

IL-15 Is Expressed by Dendritic Cells in Response to Type I IFN, Double-Stranded RNA, or Lipopolysaccharide and Promotes Dendritic Cell Activation¹

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Cytokines that are induced by infection may contribute to the initiation of immune responses through their ability to stimulate dendritic cells (DCs). In this paper, we have addressed the role of IL-15 in DC activation, investigating its expression by DCs in response to three different signals of infection and examining its ability to stimulate DCs. We report that the expression of both IL-15 and the IL-15 receptor α -chain are increased in splenic DCs from mice inoculated with dsRNA (poly(I:C)), LPS, or IFN- $\alpha\beta$, and in purified murine splenic DCs treated with IFN- $\alpha\beta$ in vitro. Furthermore, IL-15 itself was able to activate DCs, as in vivo or in vitro exposure of splenic DCs to IL-15 resulted in an up-regulation of costimulatory molecules, markedly increased production of IFN- γ by DC and an enhanced ability of DCs to stimulate Ag-specific CD8⁺ T cell proliferation. The magnitude of all of the IL-15-induced changes in DCs was reduced in mice deficient for the IFN- $\alpha\beta$ receptor, suggesting a role for IFN- $\alpha\beta$ in the stimulation of DCs by IL-15. These results identify IL-15 as a stimulatory cytokine for DCs with the potential for autocrine activity and link its effects to expression of IFN- $\alpha\beta$. *The Journal of Immunology*, 2001, 167: 1179–1187.

Dendritic cells (DCs)³ are recognized as the key APCs for the initiation of T cell-dependent immune responses. In mediating this role, DCs pass through different functional states of activation (1). Resting DCs reside in peripheral tissues in an immature state and are highly efficient in the capture and uptake of Ag. Upon receipt of various activating stimuli, they are induced to migrate to secondary lymphoid organs with a coincident reduction in their capacity for Ag uptake and a marked increase in their ability to present Ag and activate naive T cells. Activated DCs are also capable of directing the type of response made by T cells, a process that may be affected by cytokines secreted by the DCs (2).

Activation of DCs has been shown to occur in response to a number of different stimuli, including signals derived from dead or damaged cells (3) or from infection (1, 4–9). In fact, DCs are sensitive to many different indicators of infection, reflecting the key role that recognition of pathogens has played in the evolution of immunoregulatory mechanisms. Thus, DCs can be activated by exposure to whole pathogens (4–6), components of microorganisms (e.g., LPS, dsRNA, CpG DNA, toxins (5, 7–9)), and cytokines induced by infection (reviewed in Ref. 1). Of these, infection-induced cytokines have the advantage of being able to alert DCs to the presence of infectious agents that do not affect DCs

directly. Furthermore, those cytokines that can be expressed by a broad range of cell types in response to infection would be expected to be most effective as signals for DC activation; cytokines that may be of particular interest in this regard include type I IFN (IFN- $\alpha\beta$) and IL-15 (10, 11).

IFN- $\alpha\beta$ is expressed at low levels in normal or axenic mice (12), but expression is markedly increased upon infection with viruses or bacteria (13); IFN- $\alpha\beta$ is also elicited by components of infectious agents such as LPS, bacterial DNA, and dsRNA (5, 10, 14, 15). Consistent with a role in linking infection to DC activation, recent studies have shown that IFN- $\alpha\beta$ can activate DCs generated in vitro from either mouse bone marrow cells (3) or human peripheral blood precursors (16–18). Furthermore, it has been shown that IFN- $\alpha\beta$ can act as an adjuvant in the promotion of humoral immune responses through stimulation of DCs (19). In addition to its ability to activate DCs, IFN- $\alpha\beta$ has been reported to enhance NK cell cytotoxicity and activate macrophages, implicating it as a general activator of innate immune cells (20).

IL-15 was identified based on its ability to replace the activity of IL-2 in supporting the growth of the IL-2-dependent cell line CTLL-2 and can partially reproduce many of the biological effects of IL-2 (11, 21). Its ability to do so is likely related to the fact that the trimeric IL-15R, while containing a unique α -chain, shares both the β and γ subunits of the IL-2R. However, IL-15 has also been shown to have functions that are distinct from those of IL-2 (22). This is partly due to the different range of cells that can express these cytokines and their receptors. Thus, whereas IL-2 is produced exclusively by T cells, IL-15 mRNA has been detected in a wide range of cell types (21). Similarly, the IL-15R α -chain has a much broader pattern of expression than IL-2R α (23).

Like IFN- $\alpha\beta$, IL-15 is able to activate innate mediators of immunity such as NK cells and macrophages (14, 24). Furthermore, expression of IL-15 is induced by infectious agents and components thereof (24–29). The similarities may be due, at least in part, to the fact that IL-15 expression is up-regulated by IFN- $\alpha\beta$. Hence, IL-15 mRNA expression in murine peritoneal and bone marrow-derived macrophages is increased upon treatment with

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³ Abbreviations used in this paper: DC, dendritic cell; HI-IFN- $\alpha\beta$, heat-inactivated IFN- $\alpha\beta$; rm, recombinant murine; WT, wild type.

IFN- $\alpha\beta$ in vitro (29). Moreover, human DCs derived from peripheral blood monocytes by culture in GM-CSF plus IFN- $\alpha\beta$ had a markedly higher level of IL-15 expression than DCs generated in the presence of GM-CSF plus IL-4 (18). These IFN- $\alpha\beta$ -treated DCs also showed an enhanced expression of costimulatory molecules and a stronger ability to stimulate T cell proliferation in a MLR; whether IL-15 secretion contributed to the functional activity of the DCs was not assessed.

In this study, we have investigated the effects of infection-associated signals on DC expression of IL-15 as well as the capacity of IL-15 to serve as a DC activator. We report that injection of poly(I:C), LPS, or IFN- $\alpha\beta$ into mice induces up-regulated expression of both IL-15 and IL-15R α by splenic DCs. Moreover, IL-15 treatment enhanced the expression of costimulatory markers on DCs, as well as their ability to stimulate Ag-specific CD8 $^{+}$ T cell proliferation. In addition, IFN- γ secretion by splenic DC was markedly increased after treatment with IL-15, implying that IL-15 might modulate the ability of DCs to polarize T cell responses. Notably, the response of DCs to IL-15 was reduced in mice lacking a functional IFN- $\alpha\beta$ receptor, suggesting that the stimulatory effects of IL-15 were partially dependent on IFN- $\alpha\beta$. Therefore, the results provide strong evidence that IL-15 can serve as a signal linking infection to activation of DCs and suggest a possible autocrine loop for DC activation involving IFN- $\alpha\beta$ and IL-15.

Materials and Methods

Mice

C57BL/6 (B6) and BALB/c mice were purchased from the specific pathogen-free unit at the Institute for Animal Health (Compton, Berkshire, U.K.) or from Charles River-U.K. (Margate, Kent, U.K.). 129 SvEv (129) mice, 129 background mice deficient for IFN- $\alpha\beta$ receptor function (IFN- $\alpha\beta$ R $^{-/-}$) (30) (originally purchased from B&K Universal, North Humber-side, U.K.), 2C TCR-transgenic mice (31) (originally obtained from J. Sprent, The Scripps Research Institute, La Jolla, CA), and DO11.10 TCR-transgenic mice (32) were purchased from the specific pathogen-free unit at the Institute for Animal Health. All mice were used at 6–10 wk of age.

Injections

Where indicated, mice were injected i.v. with 2 μ g recombinant murine (rm) IL-15 (BioSource International, Nivelles, Belgium), 100 μ g poly(I:C) (Sigma, Dorset, U.K.), 10 μ g LPS (Sigma), or 10 5 U IFN- $\alpha\beta$ (or the equivalent volume of heat-inactivated IFN- $\alpha\beta$ (HI-IFN- $\alpha\beta$)), each in 200 μ l PBS, or with PBS alone. Doses of IL-15 and IFN- $\alpha\beta$ were chosen based on published reports on the in vivo effects of these cytokines (19, 29). High titer IFN- $\alpha\beta$ (2 \times 10 7 U/mg protein) was prepared from the C243-3 cell line following a method adapted from Tovey et al. (33). Briefly, confluent cells were primed by the addition of 10 U/ml of IFN in MEM enriched with 10% FCS and 1 mM sodium butyrate. After 16 h of culture at 37°C, C243-3 cells were infected with Newcastle Disease Virus (multiplicity of infection of 1) in MEM plus 0.5% FCS plus 5 mM theophylline. Eighteen hours postinfection, culture supernatant was collected and centrifuged at 1500 rpm for 10 min. IFN was concentrated and partially purified by ammonium sulfate precipitations and dialysis against PBS. To generate HI-IFN- $\alpha\beta$, IFN- $\alpha\beta$ was boiled for 1 h.

DC isolation and culture

Splenic DC were isolated using a method similar to that described by Vremec et al. (34). In brief, spleens from six to eight mice were pooled and cut into small fragments. Fragments were digested in RPMI 1640 containing 10% FCS (Life Technologies, Paisley, U.K.), 1 mg/ml type III collagenase (Lorne Laboratories, Reading, U.K.), and 325 KU/ml DNase I (Sigma), with periodic pipetting to break up fragments, for 25 min at room temperature. EDTA (0.1 M, pH 7.2; Sigma) was added for an additional 5 min to allow disruption of DC-T cell complexes. Cells were washed, resuspended in Nycodenz (1.077 g/ml; Life Technologies), overlaid on an additional layer of Nycodenz, and centrifuged at 1700 \times g for 20 min. The low-density fraction was collected and incubated on ice with anti-CD11c-FITC (BD Biosciences, Oxford, U.K.) followed by anti-FITC-Microbeads (Miltenyi Biotec, Biscley, U.K.). The positive fraction was recovered using a MACS separation column and checked on a FACSCalibur (BD Biosciences) for purity. The cells obtained were between 93 and 98% CD11c $^{+}$.

In some experiments, cells were then incubated with PE-anti-CD8 α (Life Technologies) and sorted into CD8 $^{+}$ vs CD8 $^{-}$ subpopulations on a MoFlo flow cytometer (Cytomation, Fort Collins, CO). The resulting CD11c $^{+}$ CD8 $^{+}$ and CD11c $^{+}$ CD8 $^{-}$ populations were >98% pure. Where indicated, DC were cultured in IMDM (Life Technologies) supplemented with 10% heat-inactivated FCS (PAA Laboratories, Linz, Austria), 100 U/ml penicillin, 100 μ g/ml streptomycin, and 100 U/ml polymyxin B (all from Life Technologies), with or without added IFN- $\alpha\beta$ (5000 U/ml) or rmIL-15 (0.5 μ g/ml).

Cytokine detection assays

A total of 10 6 purified DCs were aliquoted into each well of a 96-well culture plate in a volume of 200 μ l, with or without the addition of rmIL-15 (0.5 μ g/ml). Cultures were incubated at 37°C in 5% CO $_2$ for 18 h, after which supernatants were collected and titers of IFN- γ were measured using a Quantikine M ELISA kit (R&D Systems Europe, Abingdon, U.K.).

mAbs and flow cytometry

The following mAbs (all from BD Biosciences) were used: anti-CD54-biotin (3E2), anti-CD40-biotin (HM40-3), anti-CD80-biotin (16-10A1), anti-CD86-biotin (GL1), anti-H2D b -biotin (28-14-8), anti-I-A d /I-E d -biotin (2G9), and anti-CD11c (HL3), which was used in either FITC- or biotin-conjugated form. Biotinylated mAbs were detected with streptavidin-Red670 (Life Technologies). For staining, 2–5 \times 10 5 cells were incubated with optimal dilutions of mAbs in PBS containing 2% FCS and 0.1% NaN $_3$ (Sigma). Stained cells were analyzed on a FACSCalibur flow cytometer (BD Biosciences). Viable cells were selected for analysis based on forward and side scatter properties.

Proliferation assays

CD8 $^{+}$ and CD4 $^{+}$ responder T cells were isolated from 2C TCR-transgenic mice (31) and DO11.10 TCR-transgenic mice (32), respectively, by the following methods. Briefly, mesenteric, axillary, cervical, and inguinal lymph nodes were pooled and gently cut into small fragments. Tissues were then digested using collagenase/DNase/EDTA as described above, and nondigested fragments were removed by filtration through a cell strainer. Cells from 2C mice were washed and incubated with anti-CD8 α microbeads (Miltenyi Biotec), after which the positive fraction was isolated by passing cells through a MACS column. Purity was checked by analysis on a FACSCalibur flow cytometer and ranged from 90 to 98%. CD4 $^{+}$ cells were isolated from DO11.10 cell suspensions by negative selection by incubating cells with optimal dilutions of Abs directed against CD8 (YTS.169), B220 (RA36B2), MHC class II (TIB-120), and CD11b (M1/70) followed by sheep anti-mouse IgG- and sheep anti-rat IgG-conjugated magnetic beads (Dyna, Wirral, U.K.). Splenic DCs were irradiated with 2500 rad and plated (in triplicate) in RPMI 1640 medium supplemented with 10% heat-inactivated FCS (PAA Laboratories), 50 μ M 2-ME (Sigma), 10 mM HEPES, 5% NCTC medium, 100 U/ml penicillin, 100 μ g/ml streptomycin, 100 U/ml polymyxin B, and 250 μ g/ml gentamicin (all from Life Technologies) in 96-well flat-bottom culture plates along with 10 5 responder cells. DCs were plated at 10 4 cells per well (in the peptide titration experiments) or at the indicated numbers of cells per well. The peptide SIYRYGL (synthesized at the Institute for Animal Health, Compton, U.K.), which is recognized by CD8 $^{+}$ T cells from 2C mice in association with H-2K b (35), was added at the indicated concentrations (in the peptide titration experiments) or at 0.05 nM (in the DC titration experiments). For CD4 $^{+}$ responder cells, the peptide corresponding to aa 323–339 of OVA, which is recognized by CD4 $^{+}$ T cells from DO11.10 mice in association with I-A d (32), was used at different concentrations. After 4 days, plates were pulsed for 16 h with 1 μ Ci/well of [3 H]thymidine (Amersham Pharmacia Biotech, Little Chalfont, U.K.). Incorporation of [3 H]thymidine into DNA was analyzed following cell harvesting using a Tomtec harvester (Wallac, Turku, Finland) by liquid scintillation counting on a Microbeta Counter (Wallac).

RT-PCR and analysis of amplified products

mRNA was purified from 1.5 to 3 \times 10 6 murine CD11c $^{+}$ splenic DC using the Quickprep Micro mRNA purification kit (Amersham Pharmacia Biotech). Then 500 ng of mRNA was incubated at 25°C for 10 min with oligo-p(dT) $_{15}$ (Boehringer Mannheim, Lewes, U.K.) in the presence of 50 U RNase inhibitors (Boehringer Mannheim) and reverse-transcribed using 20 U of avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim) for 1 h at 42°C in a final volume of 20 μ l (10 mM Tris, 50 mM KCl, 5 mM MgCl $_2$, 1 mM dNTPs; pH 8.3). PCR was performed on 2 μ l of each cDNA sample using 1.25 U of Thermoprime Plus DNA polymerase

(Advanced Biotechnologies, Epson, U.K.) in a final volume of 50 μ l containing 75 mM Tris, 20 mM ammonium persulfate, 0.1% Tween 20, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 10 pmol of sense primer, and 10 pmol of antisense primer at pH 8.8). The specific primer pairs used were as follows. IFN- α_{1-2} , 5'-TGTCTGATGCAGCAGGTGG-3' (sense) and 5'-AAGACAGGGCTCTCCAGAC-3' (antisense); IFN- β , 5'-CCATCCAAGAGATGCTCCAG-3' (sense) and 5'-GTGGAGAGCAGTTGAGGACA-3' (antisense); IL-15, 5'-CATATGGAATCCAACCTGGATAGTGAAGATA-3' (sense) and 5'-CATATGCTCGAGGGACGTGTTGATGAACAT-3' (antisense); IL-15R α , 5'-CTGACATCCGGGTCAAGAAT-3' (sense) and 5'-TCTGTGTGGTCATTGCGGTA-3' (antisense); and β -actin, 5'-TGACGGGGTCACCCCACTGTGCCATCTA-3' (sense) and 5'-CTAGAAGCATTGCGGTGGAGCATGGAGGG-3' (antisense). To distinguish between IL-15 mRNA isoforms generated by alternative splicing, the following primers were used (36). For the region from exons 1 to 4: P1 (sense, positions 203–224 in murine IL-15 cDNA sequence) 5'-CGTGTGTTTGAAGGCTGAGT-3' and P4 (antisense, positions 499–520) 5'-AACACAAGTAGCAGGATGGA-3'. For the region from exons 6 to 8: P6 (sense, positions 695–714) 5'-GTGACTTTCATCCAGTTGC-3' and P8 (antisense, positions 1010–1028) 5'-ATGGAGCTGTGCTGCCTCT-3'. For the region from exons 3 to sequence upstream of exon 5: P3 (sense, positions 443–462) 5'-AGCTCTTACCTGGGCATTAA-3' and A5 (antisense, positions 56–75 in additional sequence upstream of exon 5) 5'-AAGCAACGGAACAATCAAGA-3'. All primers were obtained from Life Technologies. The samples were amplified for 30–40 cycles using the following conditions: 40 s denaturation at 94°C, 40 s annealing at 62°C, and 1 min extension at 72°C. Samples were further incubated at 72°C for 5 min. Amplified products (10 μ l) were separated by agarose gel electrophoresis on a 1.2% Tris-acetate/EDTA gel and visualized by ethidium bromide staining and UV transillumination. β -Actin RT-PCR was run in parallel to normalize the levels of mRNA in the samples. The relative density of amplified bands was determined by LKB 2202 Ultrascan densitometer (Pharmacia, Uppsala, Sweden).

Protein extraction and Western blotting

Proteins were extracted from splenic DC and assayed by Western blot, as described previously (37). Briefly, 2.5×10^6 cells were incubated for 15 min on ice in lysis buffer (50 mM Tris-HCl, pH 8.0, 120 mM NaCl, 0.25% Nonidet P-40, 0.1% SDS; Sigma) containing the protease inhibitors PMSF, aprotinin, leupeptin, and pepstatin (Roche Products, Welwyn Garden City, U.K.) (each at a final concentration of 10 ng/ml), and 1 mM DTT (Sigma). Then 60 μ g of each protein sample was applied to a 15% SDS-PAGE gel. Following separation, proteins were blotted onto a nitrocellulose membrane (Amersham Pharmacia Biotech). Membranes were blocked with 5% nonfat dry milk in TBS-0.5% Tween 20 and then probed with rabbit anti-mouse IL-15 polyclonal Ab (0.2 μ g/ml; Torrey Pines Biolabs, San Diego, CA) or anti- β -actin (Sigma) followed by HRP-conjugated anti-rabbit IgG Ab (Amersham Pharmacia Biotech). Immunoreactive protein bands were detected by using an ECL detection kit (Amersham Pharmacia Biotech).

Results

Increased expression of IL-15 mRNA in splenic DCs upon treatment with IFN- $\alpha\beta$

In view of the report that human DCs generated in vitro by culture in GM-CSF plus IFN- $\alpha\beta$ expressed higher levels of IL-15 than DCs generated in the presence of GM-CSF plus IL-4 (18), we set out to investigate whether IFN- $\alpha\beta$ treatment would also modify the expression of IL-15 by mature, in vivo-generated DCs. Initially, we examined the effects of injecting poly(I:C), a synthetic dsRNA and potent inducer of IFN- $\alpha\beta$ (38), into BALB/c mice; it has been previously reported that human monocyte-derived DCs express IFN- $\alpha\beta$ when exposed to poly(I:C) in vitro (5). Consistent with these in vitro observations, splenic DCs expressed elevated levels of IFN- α and IFN- β mRNA 4 h after injection of poly(I:C), as assessed by RT-PCR analysis (Fig. 1A). Moreover, splenic DCs isolated from poly(I:C)-injected mice had ~5-fold higher levels of IL-15 mRNA than those from control mice (Fig. 1A).

Although these results implied that IFN- $\alpha\beta$ was able to up-regulate IL-15 expression in splenic DCs, it was possible that poly(I:C) was inducing IL-15 expression independently of effects on IFN- $\alpha\beta$. To assess this possibility, we compared the ability of poly(I:C) to stimulate DC expression of IL-15 in mice lacking a functional type I IFN receptor (IFN- $\alpha\beta$ R $^{-/-}$ mice, on a 129 back-

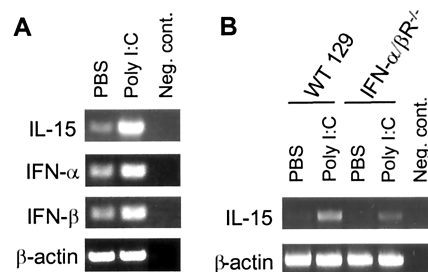


FIGURE 1. Elevated levels of IFN- $\alpha\beta$ and IL-15 mRNA in splenic DCs after in vivo treatment with poly(I:C). Poly(I:C) or PBS was injected i.v. into BALB/c (A) or WT 129 and IFN- $\alpha\beta$ R $^{-/-}$ mice (B). Four hours later, CD11c $^{+}$ DCs were purified from the spleen and mRNA was extracted. IL-15, IFN- α , and IFN- β expression was then analyzed by RT-PCR. PCR primers were used that amplify all IL-15 mRNA. In negative control lanes, no cDNA was added. These experiments were repeated twice with similar results.

ground) vs controls. As in BALB/c mice, DCs from wild-type (WT) 129 mice exhibited increased expression of IL-15 mRNA after injection of poly(I:C) (Fig. 1B). Notably, poly(I:C) injection into IFN- $\alpha\beta$ R $^{-/-}$ mice also enhanced DC expression of IL-15, although to a lesser extent than in control mice; IL-15 mRNA levels were ~2-fold higher in DCs from poly(I:C)-treated WT 129 vs poly(I:C)-treated IFN- $\alpha\beta$ R $^{-/-}$ mice. Although the latter observation suggested that IFN- $\alpha\beta$ may play a role in poly(I:C) induction of IL-15, it was clear that poly(I:C) was also able to up-regulate DC expression of IL-15 in an IFN- $\alpha\beta$ -independent manner.

Therefore, to address directly the question of whether IFN- $\alpha\beta$ can up-regulate DC expression of IL-15, we injected IFN- $\alpha\beta$ into normal mice and examined the expression of IL-15 by splenic DCs 4 h later. For comparison, we also injected LPS, which is a potent activator of DCs in vivo (39) and has been shown to induce IL-15 expression in macrophages (25, 29) and DCs generated in vitro from human peripheral blood precursors (18). As shown in Fig. 2, injection of LPS led to increased levels of IL-15 mRNA in total CD11c $^{+}$ DCs in either BALB/c (Fig. 2A) or WT 129 (Fig. 2B) mice; a smaller increase in IL-15 expression was observed after LPS injection into IFN- $\alpha\beta$ R $^{-/-}$ mice, implying that a portion of the effect of LPS was mediated through induction of IFN- $\alpha\beta$ in the host (Fig. 2B) (40). Notably, injection of IFN- $\alpha\beta$ into BALB/c or WT 129 mice induced similar levels of IL-15 mRNA as injection of LPS (Fig. 2, A and B). Confirmation that this effect was in fact mediated by IFN- $\alpha\beta$ rather than some contaminant in the preparation was provided by two observations: 1) HI-IFN- $\alpha\beta$ did not induce up-regulation of IL-15 mRNA after injection into BALB/c mice (Fig. 2A); and 2) IFN- $\alpha\beta$ injection into IFN- $\alpha\beta$ R $^{-/-}$ mice did not lead to increased levels of IL-15 mRNA. In a separate experiment, we also assessed whether CD8 α^{+} and CD8 α^{-} CD11c $^{+}$ DC subpopulations responded similarly to IFN- $\alpha\beta$ injection, because there is evidence that these phenotypically defined subsets of DCs differentially secrete cytokines, including IL-12 and IFN- γ , upon stimulation (41–43). In fact, elevated IL-15 mRNA was observed in both CD8 α^{+} and CD8 α^{-} CD11c $^{+}$ DCs after injection of IFN- $\alpha\beta$ (Fig. 2C).

mRNAs encoding secreted and nonsecreted forms of IL-15 can be generated by alternative splicing. The message for the nonsecreted form contains an alternative exon 5, which includes additional sequence upstream of the normal exon 5, and is missing exon 2 (36). The translational product of this transcript displays a shorter leader peptide that lacks a signal sequence due to stop

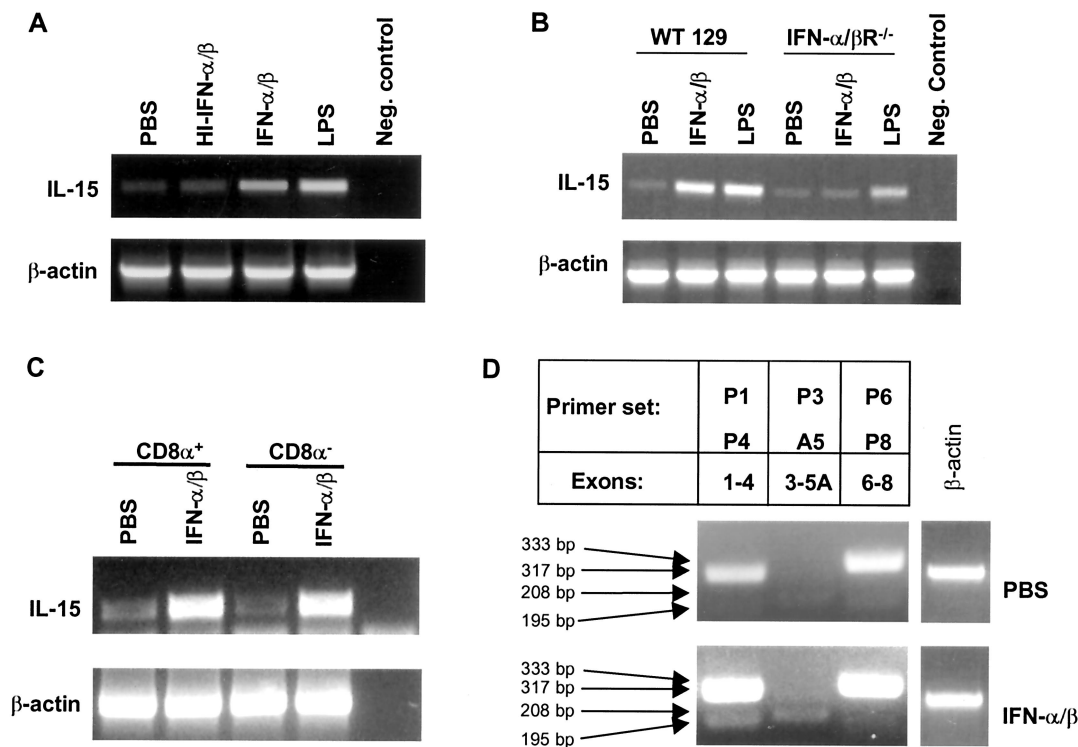


FIGURE 2. Injection of IFN- $\alpha\beta$ enhances IL-15 mRNA expression in splenic DCs. Results (from separate experiments) show IL-15 mRNA expression in total CD11c⁺ DCs (A, B, and D) or CD8 α ⁺ and CD8 α ⁻ CD11c⁺ DCs (C). BALB/c (A, C, and D) or WT 129 and IFN- $\alpha\beta$ R^{-/-} (B) mice were injected i.v. with IFN- $\alpha\beta$, HI-IFN- $\alpha\beta$, LPS (10 μ g), or PBS, and splenic DCs were isolated 4 h later. mRNA was extracted, and IL-15 expression was analyzed by RT-PCR. A–C, PCR primers were used that amplify all IL-15 mRNA. In negative control lanes, no cDNA was added. D, Three sets of IL-15 primers were used. Lane 1, P1 and P4, sense and antisense primers for exons 1 and 4, respectively, which will amplify 317- and 195-bp fragments from exon 2-containing vs exon 2-deficient transcripts, respectively. Lane 2, P3 and A5, sense and antisense primers for exon 3 and alternative exon 5, respectively, which will amplify a 208-bp fragment only from transcripts containing alternative exon 5. Lane 3, P6 and P8, sense and antisense primers for exons 6 and 8, respectively, which will amplify a 333-bp fragment from all IL-15 transcripts. β -Actin RT-PCR was run in parallel to normalize the levels of mRNA in the samples.

codons in the sequence upstream of the normal exon 5. To determine whether DCs were expressing mRNA encoding for the secreted or nonsecreted forms of IL-15 and how this was affected by IFN- $\alpha\beta$, RT-PCR analysis was performed using primers that can distinguish between the two mRNA isoforms. Paired primers for sequences in exons 1 and 4 amplified a 317-bp fragment, indicative of the presence of exon 2 and hence the secreted isoform, that predominated in DCs from both PBS- and IFN- $\alpha\beta$ -treated mice (Fig. 2D, lane 1). DCs from IFN- $\alpha\beta$ -treated mice expressed ~ 2.5 -fold higher levels of this transcript than DCs from PBS-treated mice. A minor product of 195 bp, which was the expected size for a fragment lacking exon 2, was detectable only in DCs from IFN- $\alpha\beta$ -treated mice, indicating that there was also some up-regulation of the nonsecreted isoform of IL-15 by IFN- $\alpha\beta$ (Fig. 2D, lane 1). This was also evident from the presence of a faint band at 208 bp when using primer pairs for exon 3 and alternative exon 5 to amplify mRNA from IFN- $\alpha\beta$ -treated but not PBS-treated DCs (Fig. 2D, lane 2). Overall, however, the data show that DCs express mainly mRNA encoding the secreted form of IL-15 and that this is enhanced by exposure to IFN- $\alpha\beta$.

IFN- $\alpha\beta$ up-regulates IL-15 protein expression in splenic DC

IL-15 expression is regulated at both transcriptional and posttranscriptional levels (11, 36, 44). Hence, it was important to investigate whether the IFN- $\alpha\beta$ -mediated increase in IL-15 mRNA correlated with enhanced levels of IL-15 protein. Because no reagents are currently available for the measurement of murine IL-15 by ELISA, we initially assayed for IL-15 biological activity, as mea-

sured by an ability to stimulate proliferation of the IL-2-dependent cell line CTLL-2. However, using this method we were unable to detect any IL-15 activity in the supernatants of either control DCs or DCs that had been treated with IFN- $\alpha\beta$ in vivo or in vitro. Although this might imply that the DCs were not producing IL-15 protein, it was also possible that the levels of secreted IL-15 were simply below the level of detection in this assay. This is worth considering, because it has been reported that IL-15 can act in a juxtacrine manner when secreted in very small quantities (45) and may even be present in a membrane-bound form (46).

Therefore, to further examine IL-15 protein expression by DCs we performed Western blotting of total DC proteins. DCs were purified from B6 mice 2, 4, and 6 h after injection of IFN- $\alpha\beta$ or PBS. Elevated levels of IL-15 protein were apparent in DCs within 2 h of injection of IFN- $\alpha\beta$, and by 4 h there was ~ 3 -fold more IL-15 protein in DCs from IFN- $\alpha\beta$ -treated mice than in control DCs (Fig. 3A); increased expression of IL-15 protein was still detectable 16 h after IFN- $\alpha\beta$ injection (data not shown). IL-15 protein expression was also up-regulated by ~ 2 -fold in DCs after in vitro treatment, demonstrating that IFN- $\alpha\beta$ can act directly on DCs to stimulate IL-15 expression (Fig. 3B). Therefore, these results show that IFN- $\alpha\beta$ treatment enhances not only IL-15 mRNA levels but also IL-15 protein expression in DCs.

IFN- $\alpha\beta$ enhances DC expression of IL-15R α

The expression of IL-15 by DCs raised the question of whether these cells were also capable of responding to this cytokine. As an

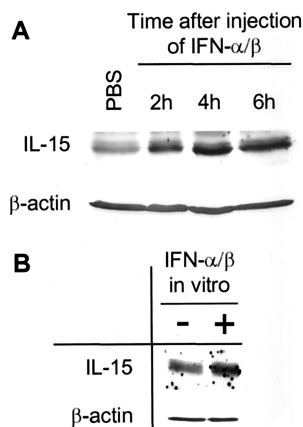


FIGURE 3. Splenic DCs up-regulate expression of IL-15 protein after in vivo or in vitro exposure to IFN- $\alpha\beta$. *A*, B6 mice were injected i.v. with IFN- $\alpha\beta$ or PBS. Splenic DCs were isolated 2, 4, and 6 h after injection of IFN- $\alpha\beta$ or 6 h after injection of PBS. *B*, DCs isolated from BALB/c mice were incubated in medium for 4 h with or without IFN- $\alpha\beta$. IL-15 protein expression was detected by Western blot analysis of cell lysates.

initial approach to address this question, we examined DC expression of high-affinity IL-15R α in control mice and in mice exposed to poly(I:C), LPS, or IFN- $\alpha\beta$; because an Ab to IL-15R α was not available we analyzed expression at the level of mRNA by RT-PCR. As shown in Fig. 4, IL-15R α expression was detectable in DCs from control (i.e., PBS-injected) 129 (Fig. 4, *A* and *C*), and BALB/c (Fig. 4, *B* and *D*) mice, suggesting that DCs can in fact respond to IL-15. Injection of either poly(I:C) or LPS resulted in an increase in IL-15R α mRNA levels within 4 h (Fig. 4, *A–C*). Notably, this was observed in both WT 129 mice and IFN- $\alpha\beta$ R $^{-/-}$ mice, implying that these substances were able to induce expression of IL-15R α in an IFN- $\alpha\beta$ -independent manner. Importantly, however, injection of IFN- $\alpha\beta$ (but not HI-IFN- $\alpha\beta$) also stimulated increased expression of IL-15R α by DCs (Fig. 4, *B* and *C*); as for IL-15 expression, increases in IL-15R α mRNA applied to both CD8 α^{+} and CD8 α^{-} subpopulations (Fig. 4*D*). Thus, in addition to

enhancing IL-15 expression by DCs, poly(I:C), LPS, and IFN- $\alpha\beta$ each augmented DC expression of IL-15R α .

Phenotypic activation of DCs by IL-15

The expression of IL-15R α by DCs suggested that they may be able to respond to IL-15. To examine this possibility, we injected rmIL-15 into normal B6 mice and isolated splenic DCs 4 h later. We then compared the phenotype of DCs from control vs IL-15-injected mice with that of DCs from mice injected with LPS, which is known to induce phenotypic maturation of DCs in vivo (39). As expected, splenic DCs from control mice exhibited a relatively mature phenotype, expressing costimulatory (CD40, CD80, CD86) and adhesion (CD54) molecules, MHC class I, and high levels of MHC class II (Fig. 5*A*). Strikingly, injection of IL-15 stimulated further phenotypic activation of DCs. A marked up-regulation of CD86 expression was observed, while CD40 and MHC class II were also expressed at higher levels after IL-15 injection (Fig. 5*A*). The magnitude of these alterations was only slightly less than that stimulated by injection of LPS, which additionally induced up-regulation of CD80. Similar phenotypic changes occurred upon culture of DCs in the presence of IL-15, indicating that IL-15 was exerting its effects by acting directly on DCs (Fig. 5*B*). Here, however, the effects of IL-15 treatment were somewhat less obvious due to the fact that DCs underwent some degree of phenotypic activation upon culture in medium alone.

Because IFN- $\alpha\beta$ -treatment up-regulates IL-15R α expression, it was of interest to determine whether DCs also become activated upon IL-15 injection into IFN- $\alpha\beta$ R $^{-/-}$ mice. As in B6 (Fig. 5*A*) and BALB/c (data not shown) mice, injection of IL-15 into WT 129 mice resulted in up-regulated cell surface expression of CD40 and CD86 on splenic DCs (Fig. 5*C*). The phenotypic changes were less marked in IFN- $\alpha\beta$ R $^{-/-}$ mice than in control mice; in particular, IL-15 induced little if any increase in CD40 expression while the magnitude of the increase in CD86 expression was also reduced. These observations suggest that low-level signaling through the IFN- $\alpha\beta$ R in normal mice can influence the ability of DCs to respond to IL-15. Nevertheless, it was clear that IL-15 was able to stimulate phenotypic activation of DCs in an IFN- $\alpha\beta$ -independent manner.

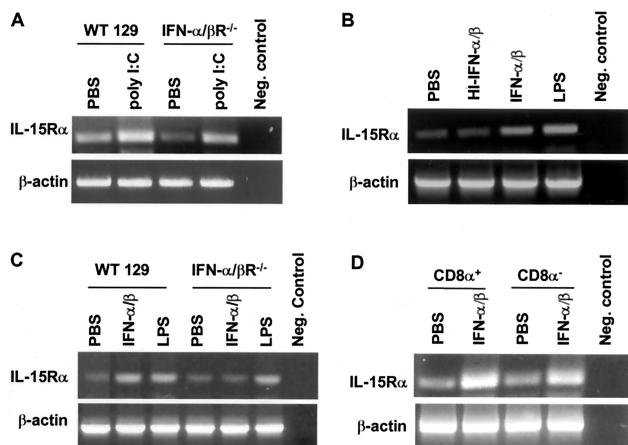


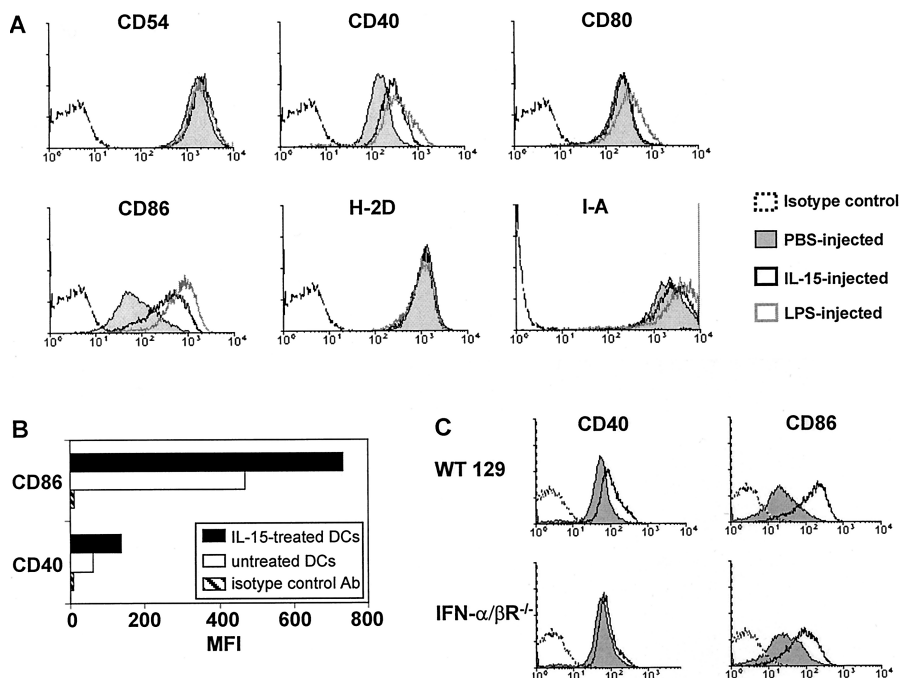
FIGURE 4. Increased IL-15R α mRNA expression by DCs following in vivo treatment with poly(I:C), IFN- $\alpha\beta$, or LPS. *A*, Poly(I:C) or PBS was injected into WT 129 and IFN- $\alpha\beta$ R $^{-/-}$ mice. *B*, IFN- $\alpha\beta$, HI-IFN- $\alpha\beta$, LPS (10 μ g), or PBS was injected into BALB/c mice. *C*, IFN- $\alpha\beta$, LPS, or PBS was injected into WT 129 and IFN- $\alpha\beta$ R $^{-/-}$ mice. *D*, IFN- $\alpha\beta$ or PBS was injected into BALB/c mice. In all cases, injections were given i.v., and DCs were isolated from the spleens 4 h later. In *D*, DCs were further separated into CD8 α^{+} and CD8 α^{-} subpopulations by cell sorting. IL-15R α mRNA expression was assessed by RT-PCR analysis.

IL-15 enhances DC secretion of IFN- γ

DCs have been shown to produce IFN- γ in response to certain stimuli, such as IL-12 (43). We investigated whether IFN- γ production was also altered by IL-15-mediated DC activation. Initially, we tested the effect on IFN- γ secretion of culturing DCs in the presence of IL-15. Splenic DC from normal B6 mice were cultured in 96-well plates with or without IL-15 for 18 h, and the supernatant was assayed for the presence of IFN- γ . As shown in Fig. 6*A*, the addition of IL-15 to the medium stimulated a marked (10-fold) increase in DC secretion of IFN- γ .

We further assessed whether exposure to IL-15 in vivo could similarly induce IFN- γ secretion by DCs. Thus, IL-15 (or PBS as a control) was injected i.v. into normal B6 or BALB/c mice, and splenic DCs were isolated 2 h later. DCs were then cultured in medium alone for 18 h, after which the supernatant was assayed for the presence of IFN- γ . The results for BALB/c DCs are shown in Fig. 6*B*; similar results were obtained for B6 DCs (data not shown). DCs from PBS-injected mice produced detectable levels of IFN- γ during the culture period. However, greater than five times more IFN- γ was present in the supernatant of DCs derived from IL-15-injected mice. As was found for phenotypic activation of DCs, induction of IFN- γ -secretion by IL-15 was reduced but not eliminated in IFN- $\alpha\beta$ R $^{-/-}$ mice compared with WT 129 mice

FIGURE 5. Exposure of DCs to IL-15 induces phenotypic activation. *A* and *C*, B6 mice (*A*) or WT 129 and IFN- $\alpha\beta$ R^{-/-} mice (*C*) were injected i.v. with rmIL-15 or PBS. Alternatively, in *A*, B6 mice were injected i.v. with LPS. Four hours later, splenic DCs were isolated, and the expression of various cell surface molecules was assessed. Dotted lines represent the background staining of isotype-matched control Abs. Filled and open (solid black line) histograms represent DCs from mice treated with PBS and IL-15, respectively. *A*, The phenotype of DCs from mice injected with LPS is shown by the open histograms with solid gray lines. Representative data from one of four experiments are shown. *B*, Splenic DCs from B6 mice were cultured for 18 h in the presence or absence of rmIL-15, and then expression of CD40 and CD86 was assessed by FACS analysis. MFI, Mean fluorescence intensity. Data are representative of four separate experiments.



(Fig. 6C), providing further evidence that DCs in these mice are less sensitive to stimulation with IL-15.

IL-15 enhances the ability of DCs to stimulate T cell proliferation

As a further test of the effects of IL-15 treatment on DC function, we compared the ability of DCs from control vs IL-15-injected mice to present peptide to naive T cells. Using OVA peptide (AA₃₂₃₋₃₃₉)-specific CD4⁺ T cells from DO11.10 TCR-transgenic mice (32) as responders and BALB/c splenic DCs as stimulators, no differences were detected between the peptide-specific proliferative responses stimulated by DCs from control vs IL-15-injected mice (data not shown). In contrast, however, IL-15-treatment did alter the ability of DCs to stimulate CD8⁺ T cells (Fig. 7). Here, splenic DCs were isolated from WT 129 or IFN- $\alpha\beta$ R^{-/-} mice that had been injected 4 h before with PBS or IL-15, while responder CD8⁺ T cells were purified from 2C TCR-transgenic mice; these T cells recognize an 8-aa peptide (SIYRYGL) presented in association with H-2K^b (35). CD8⁺ T cell proliferation was assessed in response to either constant numbers of DCs and various doses of specific peptide (Fig. 7, *A* and *B*) or various numbers of DCs and a constant low concentration (0.05 nM) of peptide (Fig. 7, *C* and *D*). In both cases, DCs from IL-15-treated WT 129 mice induced a stronger CD8⁺ T cell proliferative response than control DCs; the difference was particularly marked in the DC titration (Fig. 7C). Again, the degree of enhancement of DC stimulatory ability by IL-15 was reduced in IFN- $\alpha\beta$ R^{-/-} mice (Fig. 7, *B* and *D*). Thus, taken together with the effects on IFN- γ -secretion described above, these results demonstrate that IL-15 can serve as

an activator of DCs, modifying not only the phenotype of DCs but also their function.

Discussion

As the major, if not only, APCs capable of activating naive T cells, DCs play a critical role in regulating the immune response. In doing so, DCs interpret signals from the local microenvironment and modulate their functional activity accordingly. DCs are particularly sensitive to indicators of infection, which can both trigger DC activation and modulate the type of T cell response elicited by activated DCs. In this paper, we provide evidence that IL-15 can act as an infection-induced signal for DC activation.

This possibility was suggested initially by the observation that DC expression of both IL-15 and IL-15R α increased after exposure to three different signals associated with infection: IFN- $\alpha\beta$, dsRNA (poly(I:C)), and LPS. Although the effects of dsRNA and LPS could be partially attributed to their ability to induce IFN- $\alpha\beta$, expression of IL-15 and IL-15R α was also enhanced by these agents in an IFN- $\alpha\beta$ -independent manner. The elevated expression of IL-15 by murine splenic DCs following IFN- $\alpha\beta$ treatment reported here is consistent with a previous study showing similar effects on in vitro-derived human DCs (18). In addition, increased expression of IL-15 by DCs has also been observed after infection of mice with *Listeria monocytogenes*, although the role of bacteria-induced IFN- $\alpha\beta$ was not investigated (47). DC expression of IL-15 in response to multiple signs of infection may reflect the presence of an IFN regulatory factor element and an NF- κ B binding site in close proximity in the IL-15 promoter region; these two motifs are

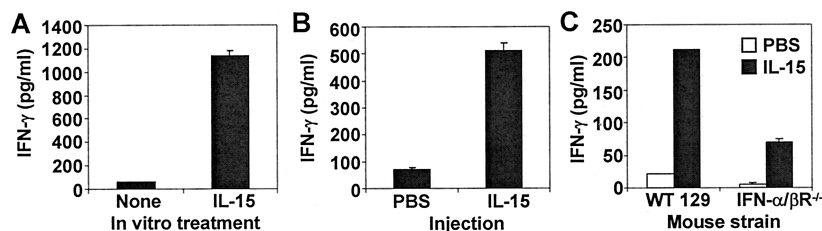


FIGURE 6. Enhanced IFN- γ production by splenic DCs after treatment with IL-15. *A*, Splenic DCs purified from B6 mice were cultured for 18 h in the presence or absence of rmIL-15. *B* and *C*, BALB/c (*B*) or WT 129 and IFN- $\alpha\beta$ R^{-/-} mice (*C*) were injected i.v. with PBS or rmIL-15. Two hours later, splenic DCs were isolated and cultured for 18 h in medium alone. IFN- γ production in the supernatant was determined by ELISA. These experiments were repeated twice with similar results.

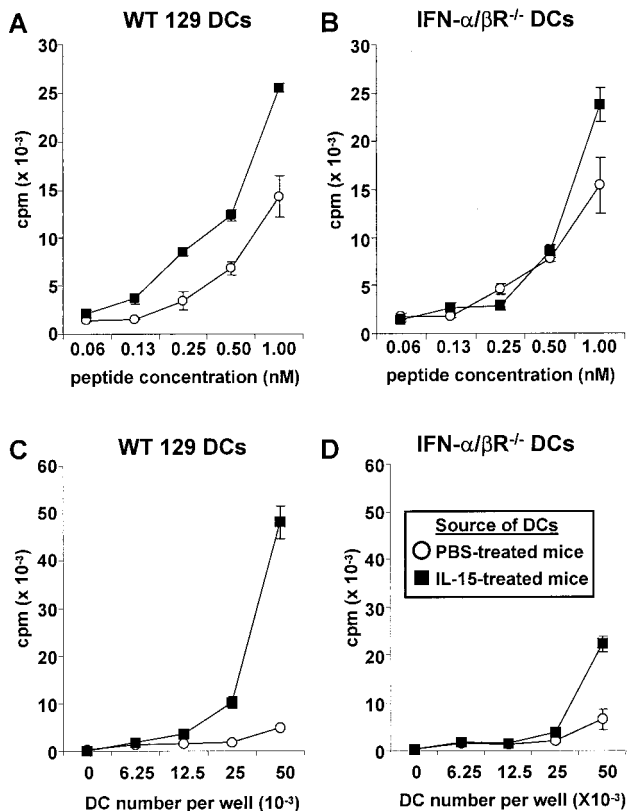


FIGURE 7. Enhanced ability of IL-15-treated DCs to stimulate Ag-specific proliferation of naive CD8⁺ T cells. mIL-15 or PBS was injected i.v. into WT 129 (A and C) or IFN- $\alpha\beta$ R^{-/-} mice (B and D), and splenic DCs were isolated 4 h later. Responder CD8⁺ T cells were purified from lymph nodes of 2C mice and cultured together with either a constant number of DCs (10⁴) and different concentrations of specific peptide (A and B) or a constant concentration of peptide (0.05 nM) and different numbers of DCs (C and D). After 4 days of culture, [³H]thymidine was added, and the cells were cultured for an additional 16 h before harvesting. Each data point represents the amount of [³H]thymidine uptake of a triplicate culture \pm SD. \circ , Splenic DC from PBS-treated mice; \blacksquare , splenic DC from mIL-15-treated mice.

also adjacent to each other in the promoter region of the IFN- β gene (48).

The relevance of IL-15 as a DC activator was shown directly in experiments examining the effects of either IL-15 injection into mice or treatment of purified DCs with IL-15 *in vitro*. IL-15 treatment stimulated increased expression of costimulatory molecules, enhanced secretion of IFN- γ , and an augmented ability to activate naive CD8⁺ T cells. With regard to this latter point, it was notable that we did not detect any increase in the ability of splenic DCs from IL-15-injected mice to stimulate CD4⁺ T cells (data not shown). With this in mind, it is tempting to speculate that IL-15-treatment functions similarly to CD4 “help” in conditioning DCs to become better stimulators of naive CD8⁺ T cells (49–51). This is worth considering, given that virus infections and immunization in the presence of CFA (which contains heat-killed bacteria) can induce Th-independent CD8⁺ T cell responses (49, 50). In any case, our results show that DCs not only express IL-15 upon receipt of infection-associated signals, but also respond to IL-15 by undergoing functional alterations. Given that expression of IL-15 appears to be a common response to infection-associated stimuli in DCs (Refs. 18 and 47 and this study) as well as other cell types (24–29), these results implicate IL-15 as an important signaling

molecule linking the presence of infection with the initiation of immune responses.

In a previous study, microarray analysis was used to demonstrate that human DCs generated *in vitro* from peripheral blood monocytes express IL-15R α mRNA after being driven to undergo maturation by treatment with a combination of IL-6, TNF- α , IL-1 β , and PGE₂ (52). In this study, we report that *in vivo*-generated splenic DCs obtained directly from untreated mice also express IL-15R α . Although reagents were not available to examine directly whether this molecule was expressed on the cell surface, the fact that DCs were clearly responsive to IL-15 both *in vivo* and *in vitro* provided strong indirect evidence that this was the case. IFN- $\alpha\beta$ appeared to regulate the sensitivity of DCs to IL-15, because DC activation in response to IL-15 injection was reduced in IFN- $\alpha\beta$ R^{-/-} mice. This may be linked to the ability of IFN- $\alpha\beta$ to up-regulate IL-15R α (see below). Alternatively, signals through IFN- $\alpha\beta$ R and IL-15R could act independently and synergize in DC activation.

The increased expression of IL-15R α that was observed after IFN- $\alpha\beta$, poly(I:C), or LPS treatment suggests that infection, in addition to augmenting production of IL-15, could enhance the ability of DCs to respond to IL-15. Small changes in expression of the α -chain, which together with the shared β - and γ -subunits of the IL-2R forms the complete IL-15R, may markedly affect cell sensitivity to IL-15 given its high affinity for IL-15R α (23). In fact, the affinity of IL-15R α for IL-15 is 1000-fold higher than the affinity of IL-2R α for IL-2. Therefore, enhanced expression of IL-15R α may allow DCs to respond to very low quantities of IL-15. In this regard, macrophages have been shown to respond to concentrations of IL-15 as low as 10⁻⁶ ng/ml (53).

Such a high level of sensitivity to IL-15 may be important, because the difficulties in detecting secreted IL-15 in most studies suggest that IL-15 is indeed released from cells in very small amounts (46, 54). Furthermore, increased expression of both IL-15 and IL-15R α in DCs after IFN- $\alpha\beta$ or poly(I:C) treatment implies that IL-15 could act in an autocrine or paracrine manner to activate DCs. This is worth considering, as IL-15 can apparently exert autocrine/paracrine effects on macrophages, melanoma cells, and myeloma cells even though secreted IL-15 cannot be detected in the supernatants of these cells (45, 53, 55). In addition to potential autocrine activity, DC production of IL-15 could modulate immune responses by acting on other cell types. In particular, the multiple effects of IL-15 on T cells, including induction of chemotaxis and enhancement of proliferation, cytokine secretion, and cytotoxic activity (28, 56–59), suggest that an increase in IL-15 expression by DCs could significantly alter the T cell response. In this respect, it has been reported that human peripheral blood-derived DCs treated with soluble trimeric CD40 ligand plus IFN- γ expressed increased IL-15 and exhibited an improved ability to stimulate Ag-specific T cells *in vitro* compared with control DCs (60). Significantly, addition of a neutralizing anti-IL-15 to the cultures reversed the enhanced activity of the treated DCs, although whether the IL-15 was acting on DCs, T cells, or both populations was not addressed.

It seems likely that IL-15 stimulation of both DCs and T cells may contribute to the reported adjuvant effects of IL-15, which have included augmenting Ab, delayed-type hypersensitivity, and CTL responses *in vivo* (61–64). Furthermore, evidence supporting a role for endogenous IL-15 in the initiation of T cell responses has been provided by studies in which an IL-15 antagonist, a soluble fragment of IL-15R α , inhibited the development of collagen-induced arthritis (65) or allograft rejection (66); again, the target cells for IL-15 action in these systems were not identified.

IL-15 treatment stimulated a marked increase in IFN- γ secretion by DCs, acting similarly in this respect to IL-12 (43). This raises the possibility that, in addition to activating DCs, IL-15 treatment may modulate the type of T cell response elicited. For example, DC-secreted IFN- γ could enhance IL-12 production by macrophages (67), ultimately promoting a Th1-type response. In this regard, it is worth noting that IL-15 has also been shown to induce IFN- γ mRNA in alveolar macrophages (68). There is in fact some evidence that IL-15 can augment Th1 responses in vivo (69, 70), although the mechanisms involved were not investigated. Of course, given the widespread expression of IL-15R, in vivo administration of IL-15 is likely to affect many different cell types, and the immune response elicited will likely reflect this fact. Nevertheless, the data presented here identify DCs as a target for IL-15 action, implicating IL-15 as an important signaling molecule linking the innate response to infection with the initiation of the adaptive immune response.

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