

Hepatic interleukin 15 (IL-15) expression: implications for local NK/NKT cell homeostasis and development

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SUMMARY

Interleukin 15 (IL-15) is critical for the development of human and murine natural killer (NK) cells and hepatic-derived NK T cells (NKT) in mice, and for the homeostatic maintenance of NK/NKT and CD8⁺ memory T cells. The lymphocyte repertoire of an adult human liver includes significant populations of NK and NKT-like cells, which may arise locally from hepatic haematopoietic stem cells (HSCs). We investigated hepatic IL-15 levels and the expression of IL-2/IL-15-receptor β -chain (IL-2/IL-15R β ; CD122) on mature hepatic lymphocytes and HSCs. Reverse transcription-polymerase chain reaction (RT-PCR) was used to detect secreted/intracellular IL-15 transcripts. IL-15 protein was localized using immunohistochemistry; levels were measured by enzyme-linked immunosorbent assay IL-2/IL-15R β expression by flow-cytometry. Normal hepatic IL-15 protein was detected at 0.43 ng/100 mg total protein ($n = 11$, range 0.10 ng–0.9 ng). There was a significant increase in HCV-infected tissue (1.78 ng, $P < 0.005$, $n = 11$, range 0.18–2.43 ng). The staining pattern suggests that infiltrating monocytes and tissue resident Kupffer cells are the main producers. IL-15 protein was detected in supernatants from cultured liver biopsy specimens in the absence of stimulation (mean 175.8 pg/100 mg wet tissue, $n = 3$), which increased significantly upon stimulation ($P < 0.05$, mean 231.21 pg). On average, 61% of hepatic HSCs expressed IL-2/IL-15R β suggesting a local lymphopoietic role. Eighty per cent of NK and 45.8% of CD56⁺ T cells expressed IL-2/IL-15R β , suggesting involvement in local CD56⁺ cell activation and expansion. Constitutive expression of IL-15 protein and IL-2/IL-15R β on hepatic lymphocytes suggests a key role in the generation and maintenance of the unique hepatic lymphoid repertoire. The significant increase observed in HCV-infected liver suggests a role for IL-15 in host antiviral responses in the liver.

Keywords human lymphopoiesis NK cells normal liver stem cells

INTRODUCTION

The lymphocyte composition of normal adult human liver (AHL) is dominated by rapid-acting innate cells. These cells are characterized by the expression of natural killer (NK) cell receptors. Up to 65% of all lymphocytes present in the normal liver consist of NK cells (CD3⁻ CD56⁺) $\gamma\delta$ T cells and T cells co-expressing NK-associated molecules (such as CD56). These populations are found in much lower proportions in peripheral blood (17%) [1], suggesting a specialized immune repertoire in the liver selected to

perform tissue-specific functions. The normal AHL also contains a large excess of CD8⁺ over CD4⁺ cells and many of these CD8⁺ T cells are activated and display a memory phenotype (CD45RO⁺) [2].

The factors responsible for the selective accumulation of CD56⁺ and CD8⁺ T and natural killer T cells (NKT) cells in the liver are unknown, but are likely to involve selective recruitment from the periphery [3–5]. The hepatic cytokine microenvironment might also influence the distribution of lymphocyte subpopulations by inducing the selective differentiation, expansion and/or survival of lymphocyte subsets derived from circulating cells. Normal AHL contains significant numbers of functional haematopoietic stem cells (HSCs) [6,7], many of which express lymphoid antigens [8], and we have postulated previously that these may develop extrathymically and contribute to the local hepatic lymphocyte pool [8], as has been shown in adult mice [9].

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Recent advances in the study of interleukin 15 (IL-15) biology have identified several key non-redundant functions for this cytokine [10,11]. IL-15 plays a wide role in the immune system, but is thought to be of particular importance in the activation of innate and tissue-associated immune responses [12]. It promotes the activation, proliferation and survival of natural killer (NK) [13–15], $\gamma\delta$ -T cells [16], CD8⁺ memory T cells and NKT cells [5,17–20]. In addition, IL-15 is an important lymphopoietic cytokine critical for the development of innate lymphocytes such as NK cells [21,22], extrathymic $\gamma\delta$ -T cells and NKT cells [17,20,23].

The role of IL-15 in the development, proliferation, survival and activation of NK cells, CD8⁺ T cells, $\gamma\delta$ T cells, NKT cells and CD56⁺ T cells prompted us to ask whether this cytokine plays a role in the liver. Despite reports of IL-15 mRNA production in a wide variety of tissues [24], only activated macrophages and epithelial cells have been shown to produce IL-15 protein [25]. IL-15 mRNA has been detected in human liver biopsy specimens [26], in hepatocyte cell lines [27] and in leucocytes isolated from University of Wisconsin solution (UW) used to perfuse human liver donor organs prior to transplantation [28]. However, direct evidence for IL-15 protein expression and secretion in the normal AHL is currently lacking.

To date, tissue expression of cytokines has been confined mainly to mRNA studies and immunohistochemical analysis. Although detection of mRNA can give us valuable information regarding the expression of genes in tissue, cytokine genes are often subjected to translational regulation [9], thus physiologically significant data regarding the expression of cytokines are more likely to come from measurement of cytokine protein. Immunohistochemistry can yield valuable information on the localization of cytokine production but gives no indication as to the actual levels expressed. A quantitative approach is required to establish organ-specific levels of cytokines. We have therefore investigated hepatic IL-15 protein levels and the expression of IL-15 receptor β chain on mature and immature hepatic lymphocytes. Our results suggest a role for IL-15 in the generation and maintenance of the distinct lymphocyte repertoire that is found in the liver.

MATERIALS AND METHODS

Tissue specimens

Normal liver wedge biopsies (50–100 mg, $n = 19$) were obtained from donor organs at the time of liver transplantation. All donors had normal serum alanine aminotransferase (ALT) levels and were considered suitable for transplantation. Donor organs were perfused extensively with UW solution prior to obtaining the biopsy. Diseased liver specimens were obtained at time of liver transplantation for end-stage hepatitis C viral (HCV, $n = 11$) infection or alcoholic liver disease (ALD, $n = 9$). As explant organs had not been perfused, diseased tissue was washed three times in Hanks's balanced salt solution (HBSS) prior to processing. For the analysis of IL-15 expression, liver samples were snap-frozen immediately in liquid nitrogen and powdered using the Braun Mikrodismembrator II (Braun Apparate, Melsungen, Germany). The powder was stored at -80°C and used subsequently for extraction of RNA (normal, $n = 9$, mean age 46 years, range 22–59, three male and six female) or protein (normal, $n = 11$, mean age 41.36 years, range 19–67, five male and nine female; HCV, $n = 11$, mean age 47.9 years, range 40–64, eight male and three female; ALD, $n = 9$, mean age 58 years, range 45–66, eight

male and one female). For the analysis of IL-15 receptor β -chain expression, hepatic mononuclear cells (HMNCs) were prepared from fresh tissue (normal, $n = 7$, mean age 30 years, range 18–56, four male and three female), as described previously [29]. Matched peripheral blood mononuclear cells (PBMCs) were prepared by standard density gradient centrifugation over Lymphoprep (Nycomed, Oslo, Norway). The Research Ethics Committee, St Vincent's University Hospital, Dublin, granted approval for this study.

Quantification of IL-15 protein

Protein was extracted from 100 mg powdered tissue using 300 μl of lysis buffer [1% detergent (Igepal), 0.5% deoxycholic acid, 0.1% sodium dodecyl sulphate (SDS) in phosphate buffered saline (PBS)] to which protease inhibitors [10 $\mu\text{g}/\text{ml}$ of phenylmethylsulphonyl fluoride, dissolved in isopropanol (PMSF) and a 1/33 dilution of aprotinin] had been added. All reagents for the extraction of protein were supplied by Sigma-Aldrich, Dublin, Ireland. The tissue powder was homogenized by passing several times through a 21-gauge needle (Beckton Dickinson, Oxford, UK) in the lysis buffer. The homogenate was then incubated on ice for 30 min, followed by centrifugation at 10 000 g for 10 min at 4°C . The supernatant was harvested and total protein quantified using the BCA Protein Assay Kit (Pierce, Rockford, IL, USA). Mean yield of soluble protein was 9.36 mg/100 mg of frozen tissue powder (range 5.87–14.6). The concentrations of IL-15 in protein preparations were determined by enzyme-linked immunosorbent assay (ELISA) using antibody pairs and recombinant human IL-15 standards purchased from R&D Systems (Abingdon, Oxon, UK). The capture (monoclonal antihuman IL-15, MAB647, clone 34505-11) and detection (biotinylated antihuman IL-15) antibodies were used at concentrations of 2 $\mu\text{g}/\text{ml}$ and 50 ng/ml, respectively. Isotype-matched IgG control antibody (Dako, Cambridge, UK) was used instead of capture antibody (2 $\mu\text{g}/\text{ml}$) to control for non-specific binding of sample to plates in the presence of antibody and for the contribution of endogenous biotin or alkaline phosphatase in the samples to the signal generated [30]. The concentrations of IL-15 protein were expressed as ng IL-15/100 mg of total protein. The detection limit of the assay under these conditions was determined as 7.8 pg/ml.

Localization of IL-15 protein expression in hepatic tissue

Sections (7 μm) were cut from frozen blocks of hepatic tissue embedded in OCT compound (Sakura, Zaeterwoude, The Netherlands), placed on glass slides coated with 2% 3-aminopropyl-triethoxy-saline in acetone (APES, Sigma Aldrich) and dried overnight at room temperature. Immunoperoxidase staining of sections was carried out using the Vectastain® Elite ABC Kit (Vector Laboratories, Peterborough, UK) according to the manufacturer's guidelines. The primary antibody used was a monoclonal anti IL-15 (R&D Systems, MAB 647, 20 $\mu\text{g}/\text{ml}$). IgG control antibody (Dako, Cambridge, UK, 20 $\mu\text{g}/\text{ml}$) was used in place of the primary antibody as a negative control. The HRP-substrate (diaminobenzidine tetrahydrochloride, DAB, Sigma Aldrich) reaction was allowed to proceed for 7 min and sections were counterstained for 30 s in Mayer's haematoxylin (BDH).

Detection of IL-15 mRNA

Total RNA was extracted from tissue powders using the RNeasy Total Pure RNA purification system (Qiagen, Crawley, UK) according to the manufacturer's instructions. The integrity of the

RNA preparations was assessed by electrophoresis in 2% agarose gels (Sigma-Aldrich). Mean yield of RNA from approximately 50 mg of hepatic tissue powder was 35.42 μ g of total RNA (range 26.3–41.7). One μ g of total RNA was reverse transcribed to cDNA using 200 units Superscript II (GIBCO-BRL, Gaithersburg, MD, USA).

The cDNA product was subjected to polymerase chain reaction (PCR) amplification of IL-15 as described previously [31]. The IL-15 specific primers (5'-GGATTTACCGTGGCTTTGAT GTAATGAG-3' and 5'-GCCTTCATGGTATTGGGAAC-3') direct the amplification of a 524 base pairs (bp) cDNA fragment that corresponds to the secreted form of the IL-15 protein. The same primers amplify a larger (650 bp) transcript, which corresponds to an intracellular form of IL-15. The size of the transcripts amplified in each reaction tube was estimated against a 100 bp DNA ladder (Promega, Southampton, UK) using RFLPSCAN™ software (Stratagene Cloning Systems, La Jolla, CA, USA). The optical density of the bands detected in the gel was determined using the EagleEye II™ still video system (Stratagene Cloning Systems) and expressed as arbitrary units which were normalized against the GAPDH-housekeeping gene product band (524/GAPDH OR 650/GAPDH OD ratio). The relative level of the two IL-15 specific transcripts was expressed as a ratio of 524/650.

IL-15 secretion assay

Liver biopsies (100 mg each) from three donors were chopped finely and cultured in triplicate in 500 μ l RPMI-1640 medium [GIBCO BRL, containing 10% fetal calf serum (FCS)] \pm exogenous activation [phorbol myristate acetate (PMA) 10 ng/ml and ionomycin 1 μ g/ml] for 72 h. Supernatants from each of the donors were pooled and IL-15 secreted into the supernatants was measured by ELISA (as described above). Culture medium alone was used to control for the presence of exogenous IL-15. Results were expressed as pg IL-15 secreted/100 mg wet tissue. Tissue measured wet is 10% heavier than the same biopsy snap-frozen.

Flow cytometric analysis

Fluorochrome-conjugated monoclonal antibodies against human CD3, CD34, CD45, CD56 and the IL-15 receptor β -chain (IL-2/IL15R β), CD122, were purchased from Becton Dickinson. Three-colour flow cytometric analysis was performed using a FACScan flow cytometer and Cell Quest software (Becton Dickinson) on single cell suspensions isolated from liver (HMNCs) and PBMCs, as described previously [29]. Levels of staining above those of the appropriate isotype-matched IgG fluorescent-labelled control MoAbs were taken as positive.

Statistical analysis

A two-tailed paired or unpaired Student's *t*-test was used where appropriate to determine the significance of differences between groups; a *P*-value of < 0.05 was taken as significant. Spearman's rank test was used for correlation analysis due to the small sample size.

RESULTS

IL-15 protein expression in liver tissue

A sandwich ELISA was used to quantify IL-15 protein levels in homogenized normal and diseased hepatic tissue. All the samples tested were positive for IL-15 protein. The mean level of IL-15 cytokine protein detected in normal liver was 0.43 ng/100 mg total

protein ($n = 11$, range 0.10–0.9 ng). There was a significant increase in IL-15 levels detected in HCV-infected tissue mean 1.78 ng ($P < 0.005$, $n = 11$ range 0.18–2.43 ng). Levels of IL-15 in ALD liver were similar to normal tissue ($n = 9$, mean 0.50 ng, range 0.32–0.84 ng, Fig. 1a). Immunohistochemical staining of hepatic tissue showed that IL-15 protein was distributed mainly within portal tracts. The cells staining positively for IL-15 have a mononuclear morphology and are therefore likely to be infiltrating monocytes (Fig. 1b). Positive staining was also observed in the parenchyma. Parenchymal cells staining positively for IL-15 are likely to be Kupffer cells, as they have a characteristic cigar-shaped morphology.

Detection of IL-15 mRNA transcripts

Nine normal donor liver samples were tested for the presence of IL-15 specific transcripts. The primers used amplify two distinct products corresponding to intracellular and secreted forms of the protein [31]. All the samples tested (100%) were positive for the smaller 524 bp product, which codes for the secreted isoform of IL-15. Five of the samples (55.6%) were also weakly positive for the larger 650 bp product, which corresponds to an intracellular form of IL-15 (Fig. 2a). On average the transcript associated with intracellular IL-15 was expressed at only 18% of the level of the transcript associated with secretory IL-15.

Secretion of IL-15

Cultured normal liver biopsies in the presence and absence of stimulation were used to determine whether hepatic IL-15 is secreted. IL-15 (above the level of culture medium alone) was detected in supernatants of biopsy material in the absence of exogenous stimulation (mean 175.8 pg/100 mg wet tissue, $n = 3$). The amount of IL-15 secreted into the medium increased in the presence of exogenous stimulation ($P < 0.05$, mean 231.21 pg, Fig. 2b).

IL-2/IL-15 receptor β -chain expression

To determine whether hepatic stem/progenitor cells expressed interleukin 2/15 receptor β -chain (CD122) (IL-2/IL-15R β), seven normal liver samples were stained with anti-CD45 (haematopoietic marker) and anti-CD34 (stem cell marker) to identify haematopoietic stem cells (HSCs, CD34⁺ CD45⁺) in combination with anti-IL-2/IL-15R β . The majority, 66%, of CD34⁺ CD45⁺ hepatic HSCs co-expressed IL-2/IL-15R β (median, range 40.8–78.82%, Fig. 3). No expression of IL-2/IL-15R β was detected on CD34⁺ CD45⁻ hepatic cells. As peripheral blood HSCs occur at very low frequency it was not possible to measure the IL-2/IL-15R β expression level on peripheral CD34⁺ CD45⁺ cells.

The expression of IL-2/IL-15R β was analysed on mature hepatic and matched peripheral blood NK and T cell populations ($n = 6$). No difference was observed for the mean expression of IL-2/IL-15R β on total CD56⁺ lymphocytes from liver or blood (68.04% versus 61.22%, respectively). However, a significantly higher proportion of hepatic CD3⁺ cells expressed this receptor chain (24.02%) when compared to matched peripheral blood (4.74%, $P = 0.03$, Fig. 4). To delineate further the subpopulations of NK/T cells that expressed IL-2/IL-15R β , the level of expression of this molecule on CD56⁺ CD3⁻ NK cells, CD56⁻ CD3⁺ conventional T cells and CD56⁺ CD3⁺ NKT-like cells was determined. Similar proportions of hepatic and peripheral NK (80.27% versus 80.87%, respectively), NKT-like (45.80% versus 40.30%, respectively) and conventional T cells (12.04% versus 3.44%, res-

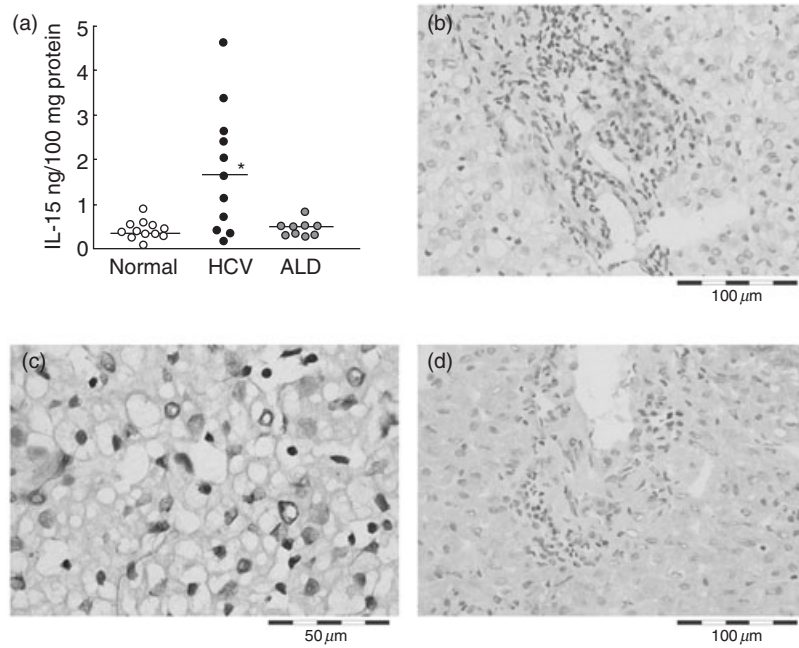


Fig. 1. Levels of interleukin-15 protein in normal liver tissue. Levels of IL-15 protein in hepatic tissue from normal ($n = 11$), hepatitis C virus (HCV) infected ($n = 11$) and alcoholic liver disease (ALD, $n = 9$) were quantified using an ELISA technique. A significant increase in IL-15 ($*p < 0.05$) was observed for HCV-infected liver (a). Immunohistochemical staining of hepatic tissue showed that IL-15 positive cells in portal tracts have a mononuclear morphology (b). Parenchymal cells staining positively for IL-15 are likely to be Kupffer cells, as they have characteristic cigar-shaped morphology (c). Staining of parenchymal cells was more intense than positively staining portal tract cells, suggesting that Kupffer cells may express more IL-15 than infiltrating monocytes. The isotype-matched negative control is shown in (d).

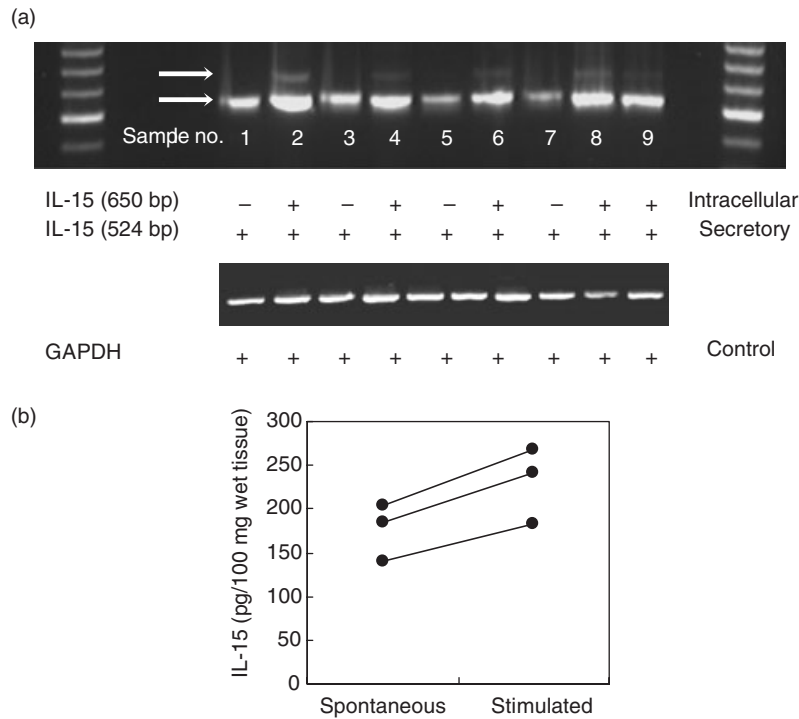


Fig. 2. Normal liver expresses the secretory isoform of the interleukin-15 gene and secretes IL-15. Electrophoresis on a 2% agarose gel of IL-15-specific PCR products for the nine normal liver samples tested are shown. The upper arrow indicates the 650 bp product and the lower arrow indicates the 524 bp product expressed constitutively in normal liver. The bright band on the 100 bp ladder represents a size of 500 bp. GAPDH controls are also shown (a). Detection of IL-15 in supernatants from three liver biopsies cultured in the absence of exogenous stimulation for 72 h suggested that IL-15 normally produced in liver tissue is secreted. A significant increase in IL-15 production ($P < 0.05$) is observed in the presence of PMA and ionomycin (b).

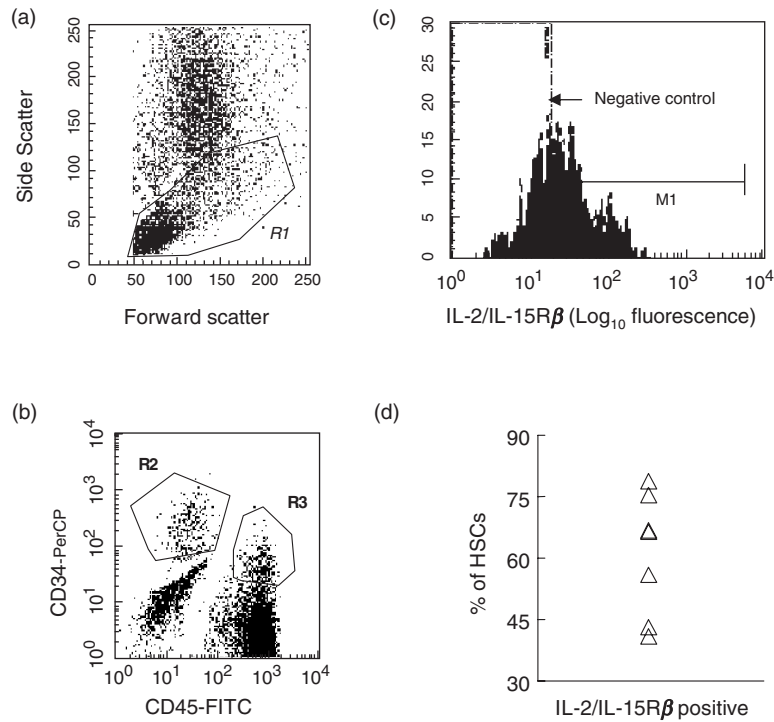


Fig. 3. IL-2/IL-15 receptor β -chain expression on hepatic haematopoietic stem cell (HSC) populations. Forward-scatter (size) and side-scatter (granularity) parameters were used to select cells of low to medium granularity (R1) (a). For assessment of the expression of IL-2/IL-15R β on hepatic haematopoietic stem cell (HSC) populations CD34⁺ CD45⁻ (R2) and CD34⁺ CD45⁺ (R3) cell populations were gated (b). The level of expression of the receptor relative to isotype-matched control antibody was assayed by histogram analysis (c). Values for each of the individual samples tested are shown (d).

pectively) were positive for IL-2/IL-15R β . Of note, the CD56⁺ NKT-like cell populations from either source contained significantly higher proportions of cells expressing this receptor chain when compared to conventional CD56⁻ T cell populations (Fig. 5a). Levels of T cells co-expressing CD56 correlated directly with IL-2/IL-15R β expressing T cells (Fig. 5b), thus the high levels of T cell positivity for this cytokine receptor chain detected in liver is due to the high proportion of NKT-like cells in the liver (Fig. 5c).

DISCUSSION

IL-15 plays a wide role in the immune system but is thought to be of particular importance in the activation of innate and tissue-associated immune responses [12]. However, data on organ-specific levels of cytokines is lacking, an important consideration when extrapolating the results of *in vitro* studies to what may be happening *in vivo* [15]. In the present study IL-15 protein was detected in all liver samples tested by ELISA. We used whole hepatic tissue, which was snap-frozen immediately and manipulated only in the presence of RNAse or protease inhibitors to avoid stimulation or breakdown during cell isolation. The expression of IL-15 protein in normal liver suggests a role for this cytokine in the generation and maintenance of the distinct lymphocyte repertoire found in the liver [1,2,5]. Indeed, IL-2/IL-15R β and IL-15 knock-out mice have a serious deficiency of NK and NKT cells [17,23], populations which predominate in normal liver [1,2]. HCV and ALD are both characterized by inflammatory infiltrates in the liver. We observed a significant increase in IL-15 protein in

HCV-infected liver but not in ALD, which suggests that this cytokine is involved in host antiviral responses but is not the only factor promoting inflammation in the liver. Using immunohistochemistry, we have demonstrated that IL-15 producing cells in portal tracts are likely to be infiltrating monocytes. There was also evidence of a sinusoidal distribution, suggesting that hepatic Kupfer cells may be the source of constitutively expressed IL-15 in the liver.

The control of IL-15 expression is complex, with regulation at the levels of transcription, translation and intracellular trafficking [10]. Constitutive expression of IL-15 mRNA, as determined by Northern blot analysis, has been demonstrated in a variety of tissues such as placenta, skeletal muscle, kidney, lung, heart, fibroblasts, epithelial cells and monocytes. However, it has been difficult to demonstrate IL-15 in supernatants of many cells that express such mRNA [24,25,32]. This observation may be explained, in part, by the more recent discovery of two IL-15 mRNA splice variants [31], which give rise to identical mature cytokine proteins but differ in their signal sequences. The signal peptides of the IL-15 isoforms direct intracellular trafficking of the protein. The isoform containing the long signal peptide (48aa) is directed to the secretory pathway while the short signal peptide (21aa) isoform is retained in the cytoplasm or the nucleus [31]. In this study we used primers which distinguish both species of the IL-15 mRNA. We found that the normal liver expresses predominantly the mRNA species associated with the long signal peptide, suggesting that IL-15 is produced and secreted in the normal AHL. The alternative transcript of IL-15 associated with intracellular forms of the cytokine was also detected at a much lower

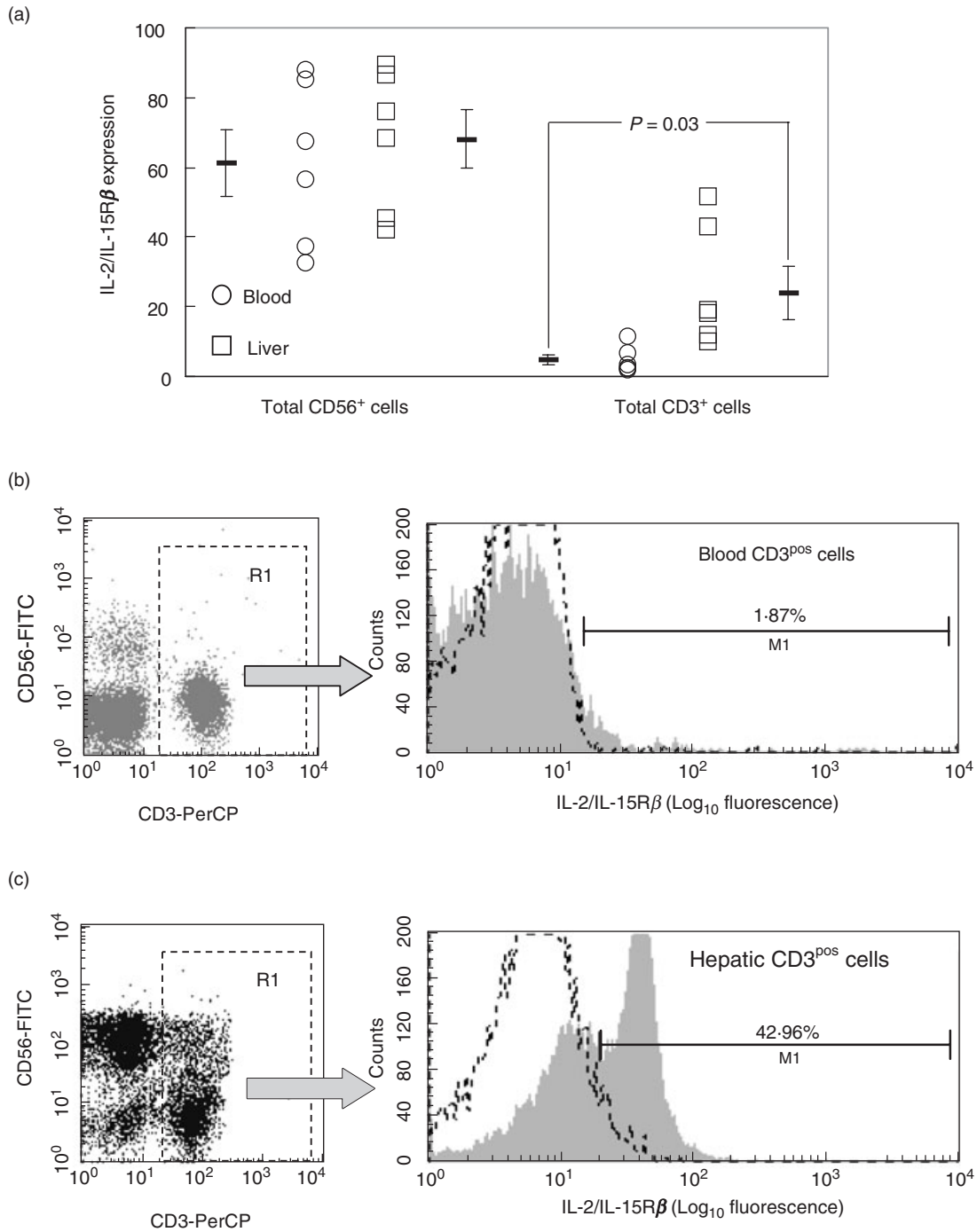


Fig. 4. IL-2/IL-15R β positive cells occur more frequently in hepatic CD3 positive T lymphocyte populations than peripheral blood. Total CD3⁺ and CD56⁺ hepatic and matched peripheral blood lymphocyte populations were gated. The scatter plot shows the percentage of CD3⁺/CD56⁺ cells co-expressing IL-2/IL-15R β for blood (○) and liver (□)-derived cells (a). Representative histogram plots of IL-2/IL-15R β expression for CD3⁺ populations are shown for blood (b) and liver (c).

intensity and in only 55.6% of samples. The physiological significance of the expression of an intracellular form of IL-15 in hepatic tissue is unknown, as a role for this form of the protein has not yet been elucidated. The scarcity of bands for the intracellular isoform of IL-15 may be due to decreased amplification efficiency of this band in the PCR assay; however, bands of equal intensity for both PCR products are detectable in NCI-H69 and NCI-H82 cell lines,

suggesting that amplification efficiency is not reduced. More importantly, detection of IL-15 in supernatants of cultured biopsies, in the presence and absence of exogenous stimulation, suggests that the cytokine protein is secreted, and therefore available, in adequate amounts to exert its effect on responsive cells.

Studies of cytokine receptor expression have proved valuable in pinpointing where specific ligand-receptor pairs have biological

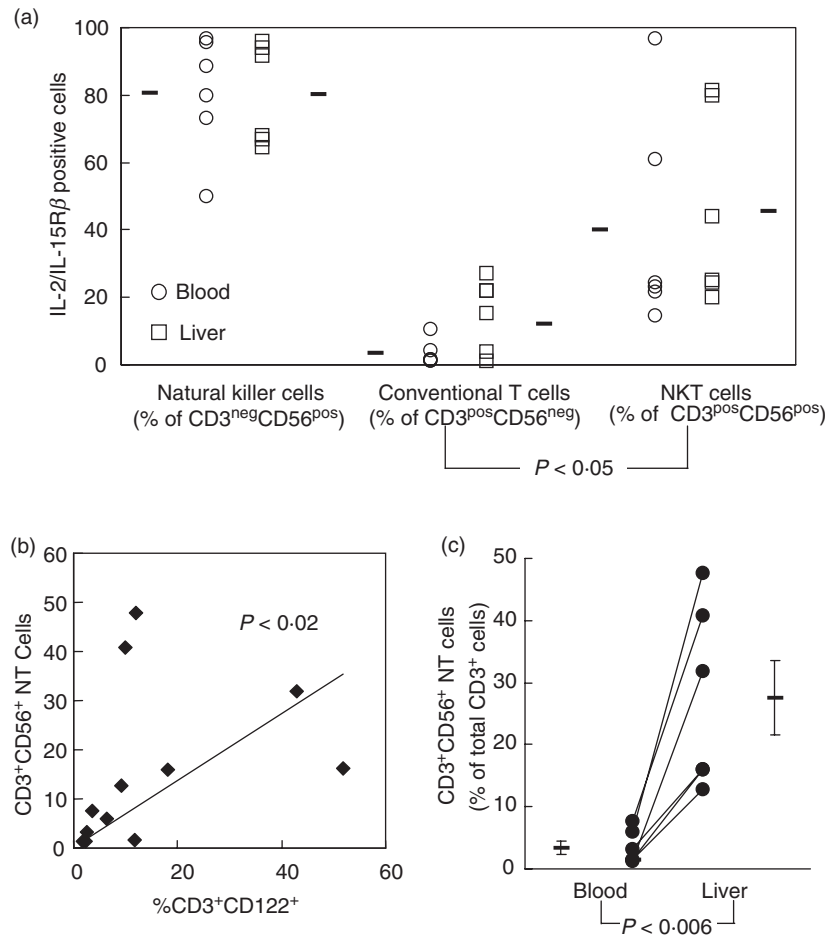


Fig. 5. Increased hepatic T cell IL2/IL-15R β expression is due to the presence of high levels of CD3⁺ CD56⁺ NT cells. A higher proportion of NKT-like cells co-express IL-2/IL-15R β than conventional T cells (a). IL2/IL-15R β expression correlated directly with levels of CD3⁺ CD56⁺ NKT-like cells (b), thus the higher expression of IL-2/IL-15R β on hepatic CD3⁺ cells may be attributed to the significantly higher proportion of CD56⁺ T cells in the liver (c).

activities. Cytokines such as KL (c-Kit ligand) and FL (flt-3 ligand) are expressed widely in different tissues, in contrast to their receptors which have a more restricted expression pattern [33]. A limitation of this study was the unavailability of a suitable antibody for the detection of the IL-15-specific IL-15R α chain; however, IL-15R β expression correlated well with IL-15 responsiveness and culture with IL-15 results in the loss of this receptor. We found that the vast majority of hepatic HSCs (CD34⁺CD45⁺) express the IL-2/IL-15R β . Furthermore; IL-2/IL-15R β was found to be expressed by significant numbers of mature lymphocytes, particularly NK cells and T cells expressing the NK-associated adhesion molecule, CD56. Therefore, HSCs and lymphocytes of the NK and NKT-like lineages are likely to be targets of endogenously produced IL-15 in the liver.

The environmental conditions within which common lymphoid progenitors develop determines their fate [34,35]. Addition of IL-15 to fetal thymic organ cultures (FTOCs) shifts differentiation away from conventional T cell receptor (TCR)- $\alpha\beta$ cells towards NK cell differentiation, while differentiation towards TCR- $\gamma\delta$ cells is unaffected [36]. The abundance of NK and TCR- $\gamma\delta$ lymphocytes in liver compared to peripheral blood [1] may be due in part to IL-15 acting on lymphoid precursors, providing them with the appropriate signals to mature locally

[17,22,23,34–36]. However, as indicated by the expression of IL-15 receptors on mature HMNC populations, IL-15 clearly has additional functions in the normal liver. Resting NK cells express IL-15 receptors constitutively [13] and IL-15 acts as an important chemoattractant and activator of NK cells [12] and NKT cells [5]. It is generally accepted that IL-15 plays an important role in the migration and maintenance of memory-type CD8⁺ T cells [25]. IL-15 also activates proliferation and MHC-unrestricted cytotoxicity in NK cells and CD56⁺ cells [15], populations that are highly enriched in the liver [1]. IL-15 is thus likely to be involved in the recruitment and regulation of hepatic NK and CD8⁺/CD56⁺ T cell populations. The IL-15 levels in the liver found in the present study are sufficient to drive CD56⁺ cell proliferation and cytotoxicity [15].

IL-15 has also been shown to effect cells of non-haematological origin, including actions on intestinal epithelial cells [37] and muscle [38]. It is possible, therefore, that hepatocytes and biliary epithelial cells may also be targets of IL-15.

In summary, the constitutive expression of IL-15 in normal AHL, and the high level of IL-2/IL-15R β on immature and mature hepatic lymphocytes, suggests that IL-15 may play a role in the generation and maintenance of the unique hepatic lymphoid repertoire. The significant increase observed in HCV-

infected liver suggests a role for IL-15 in host antiviral responses in the liver.

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