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CD40 Ligation Enhances IL-15 Production by Tubular Epithelial Cells

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Abstract. Interleukin-15 (IL-15) is a potent T-cell growth factor and activator. Acute rejection of kidney allografts strongly correlated with elevated IL-15 mRNA in the graft. A role in the rejection process is also suggested for the interaction between CD40 ligand (CD154) expressed on membranes of activated T cells and its receptor (CD40). The effect of CD40 ligation on IL-15 production in human tubular epithelial cells (TEC) was investigated. TEC were co-cultured with a cell line genetically engineered to express CD154. CD154-expressing cells (CD40L cells) bind to TEC. Addition of the CD40L cells to the TEC culture resulted in elevated IL-15 levels. This enhanced production was not observed with control cells, when anti-CD154 antibody was added, and when direct contact between CD40L-cells and TEC was prevented with the use of a Transwell system. CD40 activation resulted in a twofold increase of IL-15 mRNA transcripts as measured by reverse transcription-PCR and a concordant elevation in IL-15 protein production as measured by specific enzyme-linked immunosorbent assay. The intensity of activation by CD154 was linearly dependent on cell number, reaching plateau when the effector/target-ratio was 1:1. The increase of IL-15 levels was similar to that produced by stimulation with interferon-γ (IFN-γ). Combination of IFN-γ and activation with CD154 resulted in an additive effect. To conclude, activated T cells may enhance IL-15 expression in two ways: by secreting IFN-γ and by cell to cell contact using CD154. Each signal alone induces IL-15 in similar magnitudes, and both signals are additive. Because IL-15 is a major T-cell activator, this interaction may contribute to graft rejection.

Rejection of allografts is a major problem in organ transplantation. To achieve prolonged allograft survival, researchers have considered a recent promising therapeutic approach that involves blocking co-stimulatory signals. Co-stimulatory signals are nonantigen interactions between lymphocytes and adjacent cells. These signals are essential for optimal activation, proliferation, and cytokine release by lymphocytes (1). Many T-cell molecules may serve as receptors for co-stimulatory signals; the CD28 molecule, which binds B7 on the target cell, is the best characterized. Another important co-stimulatory pathway involves CD154, which is expressed on T cells and binds to CD40 on the target cell. The mechanism of this effect is mainly attributed to CD40 activation, which results in target cell support and induction of lymphocytes and less to the signal transduced to the T cell via CD154 (2,3).

Recent data have implicated an important role for the interaction between CD40 and CD154 in acute allograft rejection. It has been demonstrated that monoclonal anti-CD154 antibody is effective in preventing acute cardiac allograft rejection in mice (4). Also, blocking CD154-CD40 and CD28-B7 interaction, the latter using a CTLA-4-Ig fusion protein (CTLA-4 binds to B7 like CD28 but transmits an inhibitory signal), promotes long-term survival of skin and cardiac allografts in mice (5). Another study showed that combined administration of CTLA-4-Ig and anti-CD154 monoclonal antibody induced long-term renal allograft survival in nonhuman primates (6). Two recent publications reported that anti-CD154 monotherapy is effective in preventing acute rejection in allogenic islet engraftment and renal autotransplantation in nonhuman primates (7,8).

CD154 is expressed on activated CD4+ T cells. It is the ligand of CD40, a member of the tumor necrosis factor (TNF) receptor superfamily that is expressed on B cells, macrophages, dendritic cells, endothelial cells, tubular epithelial cells, and others (9). CD40 is constitutively expressed by tubular epithelial cells (TEC). Enhanced production of chemokines by TEC after CD40 activation, as previously reported, suggests a role for CD40-CD154 interaction in renal interstitial infiltration by lymphocytes (10,11).

A recent publication described production of interleukin-15 (IL-15; a T-cell activator and chemoattractant) by TEC (12). IL-15, like CD40, has been associated with allograft rejection. Elevated levels of IL-15 transcripts have been observed in posttransplant liver biopsies (13) and in mice receiving pancreatic islet cell allografts (14). More important, intragraft IL-15 transcripts were increased in patients who were rejecting

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renal allografts when compared with nonrejecting allografts (15). Tinubu *et al.* (16) demonstrated that an anti–IL-2Rβ prolongs allograft survival in monkeys.

In previous studies, we found that the Th1 cytokine interferon-γ (IFN-γ) was the only stimulant able to elevate IL-15 production by TEC (12). All other cell types reported to produce IL-15 protein respond to IFN-γ in upregulation, whereas other factors, such as lipopolysaccharides, intracellular pathogens, and ultraviolet B radiation, are only sometimes effective (17–19). Therefore, we speculated that CD40L expressed on activated Th1 cells is involved in IL-15 regulation. TEC are capable of secreting a variety of cytokines and chemokines and participate in the immune response in the kidney. Their ability to produce IL-15 and their expression of CD40 situates them as potential regional regulators of renal allograft rejection. Both CD40/CD154 and IL-15 seem to be important in the rejection process. The aim of this study was to investigate whether activation of CD40 by CD154 plays a regulatory role in the production of IL-15 by TEC.

Materials and Methods

Cell Cultures

Primary proximal TEC were prepared as described previously (20). Briefly, normal cortex tissue of human kidneys from patients with hypernephroma were immersed in ice-cold saline, cut into pieces of 1 to 2 mm^3, and washed with phosphate-buffered saline (PBS). Tissues were then continuously stirred for 1 h at 37°C in M199 medium (Biological Industries, Bet Haemek, Israel) containing 0.2% collagenase A (Boehringer Mannheim, Mannheim, Germany). After digestion, the cell suspension was filtered through a sterile gauze pad. Cells were isolated from the filtrate by centrifugation, washed twice with PBS, and incubated for 5 min at 37°C in 10 ml of trypsin-ethylenediaminetetraacetate solution B (Biological Industries). Cells were then washed twice with PBS and transferred to a 75-cm^3 tissue culture flask (Bibby, Corning, NY) in M199 medium containing 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and Biogro 2 (2%, Biological Industries). Cells were grown at 37°C in an atmosphere of 5% CO and 95% air. At confluence, cells were trypsinized and diluted (1:5) in fresh medium. Cells from passages 3 to 5 were used for experiments. Cells had a uniform appearance. Electron microscopy demonstrated the presence of intercellular junctions characteristic of epithelial cells and of microvilli characteristic of proximal TEC. Cells stained positive for cytokeratin and vimentin by indirect fluorescence (BM#6557 and BM#6079 antibodies, Sigma, Rehovot, Israel). Cells were further characterized as proximal TEC by positive staining for alkaline phosphatase (Sigma kit 86-R), acid phosphatase (Sigma kit 181-A), and nonspecific esterase (Sigma kit 91-A).

Mouse fibroblast L cells transfected with the complete human CD154 coding sequence and a neomycin resistance gene for selection of stable transfectants have been previously described (21). The parental nontransfected cells (L cells) were used as the negative control. Both parental and transfected cells were kindly supplied by Schering-Plough (Dardilly, France). Both types of cells were cultured in RPMI medium and supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Biological Industries).

Flow Cytometry

For flow cytometry analysis, TEC were harvested by ethylenediaminetetraacetate solution (1:2000, Biological Industries) then washed and incubated in 100 μl of PBS containing 0.5% bovine serum albumin (BSA) and 0.05% sodium azide for 1 h on ice with anti-CD40 monoclonal antibodies (mAb; clone EA-5, Ancell, Bayport, MN) or anti-CD154 mAb (clone 24 to 31, Ancell). Then the cells were washed and incubated with FITC-conjugated F(ab′) anti-mouse IgG (Jackson, West Grove, PA). The antibodies were diluted to recommended concentrations according to the manufacturers' instructions. Background fluorescence was adjusted with cells labeled with matching isotype control antibodies as the first antibody. TEC had no affinity to control antibodies, and fluorescence levels were always similar to that seen in nonstained cells. Analyses were done on a Coulter flow cytometer (Epics XL-MCL, Miami, FL).

Activation Protocols

TEC (10^5 cells/well) were seeded in 24-well plates (Bibby) and cultured to confluence. Cells were washed twice with medium and incubated for different lengths of time with 0.35 ml of M199 medium containing various concentrations of IFN-γ, or with CD40L cells or control L cells. In some experiments, we blocked CD154 on CD40L cells by pretreating these cells for 30 min with anti-CD154 mAb (1 μg/ml, clone 24 to 31, Ancell). Incubation of cells with the antibody was in cell medium; an isotype-matched antibody at the same concentration was used as control. At different time points, supernatant was collected and stored at −20°C for later assay of IL-15. At the end of incubation, plates were placed on ice, supernatants were collected, and cells were lysed with 0.35 ml of medium containing 0.1% NP40. Cells from two wells were counted in each plate for definition of cell number per well. All tissue culture reagents contained less than 0.025 ng/ml endotoxin.

Transwell System

A six-well Transwell system (Corning Costar Corporation, Cambridge, MA) was used to conduct co-culture experiments. The system consists of two compartments: a top well, with a porous matrix (0.4 μm), and a bottom well. This setup allows co-culture of two types of cells to grow in the same medium with soluble factors exchanged through the pores, while preventing direct contact between them. TEC were grown to confluence in the bottom well, and L cells were added either to the same well—allowing contact—or in the top well—avoiding contact.

mRNA Analysis

IL-15 mRNA was determined by reverse transcription-PCR (RT-PCR) of total RNA extracted from TEC. TEC were incubated as described above. At the end of each experiment, medium was aspirated from the wells and a guanidine isothiocyanate-containing buffer was applied. The resulting suspensions were transferred to Eppendorf tubes and stored at −70°C for later RNA extraction. Total RNA was extracted from cells using the RNeasy Mini Kit (Qiagen, Hilden, Germany). In brief, cells were lysed and homogenized, ethanol was added and samples were applied to RNeasy spin columns for absorption of RNA to membrane, contaminants were removed with wash spins, and RNA was eluted in water. RNA suspension was immediately converted to cDNA.

For cDNA generation, 13 μl of RNA sample was added to each 7 μl of reverse transcriptase reaction mixture. The reaction mixture contained 1 μl of Moloney murine leukemia virus-reverse transcriptase (200 U/μl, Life Technologies BRL, Gaithersburg, MD), 4 μl of

5x reverse transcriptase buffer (Life Technologies BRL), 0.5 μl of dithiothreitol (0.1 M, Life Technologies BRL), 0.5 μl of RNase inhibitor (40 U/μl, Sigma), 1 μl of oligo-d(T) 12 to 18 mer (40 pmol/μl, Boehringer Mannheim), and 1 μl of deoxynucleotide mix (2.5 nmol/μl each nucleotide, Sigma). The tube was incubated for 1 h at 37°C, then the volume of each sample was adjusted to 60 μl and the enzyme was inactivated by incubation for 10 min at 65°C.

IL-15 and β-actin cDNA were then amplified by PCR using specific primers (Table 1). Five μl of reverse transcription product was added to 45 μl of PCR reaction mixture containing 32.75 μl of H_2O, 2.5 μl of 5′ primer (20 μM), 2.5 μl of 3′ primer (20 μM), 2 μl of deoxynucleotide mix (2.5 nmol/μl each nucleotide, Sigma), 5 μl of 10x reaction buffer, and 0.25 μl of Dynazyme II DNA polymerase (2 U/μl, Fynnzymes Oy, Espoo, Finland). A negative control consisting of the reaction mixture without cDNA was included in each run. PCR was run for 20 to 30 cycles with β-actin primers at the following conditions: 90 s at 95°C, then 5 to 15 cycles of 45 s each at 95°C, 90 s at 60°C, and 60 s at 72°C. The last 15 cycles were run under the same conditions, but at 72°C, incubation was prolonged by 5 s in each cycle. PCR with IL-15 primers was run with the same protocol except that the annealing temperature was 55°C instead of 60°C. Every experiment was amplified with at least two different cycle numbers to ensure that amplification was in the exponential phase of PCR.

We found that 25 to 30 cycles for IL-15 and 20 to 25 cycles for β-actin were in the exponential phase of amplification, thus permitting comparison of mRNA levels in different samples. Under these conditions, we also found a linear dose response of the PCR product to increasing doses of cDNA.

Eight microliter of each sample containing amplified cDNA was loaded on an agarose gel (2%) containing ethidium bromide (0.5 μg/ml). A DNA size marker was run on the same gel (100 bp ladder, Life Technologies BRL). PCR products were quantified by video densitometry of agarose gel with the UVP GDS 5000 System (UVP Inc., San Gabriel, CA). To correct for differences in loading, we corrected densitometric values of IL-15 cDNA with corresponding values of β-actin cDNA. To allow comparison of different gels, we normalized the densitometric IL-15/β-actin ratios for the IL-15/β-actin ratio of the unstimulated TEC run in each gel.

Assay of IL-15 Protein

IL-15 in supernatants and cell lysates was measured in duplicate using enzyme-linked immunosorbent assay (ELISA) to human IL-15 made with commercially available antibodies (R&D Systems, Minneapolis, MN). Following the protocol of the manufacturer, we coated ELISA plates (type 2592, Corning Costar) overnight at room temperature with monoclonal anti-human IL-15 antibody (type MAB647) diluted in PBS to a final concentration of 2 μg/ml. Plates were then washed three times with washing buffer (0.05% Tween 20 in PBS) and incubated for 1 h with blocking buffer (1% BSA, 5% sucrose, and 0.05% NaN_3 in PBS). Plates were washed three times, and samples of rhIL-15 standards (3 to 300 pg/ml, R&D Systems) were added to wells. Wells were incubated for 2 h at room temperature. Then plates were incubated for 2 h with biotinylated monoclonal anti-human IL-15 antibody (type BAM247, 100 μl/well) diluted to a final concentration of 100 ng/ml in Tris-buffered saline (20 mM Trizma base, 150 mM NaCl [pH 7.3], with 0.1% BSA and 0.05% Tween 20). Plates were then incubated for 20 min with streptavidin horseradish peroxidase (type 43-4323, Zymed, San Francisco, CA; 1:20,000, 100 μl/well). Finally, 100 μl of tetramethylbenzidine solution (TMBSingle Solution, Zymed) was added and plates were incubated for 20 min. ELISA reaction was stopped with 100 μl of H_2SO_4 (1 N). Optical density was read with an ELISA reader at 450 nm and at 550 nm for reference.

Statistical Analyses

Results are expressed as mean ± SEM. To compare IL-15 levels between groups we used either Dunnet's method of multiple analysis and a one-way ANOVA or a two-way ANOVA. *P* values below 0.05 were considered significant.

Results

CD40 Is Expressed on TEC

We analyzed the expression of CD40 and CD154 on TEC by flow cytometry. As depicted in Figure 1, we clearly demonstrate the expression of CD40 molecules on TEC (more than 95% positively stained cells). In contrast, we were unable to detect CD154 molecules on TEC.

CD40L Cells Bind Specifically to TEC

We speculated that the interaction between TEC and T cells via CD40/CD154 would enhance IL-15 production by the TEC. The interaction between TEC and T cells involves many other molecules apart from CD40/CD154. To evaluate the isolated effect of CD40 ligation on TEC, we co-cultured the TEC with a cell murine fibroblast cell line (L cells) genetically engineered to express human CD154 (CD40L cells). As a control, we used L cells that were unaltered. TEC formed a confluent layer before L cells were introduced into the culture so that the L cells were floating in the medium, as if they were invading T cells. When TEC were co-cultured with either CD40L cells or L cells, the ability of the CD40L cells to bind to the TEC compared with L cells was noticeable after 3 to 6 h (Figure 2, A through C). CD154-expressing cells adhered to TEC while L cells formed aggregates and floated above the TEC. L cells that did not express CD154 exhibited some

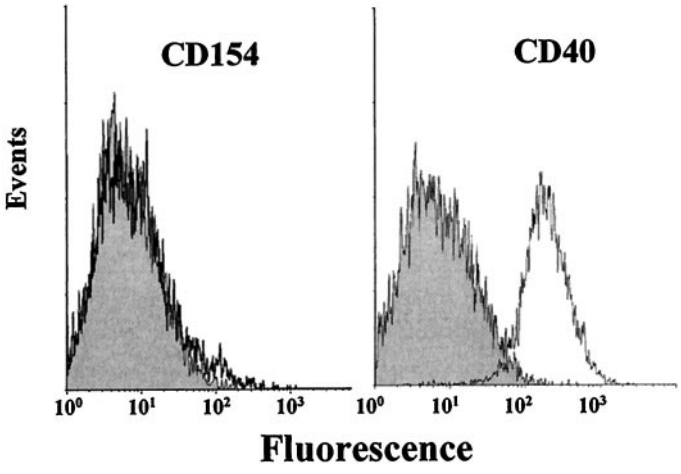

Figure 1. Flow cytometry analysis of CD40 and CD154 molecules on tubular epithelial cells (TEC). TEC were labeled by a primary antibody to CD154 or CD40 as indicated, then stained with FITC-labeled secondary antibody. The shaded histogram indicates the background fluorescence of cells labeled with an isotype-matched control antibody; the plain wide line indicates fluorescence of cells labeled with anti-CD154.

nonspecific binding, but CD40L cells showed significantly higher binding rates (data not shown) as measured by a neutral red assay described in the Materials and Methods section. Briefly, TEC were fixed and then either L cells or CD40L cells were added when specific adhesion was visible; cultures were washed to remove unbound cells, and the remaining cells were stained with neutral red. The binding of CD40L cells to TEC was blocked when CD40L cells were preincubated with an anti-CD154 antibody (data not shown). Electron microscopic view reveals a tight contact between CD40L cells and TEC (Figure 2D). We quantitated the specific adhesion of CD40L cells to TEC by neutral red dye uptake and found approximately threefold more adhesion of the CD40L cells compared with L cells (not shown).

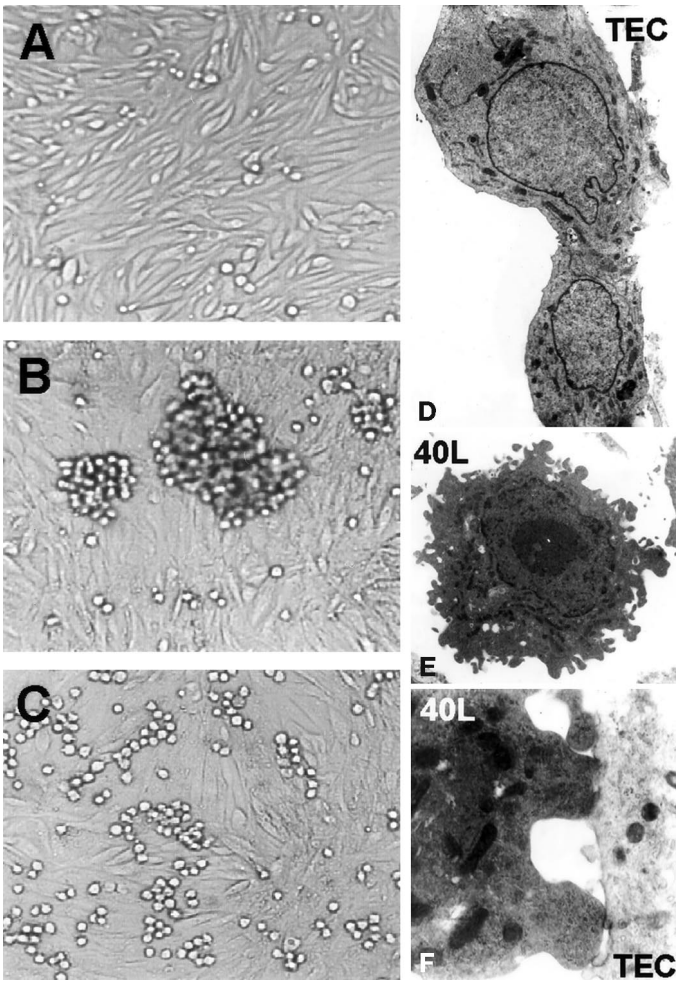

Figure 2. Co-culture of CD40L cells and TEC. Micrographic (×100) view of TEC culture (A), co-culture of TEC and control L cells (B), and co-culture of TEC and CD40L cells (C). TEC were grown to confluence and then incubated with a suspension of L cells (1.5×10^5/well) for 6 h. An electron micrographic view revealed the interaction between TEC and CD40L cells: (D) TEC monolayer, (E) CD40L-expressing cells, (F) co-culture of the two cell types.

CD154 Binding Enhances IL-15 Production

Incubation of TEC with CD154 resulted in a twofold increase in IL-15 production, as shown in Figure 3. It should be noted that the supernatants of L cells or CD40L cells had no effect on IL-15 levels (not shown). To ensure that the enhanced production of IL-15 is due to CD154-CD40 cross-linking, we used an anti-CD154 antibody and a Transwell system. We observed that preincubation of CD40L cells with CD154-blocking antibody abolished their ability to induce IL-15 production from TEC (Figure 3A). Preincubation with isotype control antibodies did not have this effect (not shown). Transwell co-culture in the same compartment resulted in induced IL-15 production, whereas co-culture in different compartments did not (Figure 3B). These observations demonstrate the contact-dependent nature of the interaction leading to IL-15 production by TEC. Co-culture of TEC with L cells did not induce IL-15 production under any conditions. Thus, we deduce that CD40 activation specifically induces IL-15 production by TEC.

CD40 Activation Increases IL-15 mRNA Levels

We investigated the effect of CD40 activation on IL-15 at the mRNA level by RT-PCR analysis. IL-15 mRNA was seen to be present at a basal level in TEC, and CD40 activation resulted in increased levels of IL-15 mRNA. IL-15 levels increased over time, reaching a peak at 6 h then dropping to almost basal levels at 12 h (Figure 4). TEC co-cultured with L cells for 6 h produced basal levels of IL-15 mRNA similar to those found at the 0 time point.

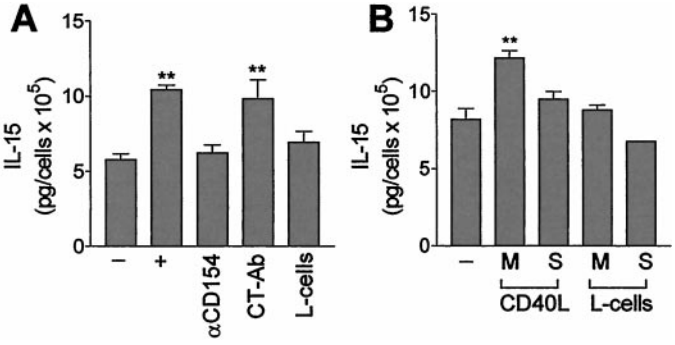

Figure 3. CD154 binding enhances interleukin-15 (IL-15) production. (A) Inhibition of IL-15 induction by αCD154-Ab. Supernatants were collected at 36 h and assayed for IL-15 from a monolayer of TEC alone (−) or from TEC co-cultured with either CD40L cells (+), or CD40L cells pretreated for 30 min with a monoclonal αCD154 or isotype control (CT-Ab) antibodies, or from TEC co-cultured with L cells. (B) Prevention of CD40L-cell contact with TEC. TEC alone (−), TEC co-cultured with CD40L cells, or TEC co-cultured with L cells in the same chamber (M) or in a Transwell system separated to two chambers (S) were assayed at 24 h for total IL-15 production (secreted and cell associated). IL-15 levels were determined by enzyme-linked immunosorbent assay. Both figures are representative of three experiments with similar results. Results are expressed as mean ± SEM. **, significance of IL-15 levels over TEC alone ($P < 0.01$).

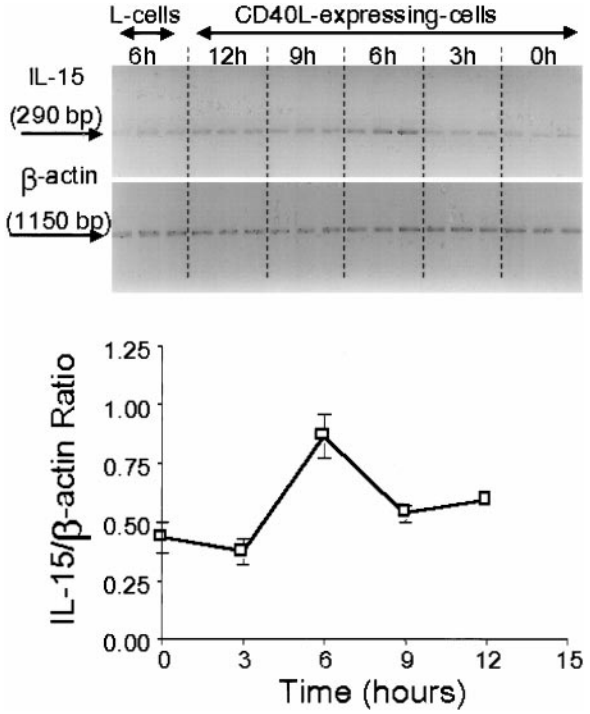

Figure 4. Time course of IL-15 mRNA levels in TEC after CD40 activation. TEC were incubated for the indicated time with CD40L cells or in the presence of L cells for 6 h. Total RNA was isolated and reverse transcription-PCR was preformed to amplify IL-15 and β-actin cDNA. The various samples were run on 2% agarose gel containing ethidium bromide. The intensity of the bands was analyzed by video densitometry and is represented graphically as the IL-15/β-actin ratio.

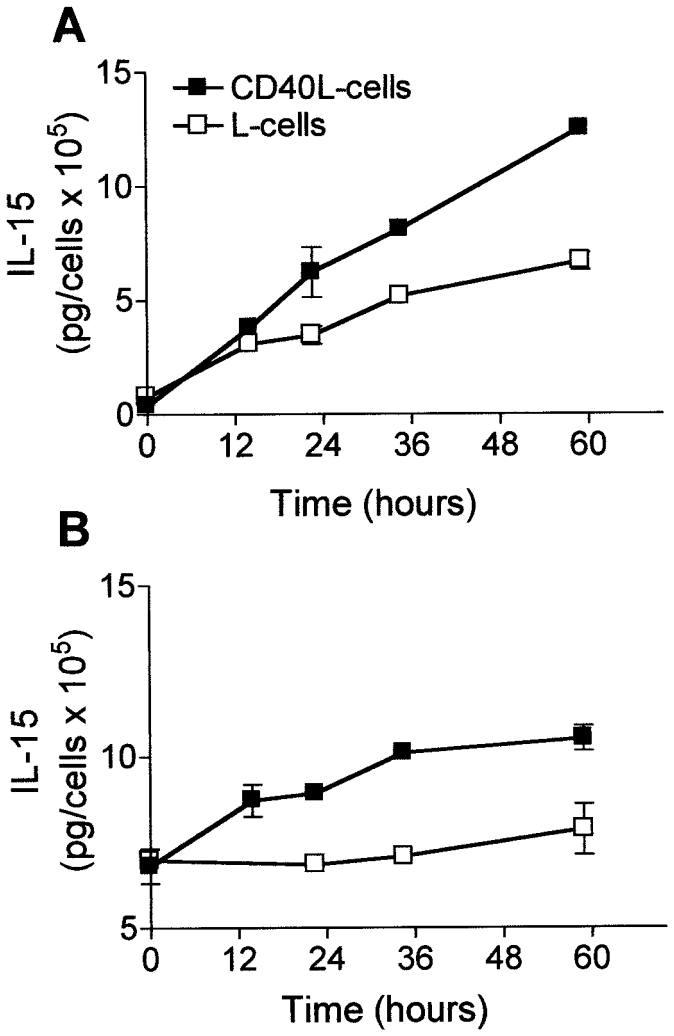

Figure 5. Time course of IL-15 protein production by TEC after CD40 activation. TEC were incubated for the indicated time with either CD40L cells or control L cells. At the indicated time points, supernatants (A) were collected and cell-associated IL-15 (B) was obtained by cell lysis with medium containing 0.1% NP40. Figures are representative of three experiments with similar results.

Time Course and Dose Response for CD40 Activation

Next, we studied the kinetics of IL-15 protein production by TEC after CD40 activation. Because IL-15 is found in the cytoplasm and membrane and not only in the supernatants, we assayed both secreted and cell-associated compartments. Both cell-associated and secreted IL-15 levels increased after CD40 activation (Figure 5). Levels of IL-15 were significantly higher 24 h after CD40 activation and accumulated steadily up to 72 h. Cell-associated IL-15 reached a plateau at 48 h when at equilibrium with the extracellular IL-15 (Figure 5B), whereas secreted IL-15 rose constantly, because it accumulates in the supernatant (Figure 5A). The increase in IL-15 production is dose dependant, reaching a plateau at a concentration of 150,000 cells/well, which corresponds with a TEC to L-cell ratio of 1:1 (Figure 6). Secreted IL-15 (Figure 6A) and cell associated IL-15 (Figure 6B) seem to be regulated in the same manner.

An Additive Effect for CD154 and IFN-γ

IFN-γ is known to enhance IL-15 production. We therefore compared CD154 and IFN-γ for their ability to induce IL-15 levels. TEC were treated with CD154 (150×10^3 cells/well), the control medium, IFN-γ (500 u/ml) or both, and IL-15 protein production was assayed by ELISA. Activation with CD154 resulted in induced IL-15 production by TEC (Figure 7). This induction did not occur with control treatment. In compliance with the mRNA levels after CD40 activation, IL-15 protein levels underwent enhancement to twice the basal levels produced by TEC, an effect similar to that produced when cells are treated with IFN-γ. The effect of stimulating TEC with both CD40 and IFN-γ resulted in an additive effect of IL-15 production. CD40L cells and control L cells were cultured alone to assay their supernatants and lysates for IL-15. The ELISA did not detect IL-15 in these samples (data not shown).

Discussion

Binding of the CD40 ligand expressed on activated T cells to its receptor is a major signal in many immune reactions. Therefore, we tested the effect of CD154 on the production of IL-15, a T-cell growth factor and activator by TEC.

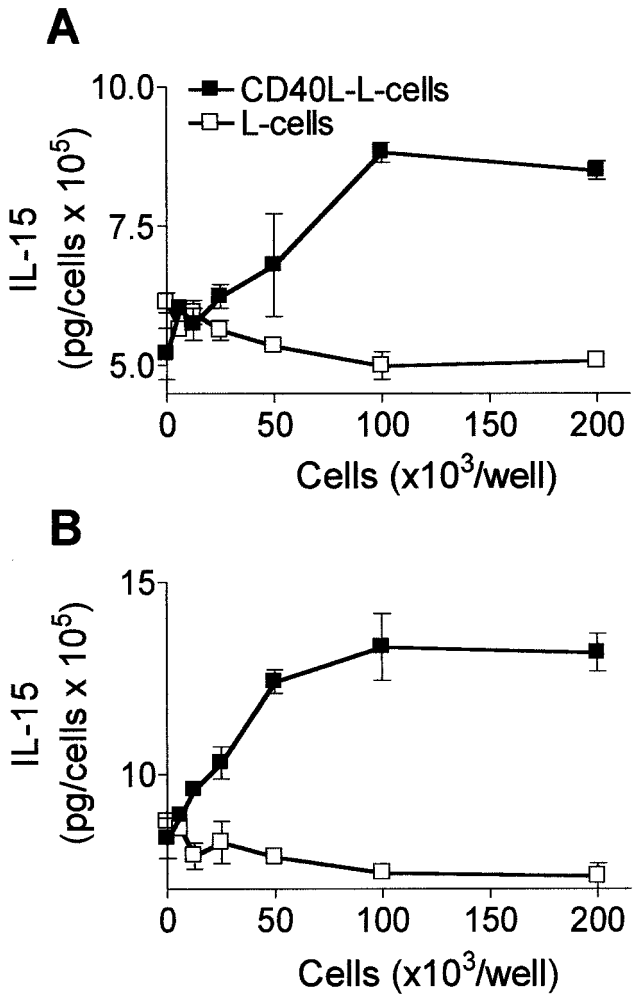

Figure 6. Dose-response curve of IL-15 protein production by TEC in response to CD40L cells. TEC were incubated with increasing numbers of CD40L cells. At 48 h, supernatants (A) were collected, and cell-associated IL-15 (B) was obtained by cell lysis with medium containing 0.1% NP40. Figures are representative of three experiments with similar results.

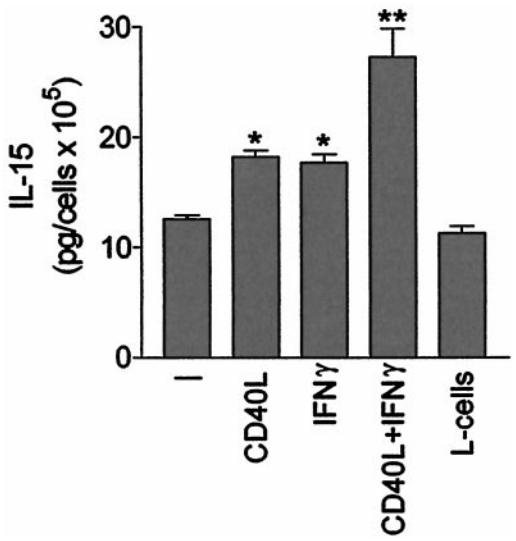

Figure 7. The combined effect of CD40L cells and interferon-γ (IFN-γ) on IL-15 production by TEC. Total IL-15 production was assayed on samples obtained at 48 h from TEC alone (−), TEC co-cultured with CD40L cells, TEC treated with IFN-γ (500 u/ml), TEC co-cultured with CD40L cells and treated with IFN-γ, and TEC co-cultured with L cells. The figure represents one of five experiments with similar results. *, significance of IL-15 levels over TEC alone ($P < 0.05$); **, significance over TEC alone ($P < 0.01$).

Soluble recombinant CD154 is considered less potent and devoid of most biologic activities (A. Douvdevani and C. Van Kooten, unpublished data). To isolate the role of the CD40 ligand from other lymphocyte signals, we used a murine cell line (L cells) genetically modified to express human CD154. This system and similar cell systems expressing CD154 are commonly used for activation of CD40 in various types of cells (10,21). In contrast to unmodified L cells, when CD40L cells are introduced to a cultured monolayer of TEC, they firmly bind to it. This specific binding of the CD40L cells suggests that it is mainly mediated by ligation of CD154 to CD40 receptors on TEC. Our observation is not surprising because CD40 is ubiquitously expressed on various cell types and we demonstrate its presence by flow cytometry on TEC similarly to previous studies on the same type of cells (2,10,22,23).

CD40 activation after the addition of the CD40L cells resulted in augmented production of IL-15 by TEC. The specificity of IL-15 induction is clear because induction is absent when the control L cells are added, induction is dependent on cell contact as demonstrated by the Transwell system, and anti-CD154 antibodies inhibit it.

CD40 activation resulted in increased levels of IL-15 mRNA, peaking at 6 h followed by an increase in IL-15 protein cell-associated levels starting at 12 h and significantly elevated secretion, as compared with unstimulated cells at 24 h. The twofold increase in IL-15 observed may represent only a partial affect of CD40 activation because there are suggestions that CD40 is expressed mainly at the basolateral site of TEC, which is not fully exposed in the culture method that we applied.

The intensity of activation by the CD154 cell was linearly dependent on cell number and reached a plateau at effector/target ratio of 1:1. This ratio complies with our observation that to activate TEC through CD40, cell to cell interaction must occur. In accordance with our findings, a recent report demonstrated that activation of CD40 by its ligand increases the production of IL-15 by dendritic cells (24).

Our data indicate that IFN-γ stimulation and CD40 activation result in an additive increase in the production of IL-15 by TEC. The additive effect of these stimulants can be attributed to their different signal transduction pathways. Data indicate that CD40 activates tumor necrosis factor receptor–associated factor proteins, which in turn activate the NF-κB/Rel family of transcription factors (25). NF-κB responsive elements were demonstrated in the IL-15 gene promoter, and there is evidence that it participates in the regulation of IL-15 gene expression (26,27). Binding of IFN-γ to its receptor results in activation of the JAK-STAT signaling pathway and later to activation of the IFN regulatory factor (IRF). The IL-15 promoter contains an

IRF response element, which is critical for its production as has been demonstrated by defective IL-15 expression in IRF double-negative mice (28,29).

IL-15 is mainly a T cell and NK cell growth factor and activator. It is interesting that both IL-15 inducers described—the soluble mediator IFN-γ and the membrane-bound inducer CD154—originate from activated T cells. During an immune response, a positive feedback loop between the kidney parenchyma and the lymphocyte invaders perpetuated by these agents can evolve. This assumption is strongly supported by the correlation that exists between acute kidney allograft rejection and elevated IL-15 mRNA in the rejected graft. The important role of CD40 and its ligand in graft rejection has been established in CD154 neutralization experiments performed in various animal models (5,6,8). CD154 neutralization therapy is now considered as a future alternative for treatment of graft rejection (30,31). The mechanism by which CD154/CD40 ligation supports graft rejection is unclear. It has been suggested that CD40 activation increases the expression of B7 molecules, ICAM-1, and CD44H on antigen-presenting cells (3,32). It has also been shown that CD40 activation strongly increases the release of the leukocyte chemoattractant IL-8, RANTES, and MCP-1 (10). In light of the present study, we suggest that blocking CD154 may also decrease IL-15 production by the graft and thus impair the support of T cells.

In conclusion, this study demonstrates that CD154, which is usually expressed on activated T cells, increases the production of IL-15 by TEC and is additive to IFN-γ in its effect. Because IL-15 is a major T-cell activator, this interaction contributes to graft rejection, a process in which both IL-15 and CD40 have been shown to be involved.

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