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IL-15 Receptor Maintains Lymphoid Homeostasis by Supporting Lymphocyte Homing and Proliferation

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Summary

The IL-15 receptor alpha subunit (IL-15Rα) mediates high-affinity binding of IL-15, a pleiotropic cytokine implicated in the development of innate immune cells. We have generated *IL-15Rα* null (*IL-15Rα*$^{-/-}$) mice to understand the role of IL-15Rα in immune development and function. *IL-15Rα*$^{-/-}$ mice are markedly lymphopenic despite grossly normal T and B lymphocyte development. This lymphopenia is due to decreased proliferation and decreased homing of *IL-15Rα*$^{-/-}$ lymphocytes to peripheral lymph nodes. These mice are also deficient in natural killer cells, natural killer T cells, CD8$^+$ T lymphocytes, and TCRγδ intraepithelial lymphocytes. In addition, memory phenotype CD8$^+$ T cells are selectively reduced in number. Thus, IL-15Rα has pleiotropic roles in immune development and function, including the positive maintenance of lymphocyte homeostasis.

Introduction

IL-15 binds to lymphocytes via a heterotrimeric IL-15 receptor (IL-15R) (Grabstein et al., 1994; Tagaya et al., 1996) comprised of IL-15Rα (Giri et al., 1995), IL-2 receptor beta (IL-2Rβ) (Bamford et al., 1994), and IL-2 receptor gamma (IL-2Rγ) (Noguchi et al., 1993; Kondo et al., 1994). The closely related trimeric IL-2R includes the latter two subunits plus IL-2 receptor alpha (IL-2Rα) (Smith, 1988). Thus, specific responses to IL-15 are conferred by IL-15Rα (Giri et al., 1995). The fact that IL-2R and IL-15R share subunits thought to mediate downstream signaling events suggests that IL-2R and IL-15R may perform similar functions. Like IL-2, IL-15 stimulates lymphocyte activation and proliferation in vitro (Bamford et al., 1994; Armitage et al., 1995). This stimulation occurs via both induction of cell cycle entry and protection against cell death (Vella et al., 1998). In addition, IL-15 and IL-2 can both induce programmed cell death (PCD) of activated lymphocytes (AICD) (Lenardo, 1991).

Despite the apparent similarities between IL-2 and IL-15, profound abnormalities in *IL-2* (*IL-2*$^{-/-}$) and *IL-2Rα*-deficient (*IL-2Rα*$^{-/-}$) mice indicate that IL-2R and IL-15R are not redundant proteins in vivo. Severe lymphadenopathy and autoimmunity in *IL-2*$^{-/-}$ and *IL-2Rα*$^{-/-}$ mice suggest that IL-2R is critical for downregulating activated lymphocytes (Sadlack et al., 1993; Willerford et al., 1995). This function of IL-2R is not compensated for by IL-15R or by other cytokine receptors sharing the IL-2Rγ subunit (e.g., IL-4R, IL-7R). Thus, IL-15R likely performs distinct immune functions from IL-2R in vivo despite indications that both proteins induce the same cytoplasmic signals in lymphocytes.

IL-15 may also play a unique role in the differentiation of innate immune cells. Innate immune cells are phylogenetically primitive cells that use restricted or monomorphic receptors to effect rapid, stereotypical responses against conserved antigens on foreign pathogens (Medzhitov and Janeway, 1997). Deficiencies in the numbers of natural killer (NK) cells (Suzuki et al., 1997), natural killer T (NKT) (Ohteki et al., 1997) cells, and intraepithelial lymphocytes (IELs) (Suzuki et al., 1997) in *IL-2Rβ*$^{-/-}$ and not *IL-2*$^{-/-}$ mice suggest that IL-2Rβ-mediated signals that do not involve IL-2 may be important in the differentiation of these innate immune cells. As IL-15R utilizes the IL-2Rβ subunit, it has been proposed that IL-15Rα may be essential for the development of these innate immune cells. However, the spontaneous lymphadenopathy, autoimmunity, and premature mortality which occurs in both *IL-2*$^{-/-}$ and *IL-2Rβ*$^{-/-}$ mice may complicate conclusions derived from these mice (Sadlack et al., 1993; Suzuki et al., 1995).

Finally, the expression of IL-15 and IL-15Rα in virtually all tissues tested contrasts sharply with the highly restricted expression of IL-2 and IL-2Rα to select subsets of lymphocytes (Giri et al., 1995). Hence, the in vivo functions of IL-15Rα and IL-2Rα are likely to differ in ways not readily discernible using in vitro studies of isolated lymphocytes. As the roles of IL-15Rα have not been directly studied in vivo, we have generated *IL-15Rα*-deficient mice (*IL-15Rα*$^{-/-}$) by gene targeting in embryonic stem (ES) cells.

Results

Generation of *IL-15Rα*$^{-/-}$ Mice

To generate *IL-15Rα*$^{-/-}$ mice, we created a targeting construct designed to delete two exons of the *IL-15Rα* gene encoding the ligand binding and proline/threonine rich extracellular domains (Figure 1A). Transfection of this construct into ES cells yielded five targeted ES cell clones, and injection of two clones into C57Bl/6 blastocysts generated >95% chimeric male offspring, which were bred to obtain germline transmission (Figure 1B).

IL-15Rα$^{-/-}$ mice were obtained at approximately Mendelian frequencies from multiple *IL-15Rα*$^{+/-}$ × *IL-15Rα*$^{+/-}$ matings (30 *IL-15Rα*$^{+/+}$, 70 *IL-15Rα*$^{+/-}$, and 25 *IL-15Rα*$^{-/-}$ mice), indicating that IL-15Rα is not required for embryonic development. Hybridization of liver DNA from *IL-15Rα*$^{-/-}$ mice with a cDNA probe representing the deleted regions of the *IL-15Rα* gene confirmed that these exons were absent (data not shown). Hybridization of liver RNA from *IL-15Rα*$^{-/-}$ mice to a cDNA probe representing the entire *IL-15Rα* coding region failed to

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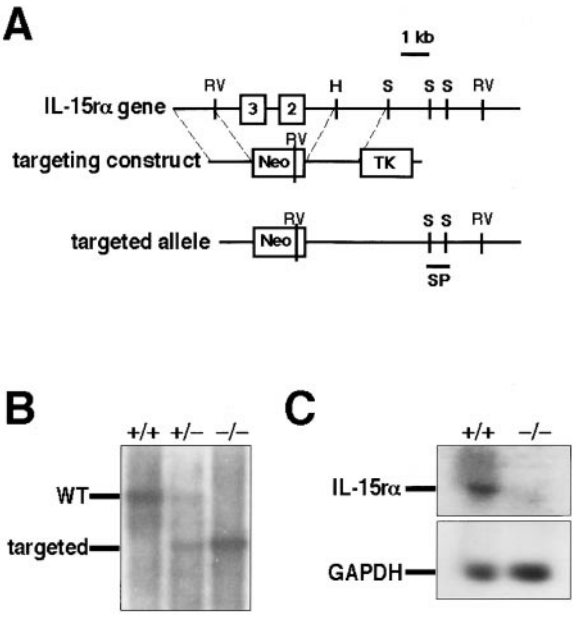

Figure 1. Generation and Analysis of *IL-15Rα*$^{-/-}$ Mice

(A) Endogenous *IL-15Rα* gene and targeting construct (RV, EcoRV; H, Hind3; S, SacI; and SP, screening probe). A 2.5 kb EcoRV–Hind3 fragment containing exons encoding the ligand binding and proline/threonine rich extracellular domains (exons 2 and 3) was replaced with a phosphoglyceraldehyde kinase–directed neomycin cassette (*pGKneo*) in a gene targeting construct. ES cell clones that survived selection were screened by Southern analysis of EcoRV digested genomic DNA, using a 600 bp SacI–SacI genomic fragment (SP) of *IL-15Rα*. This analysis yields a 10 kb or 8 kb EcoRV band for the endogenous or targeted allele, respectively.

(B) Southern analysis of *IL-15Rα*$^{-/-}$ mice. Genomic DNA from tail tips (15 μg) was digested with EcoRV and analyzed by Southern blotting as above.

(C) Northern analysis of liver RNA from *IL-15Rα*$^{-/-}$ mice. Total liver RNA from *IL-15Rα*$^{-/-}$ and IL-15Rα$^{+/+}$ littermates was analyzed by Northern blotting for hybridization to ^{32}P-labeled *IL-15Rα*- and *GAPDH*-specific probes.

detect expression of *IL-15Rα* mRNA, indicating that IL-15Rα proteins cannot be made (Figure 1C). Histological analyses of brains, lungs, livers, spleens, kidneys, stomachs, and intestines revealed no gross differences between *IL-15Rα*$^{-/-}$ and control mice, even though *IL-15Rα* expression is widespread in these tissues (Giri et al., 1995; data not shown).

Innate Immune Cell Deficiency in *IL-15Rα*$^{-/-}$ Mice

As NK cells may depend upon IL-15 (Suzuki et al., 1997), we investigated the development and function of NK cells in *IL-15Rα*$^{-/-}$ mice. To assess NK cell cytolytic function, splenocytes from polyinosine/cytosine (poly I/C)-primed *IL-15Rα*$^{-/-}$ and control mice were incubated with YAC-1 target cells. *IL-15Rα*$^{-/-}$ splenocytes displayed essentially no killing activity against YAC-1 target cells as compared to control littermates (Figure 2A). To distinguish between developmental and functional NK cell defects, splenocytes were assayed for the presence of the NK cell–specific markers DX5 and NK1.1. Flow cytometric analyses revealed that *IL-15Rα*$^{-/-}$ splenocytes are devoid of both CD3$^-$DX5$^+$ and CD3$^-$NK1.1$^+$ cells (Figure 2B; data not shown), suggesting that IL-15Rα is required for NK cell development.

NKT cells are innate immune cells that are positively selected in the context of CD1d major histocompatibility complex (MHC) antigens (Bendelac et al., 1997). NKT cells in the thymus express high levels of the adhesion molecule CD44 as well as NK1.1 (Fowlkes et al., 1987). To assess the role of IL-15 in NKT cell development, thymi from 1- to 4-week-old *IL-15Rα*$^{-/-}$ and control mice were harvested. Flow cytometric analysis of these thymocytes revealed consistently reduced numbers of CD8$^-$CD44^{Hi}NK1.1$^+$ thymocytes in *IL-15Rα*$^{-/-}$ mice, suggesting that IL-15Rα mediates NKT cell differentiation (Figure 2C).

IELs include a diverse array of T cell subsets bearing either T cell receptor (TCR) α/β or TCRγ/δ receptors. Many TCRγ/δ IELs bear CD8α/α homodimers rather than the CD8α/β heterodimers present on most peripheral T lymphocytes. These TCRγ/δ CD8α/α IELs are thought to differentiate extrathymically (Guy-Grand et al., 1991), perhaps from intestinal cryptopatches (Saito et al., 1998), and recognize microbial lipoglycans conserved on bacterial cell walls (Sieling et al., 1995). While total IEL numbers were approximately 2-fold depressed in *IL-15Rα*$^{-/-}$ mice, a 5- to 10-fold reduction of TCRγδ CD8αα IELs was observed (Figure 2D). Taken together, these data indicate that IL-15Rα is essential for the development and/or maintenance of several innate immune cell lineages.

Development of Conventional *IL-15Rα*$^{-/-}$ T and B Lymphocytes

To assess the role of IL-15Rα signals in the differentiation of conventional T and B lymphocytes, cells from thymi, fetal and neonatal livers, and bone marrows were analyzed by flow cytometry. Thymi from newborn to 4-week-old *IL-15Rα*$^{-/-}$ mice possessed approximately 25% fewer cells than control littermates (Figure 3A). Analysis of thymic subsets from multiple mice revealed that this hypocellularity was associated with reductions of all single-positive (SP) CD4$^+$ and CD8$^+$ and double-positive (DP) CD4$^+$CD8$^+$ thymocytes. However, the ratio of SP CD4$^+$ to SP CD8$^+$ thymocytes was consistently increased in *IL-15Rα*$^{-/-}$ thymi (Figure 3B). These data suggest that CD8$^+$ T cell development is selectively compromised in *IL-15Rα*$^{-/-}$ mice.

To evaluate the role of IL-15Rα in B cell development, E18.5 fetal and newborn livers as well as bone marrows were studied for the presence of B220$^+$ lymphocytes. Normal numbers of B220$^+$ lymphocytes were observed in fetal livers (Figure 3A) and normal numbers of B220$^+$ IgM$^+$ B lymphocytes were observed in bone marrows (Figure 3C) from *IL-15Rα*$^{-/-}$ mice. In addition, splenic B lymphocytes were not reduced in *IL-15Rα*$^{-/-}$ mice (Figures 3A and 3D). Therefore, B cell development is intact in these mice.

Loss of CD8$^+$ Peripheral Lymphocytes in *IL-15Rα*$^{-/-}$ Mice

As we observed a decrease of SP CD8$^+$ cells in *IL-15Rα*$^{-/-}$ thymi, we investigated the status of T lymphocyte subsets in peripheral lymphoid tissues. Flow cytometric analyses revealed that CD8$^+$ T lymphocytes were

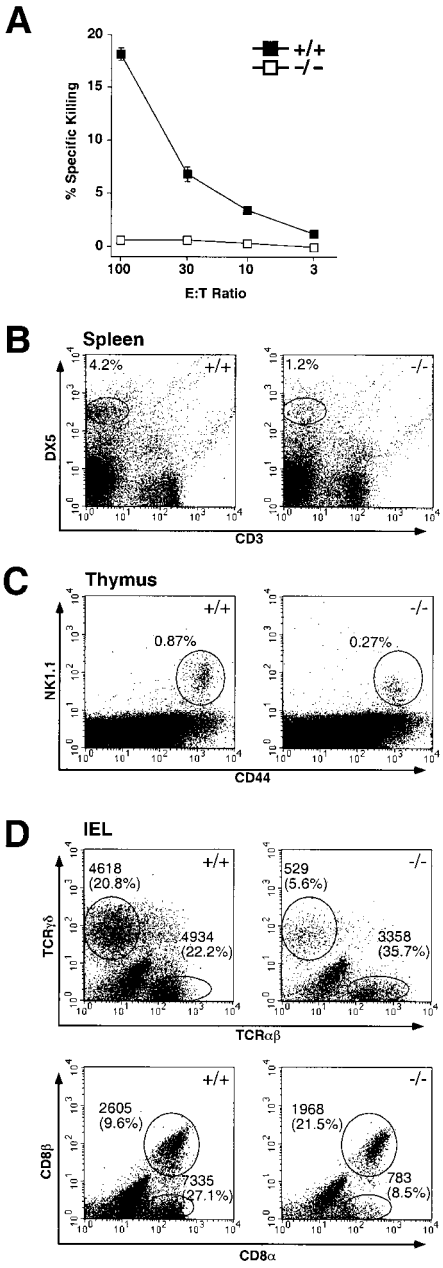

Figure 2. Innate Immune Cell Deficiency in *IL-15Rα*$^{-/-}$ Mice

(A) NK cell killing of YAC-1 target cells. Splenocytes from poly(I):(C)-injected mice were harvested and used as effector cells. Effector (E) and target (T) cells were mixed at the indicated ratios and incubated for 4 hr. ^{51}Cr release was measured and specific killing calculated as described in Experimental Procedures. Closed symbols, *IL-15Rα*$^{+/+}$ splenocytes; open symbols, *IL-15Rα*$^{-/-}$ splenocytes. Data represent one of two experiments.

(B) NK cell deficiency in *IL-15Rα*$^{-/-}$ mice. RBC-depleted splenocytes were incubated with MAbs specific for CD3 and DX5. Analysis was performed on lymphocyte-gated events after 10^5 total events were collected. The percentage of NK cell splenocytes bearing DX5 but not CD3 is indicated.

(C) NK T cell deficiency in *IL-15Rα*$^{-/-}$ mice. Thymocytes were incubated with MAbs specific for CD8, CD44, and NK1.1. Live gating on CD8-negative cells was performed, and 10^5 events were collected. The percentage of CD44^{Hi}NK1.1$^+$ cells, representing NKT cells, is indicated.

(D) IEL TCRγδ and CD8αα T cell deficiency in *IL-15Rα*$^{-/-}$ mice. IELs were incubated with MAbs specific for TCRδγ, TCRαβ, CD8α, and CD8β. Numbers represent lymphocyte-gated events in each region collected during a fixed time. Percentages of IELs for each subset are indicated in parentheses. Cytometric data are representative of four pairs of mice.

consistently decreased relative to CD4$^+$ T and B lymphocytes in both spleens and lymph nodes of *IL-15Rα*$^{-/-}$ mice (Figures 3D and 3E), suggesting that the differentiation and/or maintenance of CD8$^+$ T lymphocytes, like that of the innate immune cells described above, depends upon IL-15Rα.

As IL-15 induces the expansion of mature CD8$^+$CD44$^+$ lymphocytes in vivo (Zhang et al., 1998), we determined the activation status of CD8$^+$ T lymphocytes in non-immunized animals. CD8$^+$CD44^{Hi} and CD8$^+$CD44^{Int} lymphocytes were markedly reduced compared with CD8$^+$CD44^{Lo} lymphocytes (Figure 4). The reduced activation status of *IL-15Rα*$^{-/-}$ CD8$^+$ T lymphocytes was confirmed by assaying these cells for the presence of IL-2Rβ, CD62L (L-selectin), and Ly-6C antigens. CD8$^+$ *IL-15Rα*$^{-/-}$ T lymphocytes displayed lower IL-2Rβ and Ly-6C staining as well as higher CD62L reactivity (Figure 4). The expression of CD44 on CD4$^+$ T lymphocytes was variably reduced by IL-15Rα deficiency (data not shown). Hence, IL-15Rα is important for both the differentiation of CD8$^+$ T lymphocytes and the maintenance of activated, memory phenotype CD8$^+$ T lymphocytes.

Failure of Lymph Node Homeostasis in *IL-15Rα*$^{-/-}$ Mice

Spleens from *IL-15Rα*$^{-/-}$ mice possess similar numbers (only 10%–15% fewer) of lymphocytes compared with normal littermates. It was thus surprising to find that peripheral lymph nodes from *IL-15Rα*$^{-/-}$ mice were visibly smaller than control lymph nodes. Cell counts revealed that *IL-15Rα*$^{-/-}$ lymph nodes contained 30%–80% fewer cells than littermate controls (Figure 3A). No selective loss of T or B lymphocytes was apparent in flow cytometric analyses of *IL-15Rα*$^{-/-}$ lymph nodes (Figure 3E). Thus, the profoundly reduced cellularity of *IL-15Rα*$^{-/-}$ lymph nodes suggests that IL-15Rα supports the homeostasis of all lymph node lymphocytes.

IL-15Rα might mediate lymph node homeostasis by supporting (1) homing, (2) proliferation, and/or (3) survival of lymph node cells. To directly address the homing capacity of *IL-15Rα*$^{-/-}$ lymphocytes and to determine whether IL-15Rα expression on lymphocytes or endothelial cells is essential for homing, splenocytes from normal and *IL-15Rα*$^{-/-}$ littermates were labeled with either green or orange fluorescent dyes and injected into normal mice. Flow cytometric analyses of spleens and lymph nodes from recipient mice revealed that the ratio of labeled *IL-15Rα*$^{-/-}$ to normal lymphocytes recovered from spleens 1 and 16 hr after injection were similar to the original mix of injected lymphocytes (Figure 5A). These data indicate that the survival and ability of *IL-15Rα*$^{-/-}$ lymphocytes to migrate to spleens was normal during the course of these experiments. In contrast, the ratio of *IL-15Rα*$^{-/-}$ to normal cells found in recipient lymph nodes was consistently 1:2 to 1:4, indicating that *IL-15Rα*$^{-/-}$ lymphocytes migrate poorly to lymph nodes

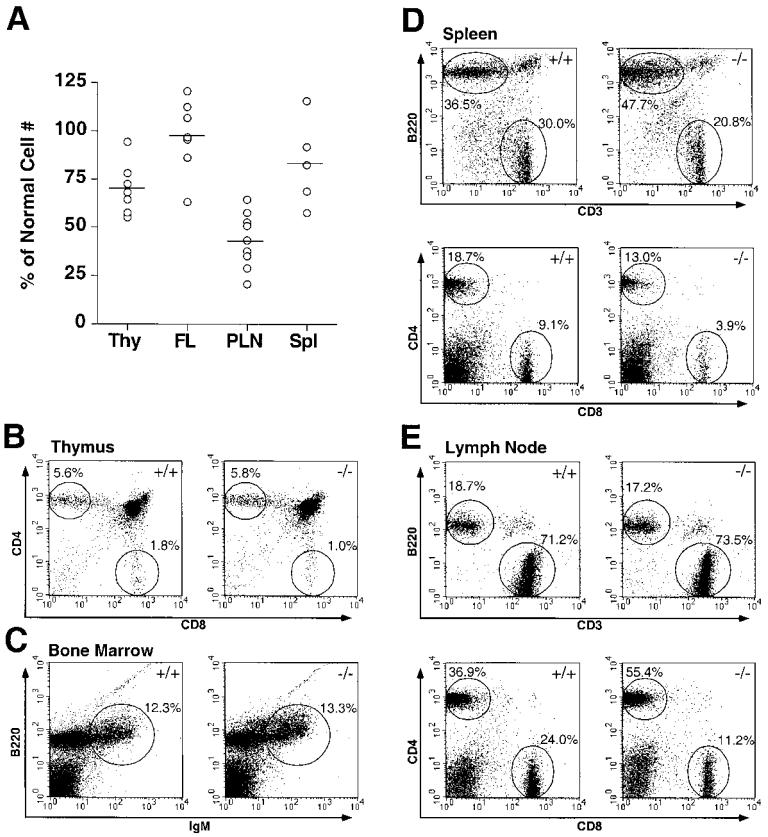

Figure 3. Lymphopenia in *IL-15Rα*$^{-/-}$ Mice

(A) Lymphocytes from various tissues (Thy, thymus; FL, fetal/neonatal liver; PLN, peripheral lymph nodes; and Spl, spleen) were counted. Thy and PLN counts include all cells, Spl were counted after RBC depletion, and FL reflect all B220^{+} cells. Each circle represents the percentage obtained by dividing the cell yield from an *IL-15Rα*$^{-/-}$ organ by an age-matched control mouse organ, and the horizontal line represents the mean. Flow cytometric analyses of *IL-15Rα*$^{-/-}$ thymocytes (B), bone marrow (C), spleens (D), and lymph nodes (E) are displayed. Equal numbers of events were collected for each set of plots. Numbers represent the percentage of lymphocyte-gated events in each region. Cytometric data are representative of at least six pairs of mice.

(Figure 5A). Exchanging the dyes used for labeling *IL-15Rα*$^{-/-}$ and normal lymphocytes confirmed that the biased homing was not due to a selective effect of one dye upon homing (data not shown). Thus, lymphocyte expression of IL-15Rα is important for the homing of lymphocytes to peripheral lymph nodes.

In addition to homing, homeostasis of lymph nodes also depends upon lymphocyte proliferation and cell death. Thus, the reduced cellularity of *IL-15Rα*$^{-/-}$ lymph nodes may also be partly due to a decreased capacity of *IL-15Rα*$^{-/-}$ lymphocytes to proliferate. This possibility was examined by injecting 5-bromo-2-deoxyuridine (BrdU) into *IL-15Rα*$^{-/-}$ and control littermates and harvesting lymph node cells after several days of treatment (Tough and Sprent, 1994). The percentage of BrdU^{+} cells in *IL-15Rα*$^{-/-}$ lymph nodes was 20% lower than in control lymph nodes throughout five days of BrdU labeling (Table 1). Because *IL-15Rα*$^{-/-}$ lymph nodes have 30%–80% fewer cells than control lymph nodes (Figure 3A), the reduced percentages of BrdU^{+} cells in *IL-15Rα*$^{-/-}$ lymph nodes represent a large reduction in the total number of dividing cells. Therefore, lymphopenia in *IL-15Rα*$^{-/-}$ mice is associated with a reduction of proliferating cells.

As IL-15 may protect lymphocytes against PCD (Vella et al., 1998; Bulfone-Paus et al., 1998), we asked whether the profound lymphopenia in *IL-15Rα*$^{-/-}$ mice was partly due to an increased susceptibility of *IL-15Rα*$^{-/-}$ lymphocytes to die. We utilized flow cytometry and FITC-conjugated annexin to detect apoptotic cells. Freshly isolated lymphocytes from *IL-15Rα*$^{-/-}$ and control littermates displayed no significant difference in the percentage of annexin^{+} cells (Figure 5B). Thus, no increased susceptibility to PCD was evident in *IL-15Rα*$^{-/-}$ lymphocytes. Therefore, the hypocellularity of *IL-15Rα*$^{-/-}$ lymph nodes appears to be due to compromised homing and proliferation of *IL-15Rα*$^{-/-}$ lymphocytes.

In Vitro Proliferation of *IL-15Rα*$^{-/-}$ Lymphocytes

IL-15 supports proliferation of activated T lymphocytes in vitro (Grabstein et al., 1994). To evaluate the role of IL-15Rα in T lymphocyte activation and proliferation, resting T lymphocytes from *IL-15Rα*$^{-/-}$ and control mice were cultured in the presence of media alone, anti-CD3 MAb, or anti-CD3 plus either IL-2 or IL-15. As expected, *IL-15Rα*$^{-/-}$ T lymphocytes failed to expand in response to anti-CD3 plus IL-15, confirming the dependence of IL-15 signaling upon IL-15Rα (Figure 6). Surprisingly, *IL-15Rα*$^{-/-}$ T lymphocytes showed less response than control T lymphocytes to anti-CD3 with or without IL-2, suggesting that IL-15Rα is important for early T cell activation and for inducing IL-2 responsiveness (Figure 6). Stimulation with ionomycin and phorbol myristyl acetate (PMA) showed that the inherent proliferative capacity of *IL-15Rα*$^{-/-}$ T lymphocytes was comparable to normal cells when IL-2Rβγ signals were bypassed (Figure 6). Thus, IL-15Rα mediates critical steps in early lymphocyte activation and proliferation.

Discussion

IL-15Rα$^{-/-}$ mice reveal several unique roles for this receptor in immune cell development and function. Most prominently, IL-15Rα mediates proliferative and homing

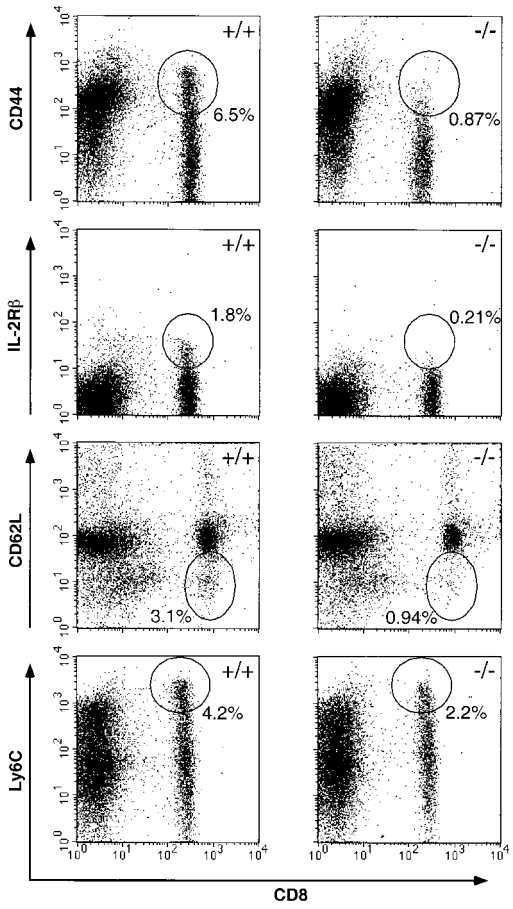

Figure 4. Reduced Activation Status of $CD8^+$ T Lymphocytes in *IL-15R*$\alpha^{-/-}$ Mice

Lymphocytes from lymph nodes of *IL-15R*$\alpha^{-/-}$ and control littermates were assayed for the presence of activation-specific markers. Numbers represent the percentage of lymphocyte-gated events in each region. Cytometric data are representative of at least four pairs of mice.

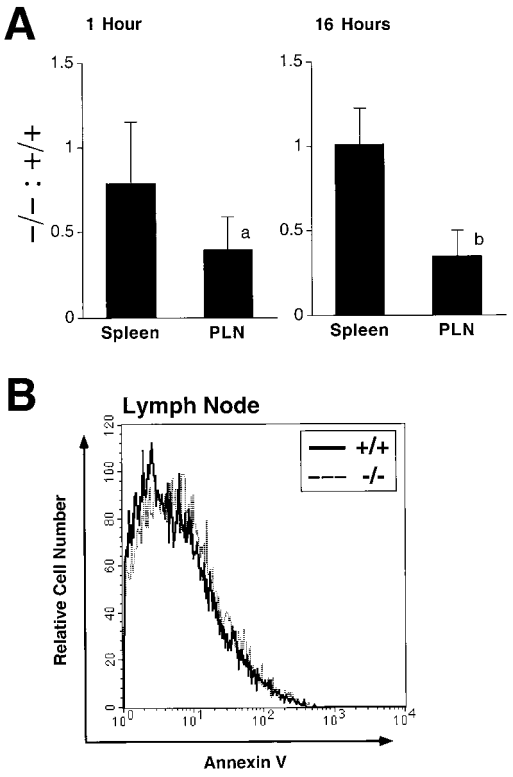

Figure 5. Failure of Lymph Node Homeostasis in *IL-15R*$\alpha^{-/-}$ Mice

(A) Homing defect of *IL-15R*$\alpha^{-/-}$ splenocytes. One and sixteen hours after tail vein injection of labeled splenocytes (see Experimental Procedures), the ratio of *IL-15R*$\alpha^{-/-}$ to normal lymphocytes recovered from spleens and lymph nodes was determined by flow cytometry. Displayed ratios are normalized to the ratios of the preinjection mixtures. $^a p = 0.0296$ at 1 hr and $^b p = 0.0206$ at 16 hr in comparing spleen to lymph node yields (paired t test, $n = 4$).

(B) Normal survival of lymphocytes in *IL-15R*$\alpha^{-/-}$ mice. Freshly isolated lymphocytes were analyzed for apoptotic cell death by annexin V staining. The histogram shows the relative number of lymphocyte-gated cells positive for annexin staining in normal (solid line) versus *IL-15R*$\alpha^{-/-}$ (dashed line) mice.

functions that are essential for the homeostasis of mature lymphocytes. IL-15Rα also facilitates the differentiation of multiple innate immune cell lineages, including $CD8^+$ T lymphocytes. Finally, IL-15Rα supports the development of memory phenotype $CD8^+$ T lymphocytes. These pleiotropic functions indicate that IL-15Rα is essential for a wide range of immune functions in vivo.

Distinction of IL-15R from IL-2R Function

The lymphopenia and innate immune deficiency of *IL-15R*$\alpha^{-/-}$ mice contrasts sharply with the lymphadenopathy and autoimmunity seen in *IL-2*$^{-/-}$ and *IL-2R*$\alpha^{-/-}$ mice, suggesting that IL-2Rα and IL-15Rα have opposing functions in vivo. This finding is remarkable in light of the fact that they share the IL-2Rβ and IL-2Rγ subunits thought to mediate cytoplasmic signaling. One tantalizing mechanism for the divergent functions may be that dissimilar association kinetics of IL-2Rα and IL-15Rα with IL-2R$\beta\gamma$ could cause IL-2R$\alpha\beta\gamma$ and IL-15R$\alpha\beta\gamma$ to initiate quantitatively distinct signaling cascades leading to qualitatively diverse cellular signals. Similar paradigms have recently been described for T cell receptor (Kersh et al., 1998) and IgE Fc receptor (Torigoe et al., 1998) complexes. Alternatively, IL-15Rα may induce different signals than IL-2Rα via the short (37 amino acid) cytoplasmic domain of IL-15Rα. Since the IL-15 ligand is ubiquitously expressed while IL-2 is expressed only

Table 1. Decreased In Vivo Proliferation of *IL-15R*$\alpha^{-/-}$ Lymphocytes

	$BrdU^+$ Cells (%)		
Days Treated	NL	*IL-15R*$\alpha^{-/-}$	% of Normal
2	6.0	4.8	79.3
2	4.1	3.3	80.3
2	6.8	5.7	83.8
3	10.8	7.4	69.1
5	15.5	12.4	79.7
5	15.9	14.2	89.1

Peripheral lymph nodes from BrdU-injected mice were harvested, and the percentage of BrdU-positive lymphocytes is displayed. The mean percentage of normal for all pairs of mice analyzed was 80% ± 6% ($n = 6$).

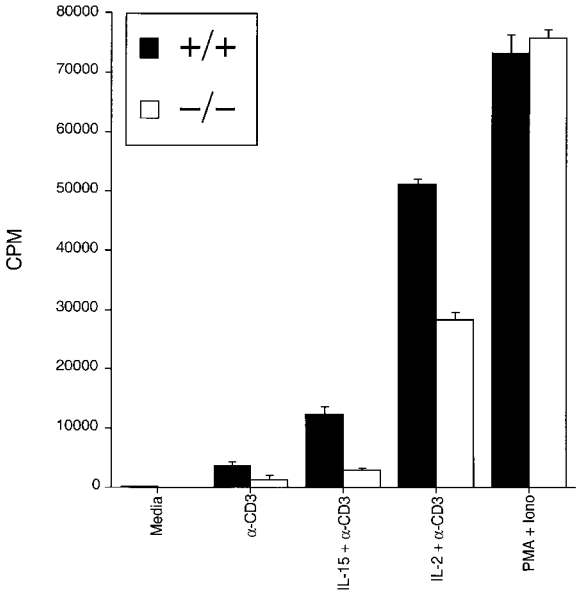

Figure 6. Activation Defect of *IL-15Rα*$^{-/-}$ T Cells

Resting T lymphocytes were purified as described in Experimental Procedures. Normal (shaded bars) or *IL-15Rα*$^{-/-}$ T cells (open bars) were cultured in media alone, anti-CD3 (1 μg/ml), anti-CD3 plus IL-15 (10 ng/ml), anti-CD3 plus IL-2 (5 ng/ml), or ionomycin (Iono; 500 ng/ml) plus PMA (10 ng/ml). Proliferation was assessed by measuring ^{3}H-thymidine incorporation as described in Experimental Procedures. Data is representative of four experiments.

by activated T lymphocytes in vivo, the divergent signals mediated by IL-15R and IL-2R could readily lead to distinct (and in many cases, opposing) functions in lymphocytes bearing both receptors.

IL-15Rα and Innate Immunity

Our results demonstrate that IL-15Rα is important for the differentiation of multiple innate immune cells. Deficiencies of NK, NKT, and TCRγ/δ IELs in *IL-15Rα*$^{-/-}$, *IL-2Rβ*$^{-/-}$, and *IL-2Rγ*$^{-/-}$ mice confirm that all three subunits of IL-15R are required for developmental IL-15 signals to these cells. Our data are also consistent with studies of interferon regulatory factor 1–deficient (*IRF-1*$^{-/-}$) mice, which demonstrate that bone marrow–derived IL-15 is essential for NK cell differentiation (Ogasawara et al., 1998). The dependence of NKT lymphocytes and TCRγ/δ IELs upon IL-15Rα appears intermediate between NK cells and CD4^{+} T lymphocytes, suggesting that other cytokines (e.g., IL-7) may partly compensate for IL-15 in the differentiation of NKT lymphocytes and TCRγ/δ IELs. In addition, it is possible that IL-15Rα performs essential functions in mature NKT and TCRγ/δ IELs so that the remaining *IL-15Rα*$^{-/-}$ NKT and TCRγ/δ IELs observed in our mice may be functionally compromised. Finally, these innate immune cells have been ascribed roles in antitumor immunity (Cui et al., 1997), as well as other immune functions. Thus, the fact that *IL-15Rα*$^{-/-}$ mice appear healthy for at least 12 weeks of age (as opposed to *IL-2Rβ*$^{-/-}$ mice, which develop autoimmunity within 6–8 weeks [Suzuki et al., 1995]) suggests that the loss of these lineages in *IL-15Rα*$^{-/-}$ mice may allow studies that reveal essential functions for these cells in vivo.

Dependence of CD8^{+} T Lymphocytes upon IL-15Rα

The reduction of thymocyte numbers in *IL-15Rα*$^{-/-}$ mice indicates that intrathymic T cell differentiation depends upon IL-15Rα. As SP CD8^{+} thymocytes were consistently reduced to a greater degree than SP CD4^{+} thymocytes, it appears that CD8^{+} lymphocytes may share a developmental requirement with innate immune lymphocytes. In this context, it is worth noting that CD8^{+} T lymphocytes resemble innate immune lymphocytes in possessing limited effector functions.

The loss of CD8^{+} CD44^{Hi} compared with CD4^{+} CD44^{Hi} T lymphocytes suggests that CD8^{+} T cells are particularly dependent upon IL-15Rα for activation, while other cytokine receptors support CD4^{+} T lymphocyte activation. This hypothesis is consistent with the recent finding that IL-15 selectively induces expansion of CD8^{+}CD44^{Hi} versus CD4^{+}CD44^{Hi} T lymphocytes (Zhang et al., 1998). However, while the prior report emphasized the importance of IL-2Rβ expression in supporting CD8^{+}CD44^{Hi} T lymphocytes, our data clearly demonstrate that IL-15Rα expression is critical for these cells. Furthermore, IL-2Rβ does not compensate for the loss of IL-15Rα in CD8^{+}CD44^{Hi} T lymphocytes. Finally, it is also possible that developmental problems with CD8^{+} T lymphocytes (see above) may compromise their responses as mature lymphocytes.

IL-15Rα and Lymph Node Homeostasis

In addition to the CD8^{+} T lymphocyte deficiency noted above, we have observed marked hypocellularity of *IL-15Rα*$^{-/-}$ lymph nodes. This defect in lymphoid homeostasis likely results from defective proliferation and homing of mature *IL-15Rα*$^{-/-}$ lymphocytes in vivo. After accounting for the reduced numbers of cells in *IL-15Rα*$^{-/-}$ lymph nodes, our in vivo BrdU studies demonstrate that there are far fewer proliferating cells in *IL-15Rα*$^{-/-}$ than in normal lymph nodes. Some of this reduction is likely due to the reduced numbers of activated CD8^{+}CD44^{Hi} lymphocytes, since a high percentage of CD44^{Hi} cells are known to be proliferating (Tough and Sprent, 1994). The proliferative function of IL-15Rα appears distinct from other costimulatory molecules, since induced mutations of IL-2, IL-4, and CD28 do not result in a loss of proliferating lymphocytes in vivo (Kuhn et al., 1991; Schorle et al., 1991; Shahinian et al., 1993). Thus, IL-15Rα is essential for maintaining the pool of "spontaneously" activated, proliferating lymphocytes normally present in nonimmunized animals.

Our in vivo homing experiments demonstrate that *IL-15Rα*$^{-/-}$ lymphocytes home poorly to lymph nodes, thus explaining the lymphopenia seen in *IL-15Rα*$^{-/-}$ lymph nodes compared to spleens. The compromised migration of *IL-15Rα*$^{-/-}$ lymphocytes resembles an ameliorated version of the homing defect seen in L-selectin-deficient splenocytes (Arbones et al., 1994). However, L-selectin expression is intact on *IL-15Rα*$^{-/-}$ lymphocytes (Figure 4). Nevertheless, IL-15Rα may support homing by activating adhesion molecules such as LFA-1 (Oppenheimer-Marks et al., 1998). Our adoptive transfer experiments further demonstrate that IL-15Rα expression on lymphocytes, rather than on endothelial cells, is likely to be essential for the heterotypic interactions which mediate lymphocyte homing.

The homeostatic defects we have observed in nonimmunized animals lead to important predictions for the roles of IL-15Rα during immune responses. Immune responses require the activation, proliferation, effector responses, and properly regulated cell death of lymphocytes. Hence, compromised lymphocyte activation and proliferation in *IL-15Rα*$^{-/-}$ mice suggest that IL-15Rα may be important for these responses. Moreover, the acquisition and maintenance of immunological memory is characterized by the presence of "memory lymphocytes," cells bearing certain activation makers (i.e., CD44, Ly-6C) but not others (i.e., CD69, CD25), and which persist in the organism while recirculating between tissues and lymph nodes. In this regard, IL-15Rα's role in supporting lymphocyte homing strongly suggests that it facilitates memory cell recirculation. In addition, the ubiquitous expression of IL-15 in multiple tissues suggests that tissue-bound memory lymphocytes, such as those which persist in the intestinal mucosa, would be supported by IL-15/IL-15Rα interactions. This support would be particularly crucial in noninflamed tissues, where few immune cells and lymphocyte-derived growth factors would be available to support memory cells. Therefore, IL-15Rα performs multiple critical homeostatic immune functions that are likely to be essential for effective immune responses.

Experimental Procedures

Generation of *IL-15Rα*$^{-/-}$ Mice

An IL-15Rα genomic clone was isolated from a murine 129 Sv genomic library (Stratagene, La Jolla, CA). A 3.0 kb Hind3–EcoRV fragment containing exons 2 and 3 of IL-15Rα was replaced with a phosphoglyceraldehyde-driven neomycin resistance gene casette flanked by lox sequences (*ploxNeolox*) and ligated to a phosphoglyceraldehyde-driven thymidine kinase gene casette (*pgkTK*). This *IL-15Rα* gene targeting construct was transfected into RW-4 ES cells (Genome Systems, St. Louis, MO) by electroporation. Genomic DNA from ES cell clones that survived selection in both G418 and 2′ fluoro-5-iodo-arabinofuranosyluracil (FIAU) was digested with EcoRV, separated on 1% agarose gels, transferred to a nylon membrane, and hybridized with a ^{32}P-labeled genomic fragment flanking the targeting construct. Targeted ES clones were microinjected into C57Bl/6 blastocysts, and male chimeric offspring were bred to obtain germline mutant mice. Mice were screened by Southern analysis (as described above). For Northern analyses, 15 μg of total RNA was separated on 1% agarose/formaldehyde gels, transferred to Zetaprobe nylon membrane (Biorad, Hercules, CA) by capillary diffusion, and hybridized with an IL-15Rα-specific cDNA probe representing the entire coding region of IL-15Rα or with a *glyceraldehyde phosphate dehydrogenase* (*GAPDH*)-specific probe.

Flow Cytometric Analysis

Single-cell suspensions were prepared from freshly isolated bone marrow, lymph nodes (axillary, posterior, and inguinal), spleen, and thymus. Samples from bone marrow and spleen were red blood cell (RBC)-depleted using hypotonic lysis. IELs were harvested by washing isolated intestinal fragments in 5 mM EDTA. Cells were incubated with monoclonal antibodies (MAbs) against cell surface markers for 30 min at 4°C. Fluorescein isothiocyanate (FITC), phycoerythrin (PE), cy-chrome (CYC), or biotin conjugated antibodies specific for murine B220, IgM, CD3, CD4, CD8α, CD8β, CD44, CD62L, TCRγδ, TCRαβ, IL-2Rβ, Ly-6C, DX5, or NK1.1 (PharMingen, San Diego, CA) were used for flow cytometric analysis. Biotinylated antibodies were developed with CYC or allophycocyanin (APC) conjugated streptavidin (SA). Apoptotic cells were detected using an apoptosis detection kit as per manufacturer's instructions (RandD Systems, Minneapolis, MN). Briefly, freshly isolated lymphocytes were incubated with FITC-annexin V for 20 min at room temperature. Cells were analyzed with a FACSCalibur cytometer using Cell Quest Software (Becton Dickinson, San Jose, CA).

^{51}Chromium-Release Assay for NK Cell Function

Mice were injected i.p. with 0.1 μg poly(I):(C) (Pharmacia, Piscataway, NJ) on day 0 and day 1 prior to harvesting spleens. Splenocytes were depleted of RBCs, and T and B cells were depleted using an anti-CD3 MAb (PharMingen) and sheep anti-mouse IgG-coated magnetic beads (Dynal, Oslo, Norway). YAC-1 target cells (1×10^6) were incubated with 100 μCi ^{51}Cr for 1 hr. Effector (E) and target (T) cell were mixed at the indicated E:T ratios and incubated for 4 hr in triplicate wells. ^{51}Cr release was measured by quantitating cpm using a scintillation counter (Packard, Meriden, CT), and the percentage of specific killing was calculated as follows: [(sample cpm − spontaneous release cpm)/(maximal release cpm − spontaneous release cpm)] × 100.

In Vivo Lymphocyte Homing

Splenocytes were prepared as described above. Splenocytes (20×10^6) from *IL-15Rα*$^{-/-}$ and control littermates were incubated for 30 min at 37°C with either 0.4 μM CellTracker Green CMFDA (5-chloromethylfluorescein diacetate) or 10 μM CellTracker Orange CMTMR (5-(and-6)-(((4-chloromethyl)benzoyl) amino)tetramethylrhodamine) (Molecular Probes, Eugene, OR). Cells were then washed and incubated at 37°C for an additional 30 min to allow complete modification of the probe. *IL-15Rα*$^{-/-}$ and control splenocytes were then mixed in equal numbers in 1 ml HEPES buffered saline. An aliquot of this preinjection lymphocyte cocktail was analyzed to confirm the preinjection ratio of *IL-15Rα*$^{-/-}$ to control cells and 1×10^7 cells from this cocktail were injected into the tail vein of normal mice. After 1 hr or 16 hr, spleens and lymph nodes were removed from the recipient mice and analyzed by flow cytometry for the presence of green- or orange-labeled cells. The ratio of *IL-15Rα*$^{-/-}$ to control lymphocytes recovered from each organ was normalized to the ratio of lymphocytes in the preinjection cocktail.

In Vivo Lymphocyte Proliferation

Mice were injected with BrdU (0.4 mg i.p. twice daily) (Sigma, St. Louis, MO) for the indicated number of days. Single-cell suspensions were prepared from lymph nodes. BrdU-positive cells were detected using an in situ cell proliferation kit (Boehringer Mannheim, Indianapolis, IN) according to the manufacturer's instructions. Briefly, 5×10^6 cells were resuspended in fixative (50 mM glycine in 70% ethanol; 30 min at 4°C), washed in PBS and resuspended in denaturation solution (4 M HCl; 15 min at room temperature), then neutralized in PBS. Cells were then incubated with an anti-BrdU antibody (45 min; 37°C) washed, and analyzed by flow cytometry. For analysis, 5×10^5 total events were collected.

In Vitro Lymphocyte Activation

Single-cell suspensions were prepared from freshly isolated lymph nodes. Lymphocytes were depleted of CD44$^+$ cells using an anti-CD44 MAb (PharMingen) and anti-IgG-coated magnetic beads (Dynal) in order to obtain resting T cells. Lymphocytes were resuspended at 1×10^6 cells/ml in culture media (RPMI 1640 supplemented with 2-mercaptoethanol, penicillin, streptomycin, L-glutamine [all from GIBCO BRL, Grand Island, NY], and 10% heat-inactivated fetal calf serum [Sigma]) containing either anti-CD3 (1 μg/ml) (PharMingen), anti-CD3 plus IL-15 (10 ng/ml) (RandD Systems), anti-CD3 plus IL-2 (5 ng/ml) (PharMingen), or ionomycin (500 ng/ml) plus PMA (10 ng/ml) (Sigma). After 2 days in culture, lymphocytes were treated with ^{3}H-thymidine (1 μCi/well), and on the following day, proliferation was measured by ^{3}H-thymidine incorporation using a liquid scintillation counter.

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