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Interleukin-15 Promotes Angiogenesis *in Vivo*

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IL-15, a cytokine with biological functions on cells of lymphoid lineage similar to those of IL-2, mediates its activities through the β and γ chains of the IL-2/15R and its own α chain. Unlike IL-2, IL-15 also binds to endothelial cells with high affinity. We report here that IL-15 is a stimulator of angiogenesis in vivo. When injected subcutaneously into nude mice, IL-15 consistently induced neovascularization of Matrigel plugs. Endothelial cells were found to express the IL-15R α chain and the IL-2/15R β and common γ chains. IL-15 induced the rapid tyrosine phosphorylation of proteins in endothelial cells, but did not stimulate endothelial cell proliferation in vitro. These findings document a previously unrecognized biological property of IL-15 and emphasize the role of IL-15 as an important mediator outside the immune system.

IL-15 is a novel cytokine with biological activities similar to those of IL-2 (1-5). Like IL-2, IL-15 stimulates T-cell proliferation, including the CD4+, CD8+, $\gamma\delta$, and memory subsets (1, 2-5); induces T-cell chemotaxis (6); promotes natural killer cell growth, cytotoxicity and secretion of IFN-γ, granulocyte/macrophage colony stimulating factor, and tumor necrosis factor-α (9,10); and costimulates B-cell growth and Ig production (11). Unlike IL-2 that is produced almost exclusively by activated T cells, the IL-15 gene is not expressed in these cells (1, 4). Expression of IL-15 mRNA was detected in several human tissues, including placenta, skeletal muscle, kidney, lung and heart (1). The most abundant cellular sources of IL-15 mRNA include monocytes, fibroblasts, epithelial cells, and stromal cells from bone marrow or thymus (1). Recently, IL-15 was identified in the synovial membrane and the synovial fluid of patients with rheumatoid arthritis (8).

Although IL-15 has no significant primary sequence homology to IL-2, it mediates its functions through the β and γ chains of the IL-2R, and this might explain the high degree of functional similarity between IL-2 and IL-15 (12). Recently, an IL-15 R-specific α chain was identified which is capable of high affinity binding to IL-15, but does not appear to participate in signal transduction (13,14). Unlike the structurally related IL-2R α chain (15), the IL-15R α chain mRNA is widely expressed in cells of T, B, macrophage, bone marrow stromal lineages and in tissues, including heart, spleen, lung, skeletal muscle and liver (13,14).

The widespread expression pattern of IL-15 and IL-15R α chain contrasts with the narrow expression pattern of IL-2 and IL-2R α chain (3,16). This has suggested the possibility that a wider variety of cell types could be responsive to IL-15 than to IL-2, and that IL-15 could have some unique properties not shared with IL-2 (3). However, IL-15 signaling in cells of hematopoietic origin requires the presence of the IL-2R β and γ chains, and their expression in cells and tissues appears to be more limited than that of IL-15R α chain (15). Thus, expression of IL-15R α chain may not indicate by itself IL-15 responsiveness. Rather, it may represent an effective mechanism for sequestration of IL-15. Human umbilical cord derived endothelial cells bind IL-15 with high affinity (12). We examined whether IL-15 may exert biological properties on endothelial cells not shared by IL-2, and report that when injected in vivo, IL-15 induced neovascularization of Matrigel plugs. Thus, stimulation of angiogenesis is a previously unrecognized biological property of IL-15.

METHODS

Mice, cells, reagents, and cytokines. Four- to six-week-old female BALB/c nu/nu mice (Taconic, Germantown, NY or National Cancer Institute, Frederick, MD) maintained in pathogen-limited conditions were used throughout. Human umbilical vein endothelial cells (HUVEC) obtained from the American Type Culture Collection (ATCC, Rockville, MD), were maintained in RPMI 1640 Medium (Gibco, BRL, Grand Island, NY), 15% heat inactivated fetal bovine serum (FBS, Intergen Co. Purchase, N.Y), 20 U/ml porcine preservative free heparin (Squibb-Marsam Inc., Cherry Hill, NJ), and 100 μg/ml endothelial cell growth supplement (ECGS, a crude extract of bovine neural tissue containing basic fibroblast growth factor (bFGF), and acidic

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fibroblast growth factor (aFGF), Calbiochem-Novabiochem Corp., La-Jolla, CA). The YTN10 cell line is a kind gift of Drs. A. Yamauchi and J. Yodoi, Kyoto University, Kyyoto, Japan. The Hut102 cell line is a kind gift of Dr. T. Waldman, NCI, Bethesda, MD. Matrigel, a crude extract of the Englebreth-Holm-Swarm (EHS) tumor, was prepared as previously described (17). Human rIL-15 was provided by Immunex Corp. (Seattle, WA). Human rIL-2 was a gift of Chiron Co. (Emeryville, CA). TGF-β and bFGF were obtained from R&D Systems (Minneapolis, MN). Vascular endothelial growth factor (VEGF) was supplied by Genetech Inc. (San Francisco, CA).

Monoclonal antibodies and immunofluorescence analysis. The monoclonal antibodies anti-Tac (antihuman IL-2Rα, mouse IgG2a) (18) and Mikβ1 (antihuman IL-2Rβ, mouse IgG2a)(19) were kind gifts from Dr T.A. Waldmann (National Institute of Health, Bethesda, MD), and TUGh4 (antihuman IL-2Rγ, rat IgG2b)(20) was a kind gift from Dr K Sugamura (Tohoku University, Sendai, Japan). Isotype matched control (mouse IgG2a and rat IgG2b) were obtained from Pharmigen (San Diego, CA). PE-conjugated goat anti-mouse and anti-rat Ig were purchased from Southern Biotechnology Associate (Birmingham, AL). Immunofluorescence analysis was performed by indirect methods, and the stained cells were analyzed using FACScan flow cytometer equipped with a Cell Quest data analysis program (Becton Dickinson, San Jose, CA), as described previously (6).

RNA preparation and reverse transcriptase-polymerase chain reaction (RT-PCR). Total RNA was extracted from the cell pellets using the guanidine isothiocyanate-phenol (TRIZOL Reagent, Life Technologies, Inc., Gaithersburg, MD) method. The first strand cDNA was synthesized from 5 μg of total RNA using the SuperScript preamplification System (Life Technologies) according to the manufacturer's conditions. The resultant cDNA was immediately diluted with H_2O to a final volume of 100 μL. PCR was performed in thin-wall reaction tubes (Perkin Elmer Cetus, Norwalk, CT) in a reaction mixture (50 μl) containing 5 μl of the first strand cDNA, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each dNTP, 400 μM each primer, and 2.5 U of *Taq* DNA polymerase (Life Technologies). Primers are listed in Table I. The sequences of the IL-2Rα, IL-2/15Rβ, γc chains, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers was described elsewhere (21, 22); the sequence of the IL-15Rα primers was designed to encompass intron 6 (14). Amplifications were performed in a thermocycler (Robocycler, Stratagene, La Jolla, CA) as follows: for detection of GAPDH, an initial denaturation step of 3 min at 95°C was followed by 30 cycles of denaturation at 95°C for 60 s, annealing at 61°C for 50 s, and extension at 72°C for 60 s followed by a final extension step of 7 min at 72°C; for detection of IL-2R and IL-15R subunits, *Taq* DNA polymerase was added to the reaction mixture after an initial denaturation at 95°C for 10 min (hot start PCR), amplification was performed by 40 cycles of denaturation at 95°C for 60 s, annealing at 55-63°C for 50 s, and extension at 72°C for 60 s followed by a final extension step of 7 min at 72°C. Each amplified product was electrophoresed through 1.5% agarose gels pre-stained with 0.5 μg/ml of ethidium bromide, and visualized under ultraviolet light.

Cell proliferation. HUVEC cells were used routinely between passages 15 and 20. After trypsinization, the cells were plated in triplicate cultures of 5-10 $\times 10^3$ cells in 0.2 ml complete medium with or without additives in a 96-well flat-bottom plate. The plates were incubated for 2-3 days. DNA synthesis was determined by [^{3}H]-thymidine deoxyribose uptake (0.5 uCi/well, 6.7 Ci/mmol; New England Nuclear, Boston, MA) during the last 18 hours of culture. Cells were detached by freezing/thawing.

In vivo Matrigel assay. This assay was performed as described (23). Briefly, Matrigel (liquid at 4°C) was mixed with IL-15 (1000 or 2000 ng/ml), IL-2 (1000 or 10000 IU/ml), VEGF (3 μg/ml) or with bFGF (25 or 150 ng/ml) either alone or in combination with IL-15. Matrigel alone or with the test growth factors/cytokines (total volume 0.5 ml) was injected subcutaneously into the midabdominal region of the BALB/c nude mouse. After injection, the Matrigel polymerizes to form a plug. After 6 days, the animals were sacrificed, the Matrigel plugs removed together with the abstract epidermis and dermis, fixed in 10% neutral buffered formalin solution (Sigma Chemical Co.), and embedded in paraffin. Histological sections were stained with Masson's trichrome. The cell occupied area in the histological sections was measured using a computerized digital analyzer (Optomax, Hollis, NH).

Immunoblotting. For determination of tyrosine phosphorylation in response to cytokines, the cells were starved overnight in medium containing 0.1% FCS without growth factor. The cells were incubated with IL-15 (500 ng/ml) for various lengths of time at 37 °C. Immunoblotting was performed essentially as described (24). Briefly, the cells were lysed at 4 °C with 1% Nonidet P-40, 300 mM NaCl, 50 mM Tris (pH 7.4), leupepsin (10 μg/ml), aprotinin (10 μg/ml, 2 mM phenylmethylsulfonyl fluoride, 1 mM sodium vanadate, 25 mM sodium fluoride, 10 mM sodium pyrophosphate, and 1 mM EDTA. Cell lysate (20 μg/lane) was separated on an 8% Tris/Glycine Gel (Novex, San Diego, CA), and proteins were transferred onto Immobilon-P transfer membrane (Millipore, Bedford, MA). After blocking with 5% skim milk in PBS, phosphotyrosine monoclonal antibody 4G10 (Upstate Biotechnology, Lake Placid, NY) diluted 1:5000 in 0.5% skim milk in PBS-T (PBS with 0.1% Tween 20). After rinsing four times in PBS-T, blots were incubated for 30 min with horseradish peroxidase-conjugated streptavidin (Kirkegaard and Perry Laboratories, Gaithersburg, MD) diluted 1: 20000 in PBS-T. Immunoblots were rinsed five times in PBS-T, and then developed with an ECL chemiluminescence detection kit (Amersham, Arlington Heights, IL).

Immunohistochemistry. Histological sections of paraffin-embedded Matrigel alone, Matrigel plus bFGF, or Matrigel plus IL-15 plugs were deparaffinized and hydrated through xylene and decreasing alcohol concentrations, washed in water, digested with 20 μg/ml Proteinase K (VWR Scientific, West Chester, PA) for 5 min at room temperature, washed in PBS, and then exposed for to a rabbit polyclonal antibody to human vitronectin receptor ($\alpha_v\beta_3/\beta_5$; Chemicon International Inc., Temecule, CA) at room temperature for 20 min. After washing in PBS, the slides were stained with a peroxidase-conjugated goat anti-rabbit antiserum and developed according to the manufacturer's instructions (Vectastain Elite ABC peroxidase kit, Vector Laboratories, Burlingame, CA)

Statistical analysis. Arithmetic means, standard deviations, Student's t tests and group comparisons using Dunnett's method were calculated by conventional formulas using Systat for the Macintosh (Systat Inc., Evanston, IL).

RESULTS

IL-15 receptor expression in human endothelial cells. IL-15 is known to bind with high affinity to endothelial cells derived from the human umbilical cord, but the molecular basis for this binding has not been characterized (12). We looked for endothelial cell expression of the IL-15 R complex subunits, including the IL-2/15R β chain, the γc chain, and the IL-15 α chain. Using MAbs that specifically recognize the human IL-2/15R β chain (Mikβ1) and the γc chain (TUGh4), endothelial cells from HUVEC were found not to express these IL-15 R subunits by FACS analysis (not shown). The control cell lines HUT 102 and YTN10 cells expressed the IL-2R α, β, and γ subunits to varying levels. To examine this further and additionally assess expression of the IL-15R α chain, we looked for transcripts of the IL-15R subunits by RT-PCR analysis. In a representative

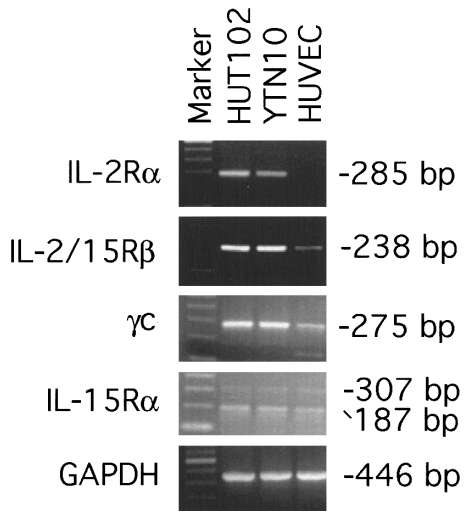

FIG. 1. IL-2/15R subunit gene expression in endothelial cells detected by RT-PCR analysis. RNA, extracted from HUT 102, YTN10, HUVEC, or FBHE cells, was reverse transcribed and subjected to PCR amplification using specific primers. The amplified products were electrophoresed through a 1.5% agarose gel.

experiment (Fig. 1), the expected size bands attributable to the IL-2/15Rβ (238 bp), γc (275 bp), and IL-15Rα (187 and 307 bp) chain transcripts were detected in the samples from HUVEC, and from the cell lines HUT102 and YTN10. It should be noted that using RT-PCR, the bands attributable to the IL-2/IL-15R β and γc subunits were less intense in samples from HUVEC compared to HUT102 and YTN10 cells, suggestive of lower levels of expression. In addition, the expected 285 bp band attributable to IL-2Rα chain transcript was derived from HUT102 and YTN10 cells, but not from HUVEC cells. These results indicated that human endothelial cells derived from the umbilical vein express all three genes known to encode for IL-15R complex, and suggest that the previously reported high affinity binding of these cells to IL-15 may be attributable to expression of the IL-15R α chain.

Effects of IL-15 on endothelial cells in vitro. To determine whether the IL-15R is functional on endothelial cells, we assessed tyrosine phosphorylation of proteins in response to IL-15. Western blot analysis with anti-phosphotyrosine antibodies demonstrated tyrosine phosphorylation of proteins in starved HUVEC after stimulation with IL-15 (Fig. 2). Within 5 minutes after exposure to 500 ng/ml IL-15, and persisting for up to 1 hour, an increase in phosphorylation of several proteins was apparent, including proteins in the 150-180, 95-150, and 70-90 kDa size ranges.

We further examined the effects of IL-15 on endothelial cell proliferation in vitro by measuring [^{3}H] thymidine incorporation and cell counting. As shown in representative experiments (Table 2), when added alone to HUVEC in 48-72 hr cultures, IL-15 (10 ng/ml) had little or no effect on DNA synthesis. Similarly, when added to HUVEC in conjunction with an endothelial cell growth supplement (ECGF, containing basic and acidic fibroblast growth factors), IL-15 (100 or 1000 ng/ml) had little effect on DNA synthesis. Time course experiments confirmed that IL-15 had little or no effect on DNA synthesis in HUVEC at earlier (1 day) or later time points (4, 5, 6, and 7 days), when added alone or in conjunction with ECGF (not shown). Cell counting performed in selected parallel cultures confirmed that IL-15 had little or no effect on HUVEC proliferation in vitro (not shown).

Effects of IL-15 on angiogenesis in vivo. To examine the potential effects of IL-15 on neovascularization in vivo, we used a previously described murine method in which subcutaneous injection of Matrigel impregnated with bFGF rapidly induces new vessel formation (23). Using this method, we tested whether IL-15 can promote or inhibit neovascularization of Matrigel plugs. Groups of mice (3-5) were injected with either Matrigel alone or Matrigel together with one of the following: IL-15 (1000 or 2000 ng/ml); bFGF (25 or 150 ng/ml); VEGF (3000 ng/ml); IL-2 (1000 or 10000 IU/ml); or bFGF (25 ng/ml) plus IL-15 (1000 ng/ml). Matrigel plugs were removed from the mice 6 days after injection, processed for histology, and evaluated for cell in-

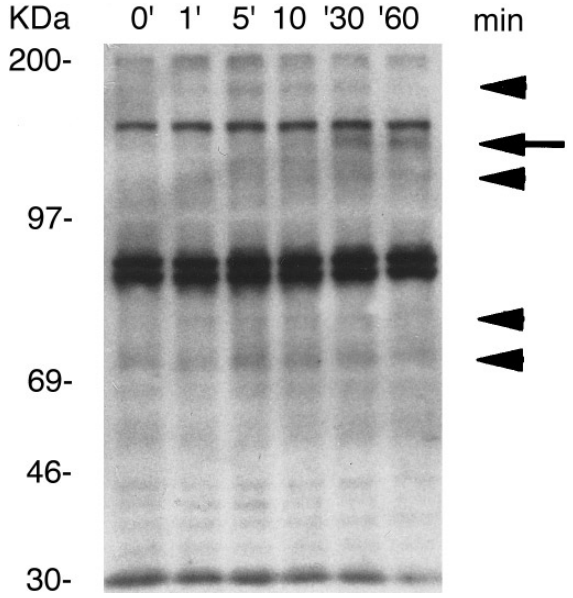

FIG. 2. Tyrosine phosphorylation of proteins in endothelial cells exposed to IL-15. HUVEC were incubated with IL-15 (500 ng/ml) for the designated times. After cell lysis, cellular proteins were separated through a Tris/glycine gel and transferred onto an Immobilon-P membrane. Phosphotyrosine-containing proteins were stained with a biotin-conjugated anti-phosphotyrosine MAb (4G10) followed by horseradish peroxidase-conjugated streptavidin and then developed by chemiluminescence. Tyrosine phosphorylated proteins that appeared after 1-5 minutes or 30 minutes incubation are indicated by arrowheads or arrows, respectively.

TABLE 1

PCR Primers, Product Sizes, and Annealing Temperatures

PCR product	Genbank Accession No.	Sequence	Product size (bp)	Annealing temp (°C)
IL-2Rα	K03122	TCTTCCCATCCCACATCCTC		
		TCTGCGGAAACCTCTCTTGC	285	57
IL-2/15Rβ	M26062	GGCTTTTGGCTTCATCATCT		
		CTTGTCCCTCTCCAGCACTT	238	55
γc	D11086	ACGGGAACCCAGGAGACAGG		
		AGCGGCTCCGAACACGAAAC	275	59
IL-15Rα	U31628	AGACCTGGGAACTCACAGCATCCG		
		GCTTCCATTTCAACGCTGGCCAGC	187/307	61
GAPDH	M32599	GCCACCCAGAAGACTGTGGATGGC		
		CATGTAGGCCATGAGGTCCACCAC	446	61

vasion by a semiautomated digitalized analyzer. As expected, Matrigel plugs impregnated with either bFGF (25 and 150 ng/ml) or VEGF (3000 ng/ml) exhibited increased cellularity compared to Matrigel plugs alone, reflective of stimulation of angiogenesis by these factors (Table 3). In 3 representative experiments, Matrigel plugs impregnated with IL-15 (1000 or 2000 ng/ml) contained 4.8-18.3 fold more cells compared to Matrigel plugs alone (IL-15 induction significantly different from none at 0.05 level using Dunnett's method). Addition of IL-15 (1000 ng/ml) to bFGF-induced Matrigel plugs (25 ng/ml) had little enhancing effect on the cellularity of the plugs (Table 3), and produced no significant change in the morphology of the plugs (not shown). In contrast to IL-15, IL-2 (1000 or 10000 IU/ml) had little or no effect on the vascularization of Matrigel plugs.

Microscopic examination of IL-15-induced Matrigel plugs stained with Masson's trichrome stain revealed the presence of abundant cellularity that was characteristically absent from Matrigel alone plugs (Fig. 3 a, b). The cells invading the IL-15 containing plugs were organized to form tubular structures, often containing red cells (Fig. 3b). To ensure that the cell population invading the IL-15 impregnated plugs consisted of endothelial cells, we looked for expression of $\alpha_v\beta_3/\beta_5$. Previous studies have demonstrated that both in human and chick, blood vessels involved in angiogenesis ex-

TABLE 2

Effects of IL-15 on Endothelial Cell Proliferation in Vitro

Culture conditions	Expt. 1	Expt. 2	Expt. 3
	HUVEC		
Medium	170	560	52
ECGF (110 μg/ml)	4,657	36,396	7,009
ECGF*	729	21,456	5,909
ECGF* + IL-15 (100 ng/ml)	517	21,741	ND
ECGF* + IL-15 (1000 ng/ml)	576	21,915	7,033
ECGF* + IL-2 (1000 IU/ml)	544	21,489	ND
IL-15 (10 ng/ml)	ND	409	47

The endothelial cells HUVEC (5×10^3 cells/0.2 ml well in experiments 1, 2, and 3; 10×10^3 cells/0.2 ml well in experiment 2) were cultured for 48-72 h with or without IL-15, TGFβ, or IL-2 in either the presence or absence of endothelial cell growth factor (ECGF). ^{3}H-thymidine was added during the final 18 h of culture. The results are expressed as mean cpm of triplicate cultures.

* ECGF at 1,10, 25 ng/ml in Expts. 1, 2, and 3, respectively.

TABLE 3

Effects of IL-15 on Angiogenesis in Vivo

Additions to Matrigel	Mean surface area (μm^2) occupied by cell field (±SD)
None	23 (20)
bFGF (25 ng/ml)	640 (140)
IL-15 (1000 ng/ml)	421 (215)
bFGF (25 ng/ml)+IL-15 (1000 ng/ml)	726 (46)
	150 (56)
None	
bFGF (25 ng/ml)	591 (96)
bFGF (150 ng/ml)	1507 (283)
IL-15 (1000 ng/ml)	723 (334)
IL-15 (2000 ng/ml)	1107 (367)
bFGF (25 ng/ml)+IL-15 (1000 ng/ml)	969 (12)
VEGF (3 μg/ml)	1327 (245)
None	93 (19)
bFGF (25 ng/ml)	705 (24)
bFGF (150 ng/ml)	1158 (563)
IL-15 (1000 ng/ml)	788 (33)
IL-2 (1000 IU/ml)	105 (30)
IL-2 (10,000 IU/ml)	62 (20)

Balb/c nu/nu female mice (3-5 per condition) were injected subcutaneously with Matrigel alone, Matrigel plus bFGF (25 or 150 ng/ml), Matrigel plus IL-15 (1000 or 2000 ng/ml), Matrigel plus bFGF (25 ng/ml) and IL-15 (1000 ng/ml), Matrigel plus VEGF (3 μg/ml), or Matrigel plus IL-2 (1000 or 10,000 IU/ml). The Matrigel plugs were removed after 6 d and processed for histology. The results reflect the mean (± SD) surface area (μm^2) occupied by cells/field (4×10^4 μm^2), as determined by a semiautomated digitalized analyzer.

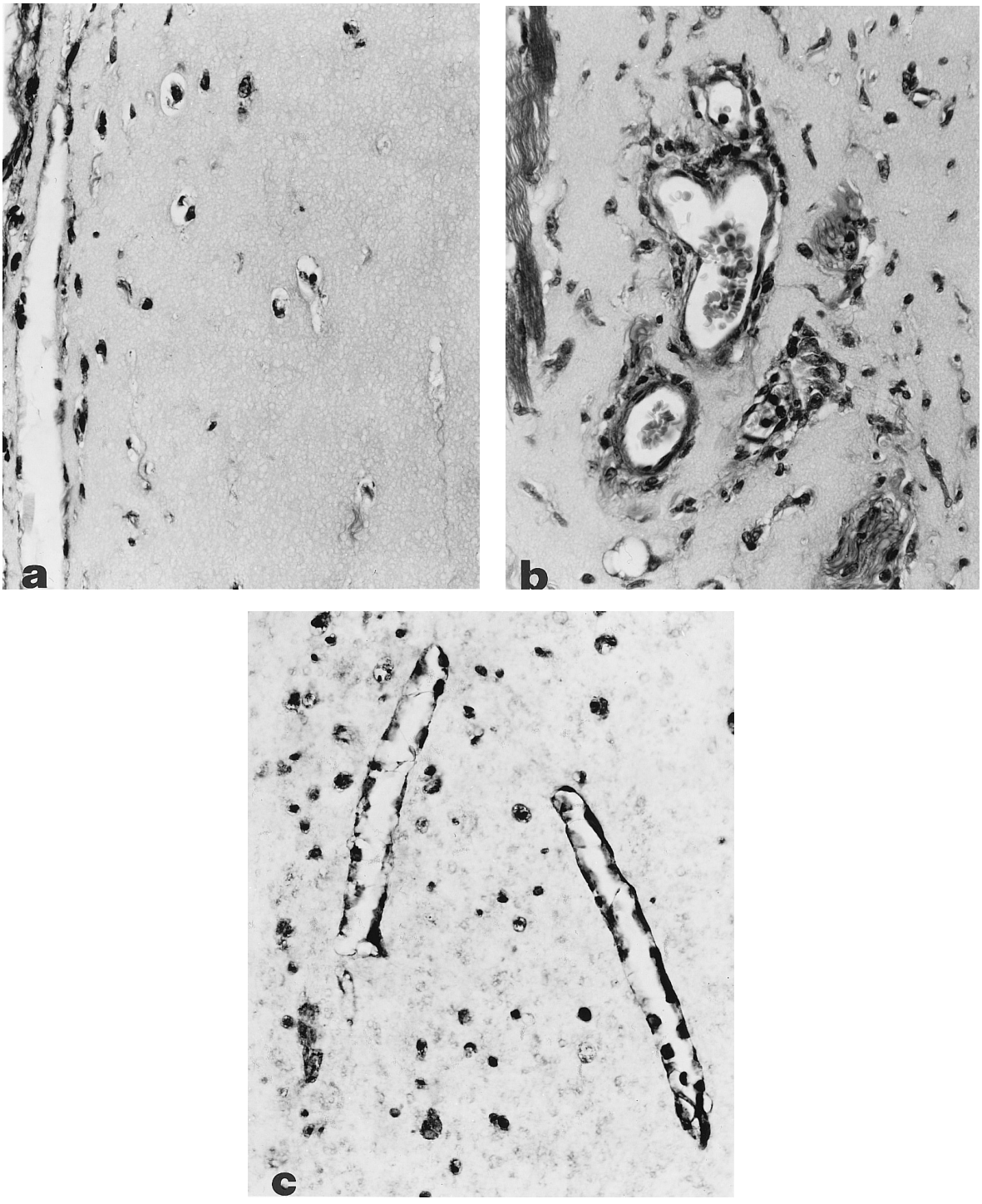

FIG. 3. Microscopic morphology of Matrigel plugs. Masson's trichrome stained Matrigel alone plug (**a**); Masson's trichrome stained Matrigel plug impregnated with IL-15 (**b**); and Matrigel plug impregnated with IL-15 stained with a rabbit polyclonal antibody to human vitronectin receptor ($\alpha_v\beta_3/\beta_5$) and a peroxidase-labelled goat anti-rabbit antiserum (**c**). Female BALB/c nu/nu mice were injected subcutaneously with either Matrigel alone or Matrigel plus IL-15 (1000 ng/ml). Plugs were removed 6 d after injection and processed for histology. Magnification (400 ×).

press the integrin $\alpha_v\beta_3/\beta_5$ at high levels, but other cells generally do not (25, 26). Using a rabbit antiserum to human $\alpha_v\beta_3/\beta_5$ that was found to cross react with the murine antigen (Passaniti, personal communication) and a peroxidase-labelled goat anti-rabbit antibody, we found that virtually all the cells in either bFGF (not shown) or IL-15 induced Matrigel plugs stained positive (Fig. 3c), while many cells immediately outside the

plug were negative. Together, these findings demonstrate that IL-15 can promote angiogenesis in vivo.

DISCUSSION

Formation of capillary blood vessels leading to neovascularization is essential to a variety of physiologic processes, including reproduction, tissue and organ development, and wound healing (27). It is also associated with, and may contribute to, an array of pathologic processes, including cancer, rheumatoid arthritis, and diabetic retinopathy. Capillary blood vessels are composed of endothelial cells and pericytes, and these cell types can multiply, migrate, organize into tubes, and form new capillaries in response to appropriate angiogenic signals (28). A number of angiogenic proteins have been identified, including bFGF, aFGF, VEGF, platelet-derived endothelial cell growth factor (PD-ECGF), angiogenin, TGF-β, TNF-α, and IL-8 (27,29). Some of these factors, including bFGF, aFGF and VEGF, are directly mitogenic and chemotactic for endothelial cells and promote tube formation in vitro. Other angiogenic factors do not exhibit these functions in vitro and their mechanisms of action are largely unknown.

We show that IL-15 is a previously unrecognized angiogenic factor. When mixed into Matrigel and injected subcutaneously into nude mice, IL-15 induced a vigorous local angiogenic response. In contrast to control plugs containing Matrigel alone which were virtually acellular, Matrigel plugs impregnated with IL-15 contained numerous cells, often organized to form tubular structures, resembling capillaries with lumens containing RBCs. The cells infiltrating the IL-15 containing Matrigel plugs stained positively for the integrin receptor $\alpha_v\beta_3$, a marker of angiogenic vascular tissue identified as the endothelial cell receptor for von Willebrand factor and vitronectin (25,26,30). The histological features of the angiogenic response induced by IL-15 were similar to those induced by bFGF, although the magnitude of the cellular response was somewhat smaller.

In vitro testing by PCR showed that endothelial cells express the IL-15R α chain, the high affinity IL-15 binding subunit in T cells, suggesting that this IL-15R subunit is responsible for the high affinity IL-15 binding to endothelial cells noted previously. In addition, exposure of endothelial cells to IL-15 was associated with activation of tyrosine phosphorylation of proteins in HUVEC, suggesting that endothelial cells can respond directly to IL-15. Previously, it was demonstrated that the γc and the IL-2R β chains are required for IL-15 signaling in T cells, but the IL-15R α chain is not (12-14). Using FACS analysis, we found no evidence of γc and IL-2R β chain surface expression on endothelial cells. However, using PCR, mRNAs for the γc and the IL-2R β chains were consistently amplified from endothelial cells, suggesting that expression of these receptor subunits, although occurring at a low level, may account for IL-15 signaling through endothelial cells. Recently, it was reported that mast cells proliferate in response to IL-15 but not IL-2 (16,31), and that this response is likely attributable to a novel IL-15 receptor/signal transduction pathway involving tyrosine phosphorylation of Janus-associated kinase (Jak)2 and signal transducers and activators of transcription (Stat)5 (16,31). This signaling pathway differs from that utilized by IL-15 in activated T cells where the γc subunit is involved and leads to the phosphorylation of Jak1/Jak3 and Stat3/Stat5 (16, 32). We have not investigated the IL-15 signal transduction pathway in endothelial cells, but if IL-15 utilizes the γc subunit in endothelial cells as it does in activated T cells, one might expect to observe phosphorylation of Jak1/Jak3 and Stat3/Stat5 in IL-15 stimulated endothelial cells.

In contrast to its angiogenic function in vivo, IL-15 did not promote endothelial cell division in vitro. When added to endothelial cell cultures either alone or in conjunction with ECGF, IL-15 consistently failed to stimulate DNA synthesis. This finding stresses the differences between in vitro and in vivo systems to study angiogenesis, and the complexities of the angiogenic response (27).

The in vitro results documenting IL-15 induction of protein phosphorylationin endothelial cells is unlikely due to contamination of endothelial cell cultures with other IL-15 responsive cells, including T cells, NK cells, mast cells, and skeletal muscle cells (3,16,33). Virtually all (>95%) of the HUVEC cells stained positive for von Willebrand factor, and were fully dependent on the presence of ECGF containing basic and acidic FGFs. These growth factors are not known to support long-term growth of the IL-15 responsive T, NK, mast cells, and skeletal muscle cells (34,35). Furthermore, the absence of T and NK cells is supported by the failure to detect expression of the IL-2 R α chain from endothelial cell cultures by RT-PCR.

The differences between results of in vitro and in vivo assays for angiogenesis, and the complexities of the angiogenic process have emerged from previous studies with other angiogenic factors, including TGF-β and TNFα (27). These cytokines stimulate angiogenesis in vivo, but profoundly inhibit the proliferation of certain endothelial cells in vitro (34,36). It was proposed that the angiogenic effect of TGF-β and TNFα in vivo are the result of monocyte/macrophage mobilization, and their secretion of endothelial cell mitogenic factors such as bFGF, PDGF, and VEGF (37, 38). The basis for the paradoxical activities displayed by IL-15 in vitro and in vivo are presently unknown. It could be that IL-15 is capable of promoting the proliferation of endothelial cells directly, but this effect cannot be appreciated under the culture conditions employed. Alternatively, IL-15 may exert other direct angiogenic ef-

fects on endothelial cells, including stimulation of cell migration. In addition, the wide distribution of mRNAs for IL-15 and its receptor in various tissues and cells raises the possibility that the angiogenic effect displayed by IL-15 in vivo is the result of the contribution of cells and factors indirectly induced by the cytokine.

Since its original descriptions as a structurally distinct cytokine with biological activities similar to those of IL-2, a number of studies have addressed the apparent redundancy of IL-2 and IL-15 functions. Recently, IL-15 was reported to promote the proliferation of mast cells and to induce fiber hypertrophy in skeletal muscle cells, functions not displayed by IL-2 (16). We report here that IL-15 is an angiogenic factor in vivo, an additional biological function not displayed by IL-2. This newly described property of IL-15 emphasizes its role as an important mediator outside the immune system.

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