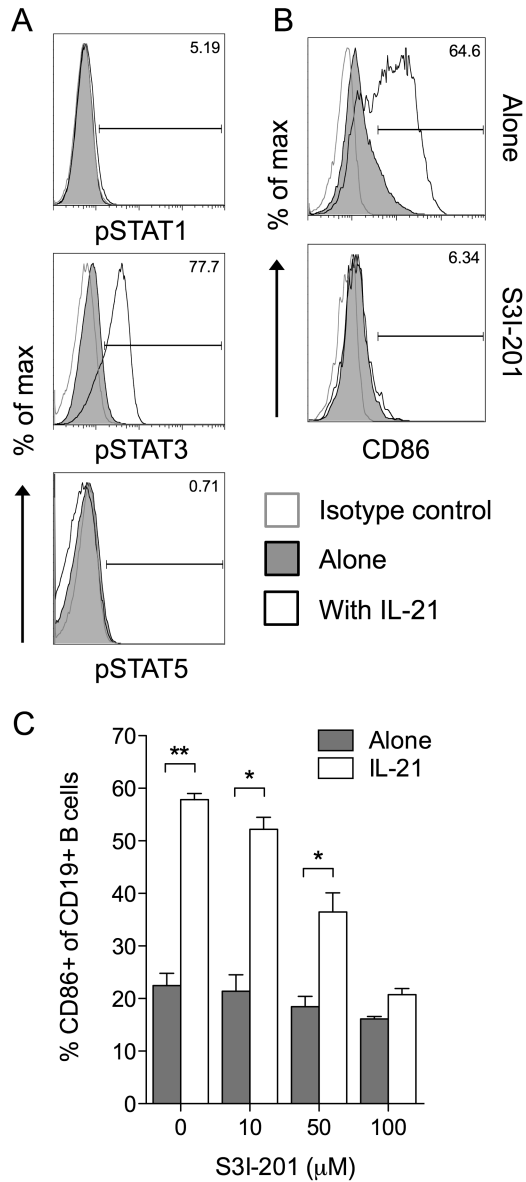


Figure 3. Induction of CD86 by IL-21 is dependent on STAT3 activation
 (A) 1×10^6 BALB/c CD19+ B cells were cultured for 2 hours alone or in the presence of 200ng/ml IL-21. Histograms show representative staining for phosphorylated STAT proteins as indicated. (B) 1×10^6 BALB/c CD19+ B cells were cultured for 16 hours alone or in the presence of 200ng/ml IL-21, 100μM S3I-201 or both. Histograms show representative CD86 expression for gated CD19+ B cells. (C) Graph shows collated CD86 expression data for cells from B when cultured with the indicated doses of S3I-201. Data are representative of 3 independent experiments. * $P < .05$. ** $P < .01$.

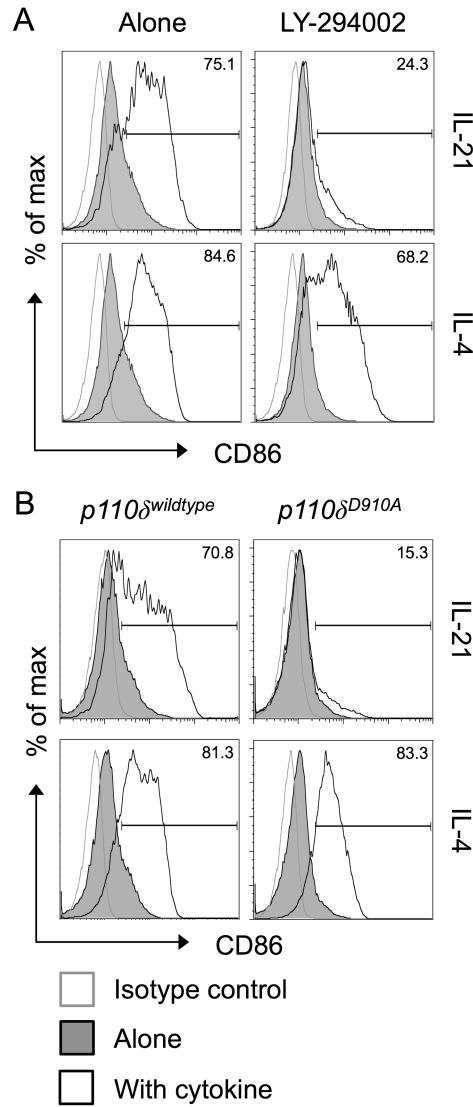


Figure 4. Induction of CD86 by IL-21 is dependent on PI3K p110δ

(A) 1×10^6 BALB/c CD19⁺ B cells were cultured for 16 hours alone or in the presence of 200ng/ml IL-21 or 10ng/ml IL-4. Where indicated cultures also contained 10 μ M LY-294002. Histograms show CD86 expression for gated CD19⁺ B cells. (B) 1×10^6 BALB/c CD19⁺ B cells were cultured for 16 hours alone or in the presence of 200ng/ml IL-21 or 10ng/ml IL-4. Cells expressed the wildtype or mutant isoforms of PI3K P110 δ as indicated. Histograms show CD86 expression for gated CD19⁺ B cells. Data are representative of 3 independent experiments.

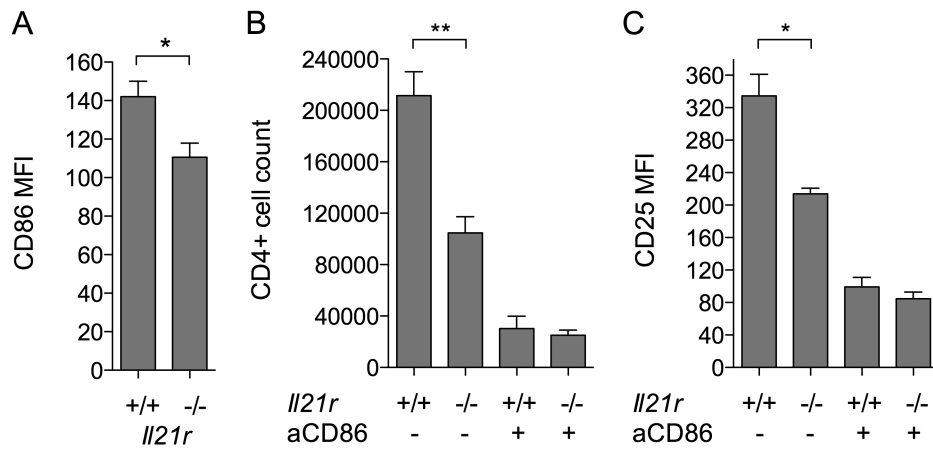


Figure 5. IL-21 signalling to B cells promotes CD86-dependent T cell proliferation
 2.5×10^4 IL-21R^{-/-} CD4⁺CD25⁻ T conv were cultured with 0.8 μ g/ml anti-CD3 and 2.5×10^4 IL-21R^{-/-} or +/+ CD19⁺ B cells alone or in the presence of 10 μ g/ml anti-CD86. (A) Graph shows collated CD86 MFI data for gated CD19⁺ B cells harvested after 16 hours in the absence of anti-CD86 blocking antibody (B) Graph shows collated data for absolute CD4⁺ T conv counts after harvest at day 3 when cultured with IL-21R^{-/-} or +/+ B cells as indicated. (C) Graph shows collated CD25 MFI data for CD4⁺ T conv from B. * P < .05. ** P < .01.

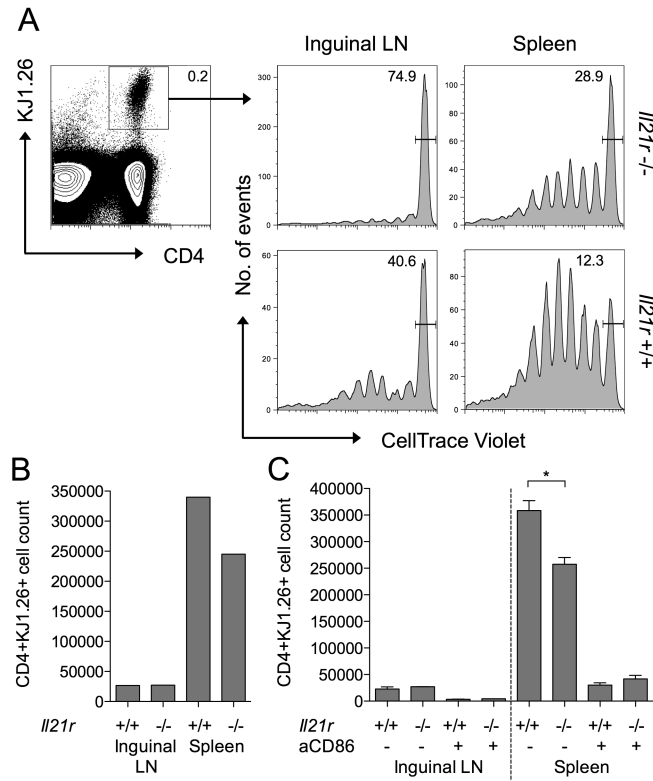


Figure 6. IL-21 signalling to B cells promotes CD86-dependent T cell proliferation in vivo (A) 3×10^6 OVA peptide loaded IL-21R^{-/-} or +/+ CD19⁺ B cells were adoptively transferred into IL-21R^{-/-} recipients. After 24 hours mice received 2×10^6 CellTrace labelled IL-21R^{-/-} DO11⁺ CD4⁺ T cells intravenously and 1 μ g IL-21 intraperitoneally. Histograms show representative CellTrace dilution for gated CD4⁺ DO11⁺ T cells harvested from spleens or inguinal lymph nodes of IL-21R^{-/-} or +/+ B cell recipients at day 7. (B) Graph shows representative absolute CD4⁺DO11⁺ T cell counts from A. (C) IL-21R^{-/-} T cells were activated in the presence of OVA peptide loaded IL-21R^{-/-} or +/+ CD19⁺ B cells (as above) and recipient mice received 100 μ g anti-CD86 or isotype control antibody intraperitoneally on day 1. Graphs show collated absolute CD4⁺DO11⁺ T cell counts. Data are representative of at least 2 independent experiments. * P < .05.