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Interleukin-2 is Present in Human Blood Vessels and Released in Biologically Active Form by Heparanase

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Abstract

Interleukin-2 is a multifaceted cytokine with both immunostimulatory and immunosuppressive properties. Our laboratory recently demonstrated that the availability of IL-2 is regulated, in part, by association with perlecan, a heparan sulfate proteoglycan. Given the abundance of perlecan in blood vessels, we asked whether IL-2 is present in vessel walls. Our results indicate that IL-2 is associated with endothelial and smooth muscle cells within the human arterial wall. This IL-2 is released by heparanase, and promotes the proliferation of an IL-2 dependent cell line. Given the presence of IL-2 in human arteries, we asked whether the large vessels of IL-2 deficient mice were normal. The aortas of IL-2 deficient mice exhibited a loss of smooth muscle cells, suggesting that IL-2 may contribute to their survival. In their entirety, these results suggest a here-to-fore unrecognized role of IL-2 in vascular biology, and have significant implications for both the immune and cardiovascular systems.

Introduction

IL-2 is a multifunctional cytokine, known to promote apoptosis, proliferation, and survival of lymphocytes[1]. The importance of IL-2 has recently resurfaced in the context of maintaining T regulatory cells[2]. Given the many functions of this cytokine, means by which the availability of IL-2 is regulated are of critical import. Our laboratory previously reported that IL-2 is retained in lymphoid tissues by association with heparan sulfate glycosaminoglycan (HS GAG)[3]. *In vivo*, heparan sulfate is usually found in proteoglycan form, in which the HS GAG chains are covalently linked to a specific core protein. We recently demonstrated that perlecan is the major proteoglycan that binds IL-2 in murine spleens[4]. Since perlecan is one of the main heparan sulfate proteoglycans (HSPGs) found in blood vessels, we asked whether IL-2 is retained in human vessels.

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Perlecan is a large HSPG typically found pericellularly or in basement membranes[5]. Both endothelial cells and smooth muscle cells, the major cells comprising vascular walls, produce perlecan [6,7]. The heparan sulfate chains of perlecan and other HSPGs play a significant role in several vascular wall functions, including the regulation of coagulation via binding to anti-thrombin III[8], recruitment of leukocytes via binding to chemokines[9], and regulation of smooth muscle cell proliferation via binding to fibroblast growth factor–2 (FGF-2)[10].

Because of their location in subendothelial basement membranes, HSPGs such as perlecan, agrin, and type XVIII collagen contribute to the barrier function of these structures[11]. Leukocytes transmigrating between endothelial cells must degrade the basement membrane prior to entering tissues. This degradation is achieved through enzymes such as various proteases[12] and heparanase[13].

Heparanase is an endo-β-D-glucuronidase capable of cleaving heparin and the HS oligosaccharides of HSPGs. Many cells of the immune system have been reported to express heparanase, including T cells, B cells, neutrophils, macrophages, platelets, dendritic cells, and endothelial cells[14]. Heparanase not only facilitates the extravasation of leukocytes, but it potentially releases chemokines and/or growth factors sequestered in basement membranes by association with HS chains[15].

In light of the critical location of perlecan with respect to leukocyte-vascular interactions, the importance of IL-2 to immune function, and our previous report regarding the association of IL-2 with perlecan, we asked whether IL-2 is retained in the vascular wall of human blood vessels. We found that IL-2 is retained along the endothelium and in the media of the arterial wall. This IL-2 is released by the activity of endogenous heparanase and induces proliferation of an IL-2 dependent cell line. Our results suggest that IL-2 may be strategically located to modulate T lymphocytes and other leukocytes expressing the IL-2 receptor as they extravasate through blood vessel walls. In addition, the identification of IL-2 in human iliac vessels prompted us to examine the aortas of IL-2 deficient mice, which were found to lose smooth muscle cells over time and become aneurysmal. These findings suggest that IL-2 may be important in the survival of vascular smooth muscle cells and may impact the development of aneurysms in humans.

Results

Localization of IL-2 within human arteries

To determine whether IL-2 is present in blood vessel walls, we examined sections of human iliac artery by immunofluorescence. To facilitate visualization of cells within the arterial wall, smooth muscle cells were labeled with an antibody recognizing α-smooth muscle actin. Endothelial cells were localized via Ulex europaeus-1 (UEA-1), and the internal elastic lamina was visualized by autofluorescence. Co-localization studies were performed by confocal microscopy. As seen in Figure 1a, IL-2 was seen along endothelial cell surfaces and within the smooth muscle cell layer. The majority of IL-2 appeared to co-localize with smooth muscle cells (see insert, Figure 1) as very little actin without IL-2 was seen (green areas). Controls, including pre-incubation of the primary antibody with a five-molar excess

of IL-2 (Figure 1b), and secondary antibody alone (not shown) were negative. A similar distribution of IL-2 was observed in murine aortas (not shown).

To ascertain whether the IL-2 seen in arteries was associated with heparan sulfate, the sections were digested with commercially available heparinase I. Like heparanase, heparinase I degrades both heparin and heparan sulfate oligosaccharides[16]. As seen in Figure 1c, digestion of the tissue section with heparinase I prior to staining abrogated detection of IL-2 in the vessel wall. Widespread binding to the tissues of an anti-Δ HS antibody (HS stubs), specific for sugar moieties exposed after digestion of HSPGs with heparinase I, confirmed the enzymatic cleavage of HS chains by heparinase I (Figure 1c). Staining for cytokeratin 19 following digestion with heparinase I did not weaken demonstrably, suggesting that the loss of IL-2 was due to the activity of heparinase I, and not potential contaminating proteases. Finally, IL-2 was easily detectable if heparinase I was preincubated with the heparanase inhibitor PI-88 (Figure 1d)[17], again indicating that the results observed were due to the enzymatic action of heparinase I. Since PI-88 is a highly sulfated HS-like carbohydrate and competitive inhibitor of heparanase, the potential for PI-88 to elute IL-2 from vessel walls was a concern. Tissue sections incubated with PI-88 alone showed robust staining for IL-2, suggesting that PI-88 was not eluting IL-2 from tissues.

In light of our recent work identifying perlecan as the major HSPG that binds IL-2 in murine spleen, we asked whether the distribution of IL-2 in human vessels co-localized with perlecan. As seen in Figure 1a, perlecan was identified along smooth muscle and endothelial cell surfaces. IL-2 co-localized with perlecan in several areas, but there were also some areas in which IL-2 was present without perlecan (red versus yellow areas). These data suggest that IL-2 may bind other HSPGs, in addition to perlecan, present in vessel walls.

Identification of IL-2 in blood vessels by Western blot analysis

As a second means of assessing the presence of IL-2 in blood vessels, we performed a Western blot analysis on tissue homogenates of human arteries. Western blot analysis revealed a doublet at 17-19 kD, consistent with IL-2[18] (Figure 2). Preadsorption of the anti-IL-2 antibody with a 5M excess of IL-2 eliminated recognition of the doublet.

We next asked whether heparinase digestion of arteries would release IL-2. To this end, small pieces of vessel were incubated with heparinase I, and the material liberated (digest) was assessed for IL-2 by Western blot analysis. Digestion with heparinase I liberated a strong 17 kD band, compared to the same band in the homogenate, which was relatively light (Figure 2a). These data suggest that the amount of IL-2 (relative to other proteins present) is fairly low in the homogenate but much higher in the heparinase digest.

In summary, these results suggest that IL-2 is retained in blood vessel walls by association with HS. Furthermore, this IL-2 is liberated by heparinase digestion. Release of IL-2 by heparinase is consistent with previous results from our laboratory demonstrating that IL-2 associates with the GAG chains, rather than the protein core, of HSPGs[3,4].

Source of IL-2

Given the substantial amount of IL-2 found in blood vessels, we began to address the source of IL-2 in these tissues. Since a portion of IL-2 produced during an immune response likely enters the systemic circulation, we first asked whether IL-2 in the bloodstream is retained in blood vessels. To address this question, we labeled commercial IL-2 with an infrared dye (infrared-IL-2). The infrared-IL-2 was then administered intraperitoneally to Balb/c mice. Aortas were harvested, proteins were extracted and separated by SDS-PAGE, then analyzed on an infrared scanner. As seen in Figure 2b, infrared-IL-2 was identified in the aortic tissues. While multiple doses of infrared-IL-2 were given in this particular experiment, a single dose of 1.5 μg of infrared-IL-2 also resulted in detectable infrared-IL-2 within vessels (Figure 2c). To determine if this IL-2 was retained by heparan sulfate, we digested the tissues with heparinase I. Digestion of the vessel tissue with heparinase I released the infrared-IL-2 (Figure 2c), suggesting that IL-2 gains access to vessels via the bloodstream, and is retained there by heparan sulfate.

We next asked whether cells within the blood vessel wall produce IL-2. To this end, we first asked whether IL-2 message is expressed in blood vessel tissue. As seen in Figure 2d, IL-2 message was detected in murine aortas. This result suggests that at least some "intravascular" IL-2 is produced within the vessel itself. Since T cells are the main producers of IL-2, we asked whether T cells expressing IL-2 were present within aortic tissues. To address this question, we used transgenic mice that express green fluorescent protein (GFP) upon activation of the IL-2 promoter[19]. To promote IL-2 expression, the transgenic mice were first stimulated with anti-CD3. Aortas were harvested 24h later and analyzed by confocal microscopy. Murine aortas did contain scattered GFP+ cells, indicating that T cells producing IL-2 are present in large vessels such as the aorta (Figure 2e).

In total, these results suggest that IL-2 in blood vessels comes both from systemic sources and local production, and that T cells provide at least one source of vascular IL-2. Whether cells comprising the vessel wall contribute to intravascular IL-2 is an interesting question that will require further study.

IL-2 in blood vessels promotes proliferation upon release by endogenous heparanase

In light of the above results, we next asked whether the IL-2 released from human iliac arteries by heparinase is biologically active. To this end, we developed a bioassay using the IL-2 dependent T cell line CTLL-2, in which responses to IL-2 would be dependent upon its release from arterial tissue. The first step in developing this assay was to determine whether CTLL-2 cells express the heparanase enzyme. Western blot analysis was performed on both cell lysates and media from CTLL-2 cultures. As seen in Figure 3a, both the pro- (inactive, 65 kD) and active (51 kD) forms of the heparanase enzyme were present in the CTLL-2 cell lysates and culture supernatants. This result is consistent with reports that heparanase is processed intracellularly[20]. To ensure that the heparanase expressed by CTLL-2 cells was active, a heparanase activity assay was performed using media from CTLL-2 cultures. As seen in Figure 3b, supernatants from CTLL-2 cultures exhibited heparanase activity that was inhibited by PI-88 (a heparanase inhibitor[17]), as well as two distinct anti-heparanase

antibodies[21]. In a tandem assay, an isotype control for the heparanase antibody was negative (CTLL-2 conditioned media 6132 ± 190 cpm, CTLL-2 conditioned media plus rabbit IgG 5565 ± 468 cpm). In a second tandem assay, the commercially available anti-heparanase antibody (anti-hep#1) was demonstrated to have blocking activity, as heparanase from a heparanase-producing cell line elicited 2600 ± 830 cpm, and heparanase plus anti-heparanase antibodies generated only 91 ± 21 cpm.

Having confirmed that CTLL-2 cells produce active heparanase, we proceeded to ask whether this heparanase would release sufficient quantities of biologically active IL-2 to induce proliferation. To this end, CTLL-2 cells were cultured with a small piece of human iliac artery placed in a transwell insert. The transwell membrane ensured that the cells were physically separated from the tissues and could, therefore, respond only to soluble IL-2 liberated by the actions of heparanase and/or other enzymes released by the CTLL-2 cells. As seen in Figure 3c, CTLL-2 cells proliferated when co-cultured with, but separated from, pieces of blood vessel. Inhibition of heparanase by an anti-heparanase antibodies or PI-88 abrogated the proliferative response. A blocking anti-IL-2 antibody also inhibited proliferation, indicating that the proliferation observed was due to IL-2. Isotype controls for both the anti-heparanase and anti-IL-2 antibodies had no impact on proliferation (not shown). In addition, PI-88 did not influence the proliferation of CTLL-2 cells cultured with IL-2 (3.2 ng/ml IL-2 146,285 ± 1088 cpm, 3.2 ng/ml IL-2 + 3 ng/ml PI-88 151,320 ± 7497 cpm).

Given the widespread presence of IL-2 along endothelium and in the parenchyma of lymphoid tissues *in vivo*[3,4,22] (Figure 1), lymphoid cells may be in frequent contact with IL-2. Therefore, we asked whether proliferation of CTLL-2 cells in direct contact with blood vessel tissue (i.e., in the absence of the transwell) was dependent on release of IL-2 by heparanase. As seen in Figure 3d, proliferation of CTLL-2 cells in direct contact with vessel tissue was inhibited by anti-heparanase antibodies, suggesting that IL-2 must be released by heparanase to promote proliferation. Although our findings indicate that heparanase releases IL-2, they do not preclude the possibility that other enzymes, such as matrix metalloproteinases, release IL-2 as well. The extent to which inhibition of heparanase decreased proliferation of the CTLL-2 cells, however, suggests that heparanase is the main enzyme responsible for releasing IL-2 from HSPGs.

IL-2 knock out mice exhibit aneurysmal aortas

Although IL-2 is typically thought of as cytokine that affects lymphocytes, in light of the significant amount of IL-2 found in human iliac arteries, we began to determine whether IL-2 might influence the cells comprising the vascular wall. To this end, aortas of IL-2 KO mice were examined histologically and compared to age-matched, wildtype (WT) controls. The IL-2 KO mice used were DO11.10/IL-2 KO and DO11.10/RAG-1 KO/IL-2 KO mice. The latter mice express only T cells bearing the transgenic T cell receptor, DO11.10, and therefore lack autoimmunity. Using these mice alleviates the potential influence of autoimmune-mediated inflammatory changes on vessels.

At relatively young ages (6 – 10 weeks) the aortas of IL-2 KO mice were similar to WT mice (not shown). By approximately 20 weeks of age and older, the aortas of the IL-2 KO

mice exhibited a loss of smooth muscle cells (Figure 4a). In addition, the morphology of the smooth muscle cells was different. In the IL-2 KO mice, the cell bodies of the smooth muscle cells were smaller and more compact, whereas in WT mice the smooth muscle cells were larger with more extended processes (see 40× and inserts, Figure 4). In severe cases (Figure 4a, DKO), the aortas became extremely thin with very few smooth muscle cells remaining. Morphometric analysis of Van Gieson-stained WT and IL-2 KO aortas revealed that the internal elastic laminae within the IL-2 KO vessels were closer together than the WT, likely because of the smaller smooth muscle cell bodies in the KO mice (Figure 4b). Elastin fibers in the adventitia were abnormal as well. In the WT mice, these fibers appeared as thin, pale strands. In the KO mice, these fibers had a thick, beaded appearance and were much darker in color.

The endothelial cell layer also appeared abnormal (Figure 4a). Whereas in the WT mice the endothelial layer was smooth and without breaks, the IL-2 deficient mice exhibited irregular endothelial cells layers with gaps in which no endothelial cells were present. Aortas from IL-2Rβ KO mice also exhibited similar changes (not shown). While this finding is likely an artifact of tissue preservation, it does suggest that endothelial cells or the endothelial cell – basement membrane associations of IL-2 deficient mice are fragile, as compared to WT mice whose endothelial layer remained intact following vessel harvest and histologic processing.

These findings suggest that IL-2, either directly or indirectly, influences vascular smooth muscle cells. Given the abnormalities seen throughout the vessel wall, IL-2 may affect other cell types as well. Whether this represents an impact on factors such as survival, proliferation, adhesivity, apoptosis, or response to mechanical stress, remains to be determined.

Murine vascular smooth muscle cells express IL-2 receptor β

In order for IL-2 to exhibit a direct effect on cells comprising the vascular wall, these cells must express the IL-2 receptor (IL-2R). While reports in the literature indicate that both endothelial cells[23] and fibroblasts[24] express IL-2Rα and β, there are no convincing studies regarding the expression of IL-2R on vascular smooth muscle cells. Therefore, we cultured smooth muscle cells from murine aortas, and tested the cell lysates for expression of IL-2Rβ by Western blot analysis. As seen in Figure 5a, murine vascular smooth cells expressed IL-2Rβ. These results were not due to contamination of the cultures with endothelial cells or fibroblasts, as expression of UEA-1 (endothelial cell marker) and S100A4 (fibroblast marker) were negative. Human smooth muscle cells in aortic tissue (Figure 5c) and cultures (Figure 5d) were also found to express IL-2Rβ protein and message, respectively. These results suggest that IL-2 has a direct effect on smooth muscle cells via the IL-2 receptor. Whether this effect is via the tripartite receptor or IL-2 receptor βγ is a question for future studies.

Discussion

Lymphocytes must pass through blood vessels to enter inflamed tissues. In order to cross the subendothelial basement membrane, extravasating lymphocytes release proteases and

heparanase[14]. Our finding that IL-2 is present on the surface of endothelial cells and smooth muscle cells lining blood vessels, and that biologically active IL-2 is released by heparanase, suggests that IL-2 plays an unanticipated early role in modulating immune responses in inflamed tissues. Although for logistic reasons our studies utilized large vessels, IL-2 is retained along the endothelium lining entry points into the spleen, including both the marginal zone and central arteriole (Miller et al., 2008).

In their review on endothelial injury, Tesfamariam and DeFelice state, "The endothelium comprises the largest homogeneous surface of the body for actively mediating immune defense"[25]. The localization of IL-2 along vascular endothelium, therefore, suggests that IL-2 may be a critical "first responder" in immune defense mechanisms. IL-2 is typically not thought of in these terms since, depending on the antigen and route, significant IL-2 production by T cells is not observed until anywhere from 15 – 45 hours after antigen exposure[26,27].

Although the functions of IL-2 are usually associated with lymphocytes and NK cells, IL-2 is known to influence other cells important to normal immune responses, including neutrophils[28], dendritic cells[29], macrophages[30], and platelets[31]. Interestingly, IL-2 has been shown to increase the adhesion of neutrophils, NK cells, and lymphocytes to endothelial cells[28,32,33]. Administration of IL-2 *in vivo* has been shown to induce microvascular platelet thrombi via increased endothelial-platelet interactions[31]. Therefore, IL-2 localized to blood vessels may influence cells responsible for both innate and acquired immune responses as they enter inflamed tissues. In addition, local release of IL-2 in vessels may potentially contribute to the development of microthrombi in pathologies such as hemolytic uremic syndrome and antibody-mediated rejection, which are characterized by endothelial cell damage[25,34].

Our studies suggest that the IL-2 retained in blood vessels must first be released by heparanase to stimulate proliferation, since anti-heparanase antibodies inhibited the proliferation of CTLL-2 cells cultured directly with vessel tissue (see Figure 3d). As mentioned in the introduction, heparanase is produced by most immune cells, including lymphocytes, platelets, neutrophils, endothelial cells, and macrophages[14]. Heparanase is stored in active form in lysosomes, then released by degranulation in response to chemoattactants or inflammatory stimuli[20].

Despite its production by multiple cell types, heparanase is tightly controlled by several mechanisms, including (1) regulated secretion, (2) uptake of secreted heparanase by low-density lipoprotein-related receptor protein[14] and syndecan-4[35] and (3) expression, in that only one functional isoform of mammalian heparanase has been identified to date[14]. Therefore, even though IL-2 is apparently widely distributed along both major vessels and smaller, intraparenchymal vessels[3,4,22] its release by heparanase would be tightly controlled by the above mechanisms. Conversely, dysregulation of heparanase contributes to the metastatic capabilities of many cancers. For this reason, heparanase is being investigated as target of chemotherapeutic agents in both animal models and human clinical trials[36]. Whether release of IL-2 by heparanase contributes to the metastatic potential of certain cancers, especially leukemias and lymphomas, remains to be determined.

Heparan sulfate binds several types of biologically active proteins, including specific cytokines, chemokines, and growth factors[15]. Although it is logical to postulate that these various mediators are released by heparanase, only a few examples in the literature validate this assumption. The primary example of a heparanase-mediated release of a growth factor is that of bFGF[37]. Basic FGF is sequestered and protected in the subendothelial matrix by HSPGs. Heparanase, released by activated platelets (and likely other cells) at the site of tissue injury, releases bFGF, which in turn stimulates smooth muscle cell migration and proliferation[37]. Despite a paucity of information regarding release of growth factors by heparanase, several examples of protease-mediated release of growth factors exist. Some of these include: the release of transforming growth factor β1 by chymases and elastases[38], the release of colony-stimulating factor-1 by proteases[39], and the release of insulin-like growth factor II by matrix metalloproteinase 7[40]. While our data demonstrate that HSPG-bound IL-2 is released by heparanase, other enzymes, including various proteases, may release IL-2 via cleaving the protein core of the proteoglycan.

Since T cells are the primary producers of IL-2 in the immune system, these cells are likely the main source of the IL-2 observed in human arteries. Although the presence of T cells in atherosclerotic vessels is well documented, Galkina et al. showed that a substantial number of T cells, B cells, dendritic cells, and macrophages are present in normal vessels[41]. Our studies indicate that IL-2-producing T cells are present within arterial walls (Figure 2d). It is possible, however, that some IL-2 comes from the cells comprising the blood vessel wall, as both endothelial cells[42] and smooth muscle cells[43] have been reported to express IL-2 under certain conditions. Our studies with infrared-IL-2 suggest that circulating IL-2 is readily taken up in vessels, indicating that some portion of IL-2 in vessels comes from IL-2 that enters the micro- or macro-circulation. This uptake of IL-2, evident in vessels and other tissues (Figure 2 and unpublished data), likely contributes to the short half-life of IL-2 in the bloodstream[44].

The presence of IL-2 in both large and small vessels raises the question as to whether IL-2 has a direct influence on endothelial cells and smooth muscle cells. Previous studies have shown that IL-2 has a direct effect on the permeability of endothelial cells[45]. A recent study by Bae et al. indicated that IL-2 promotes angiogenesis, and demonstrated that endothelial cells express IL-2Rα and β[23]. IL-2 has also been shown to stimulate GAG synthesis in vascular smooth muscle cells and enhance their responsiveness to angiotensin II[46]. Our histologic examination of aortas from IL-2 deficient mice suggests that IL-2 plays a role in maintaining vascular smooth muscle cells. Since the loss of smooth muscle cells was also seen in DO11.10/RAG-1 KO/IL-2 mice, which do not exhibit autoimmunity, this pathology is not the result of nonspecific, autoimmune-mediated inflammation. Our finding that vascular smooth muscle cells express IL-2Rβ suggests that IL-2 has a direct impact on these cells; however, the mechanism(s) by which IL-2 mediates this impact remains to be determined. Clearly, an influence of IL-2 on smooth muscle cells suggests exciting possibilities and warrants further study.

In summary, the identification of HS-bound IL-2 in human blood vessels is unprecedented and has several implications for the role of IL-2 in initiating immune responses. In addition, this finding leads to the consideration of alternative functions for IL-2 outside the immune

system. It is both daunting and refreshing to discover that new biology is yet to be learned regarding one of immunology's oldest cytokines.

Materials and methods

Materials and tissues

Small sections of human iliac artery and spleen were obtained from deceased donor organs. Consent for research was provided by next of kin. Pieces of murine aorta were obtained from DO11.10, DO11.10/IL-2 knock out (KO), and DO11.10/RAG-1 KO/IL-2 KO mice present within our colony. BALB/c DO11.10 mice expressing a transgenic IL-2 promoter/green fluorescent protein (GFP) reporter were the kind gift of Dr. Casey Weaver[19]. Mice were housed in specific pathogen free facilities and all experiments were in accordance with protocols approved by the University of Nebraska Institutional Animal Care and Use Committee. Chemical reagents, heparin-sepharose columns, and heparinase I were obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise indicated. The following antibodies were used. A hamster anti-mouse CD3 (clone 145-2C11), and a biotinylated anti-CD31 were obtained from BD Pharmingen (San Diego, CA). A chicken polyclonal antibody recognizing human IL-2, a rabbit polyclonal antibody recognizing a smooth muscle cell actin, a rabbit polyclonal antibody against cytokeratin 19, a rabbit polyclonal recognizing S100A4, and a biotinylated goat polyclonal anti-GFP antibody were from Abcam (Cambridge, MA). A mouse monoclonal antibody recognizing heparan sulfate Δ (clone 3G10) was obtained from U.S. Biological (Swampscott, MA). A rat anti-mouse IL-2Rβ was from eBioscience (San Diego, CA), and a goal anti-human IL-2Rβ polyclonal was from R & D Systems (Minneapolis, MN). A polyclonal rabbit anti-perlecan antibody, which recognizes perlecan isolated from Engelbreth Holm Swarm tumor matrix, was the kind gift of Dr. Gerardo Castillo (paid consultant, ProteoTech, Seattle, WA).[4] Two distinct polyclonal anti-heparanase antibodies were used. One rabbit polyclonal was the kind gift of Dr. Jeffrey Platt (University of Michigan, AnnArbor, MI)[21]. A second rabbit polyclonal, and a purified heparanase standard, were obtained from Insight Biopharmaceuticals (Rehovot, Israel). PI-88, a competitive inhibitor of heparanase[17] was generously provided by Progen Industries Ltd. (Darra, Queensland, Australia). UEA-1-FITC was purchased from Sigma. CTLL-2 cells and human aortic smooth muscle cells were obtained from American Tissue Type Collection (Manassas, VA). CAG myeloma cells, transfected with heparanase, were the kind gift of Ralph Sanderson (University of Alabama, Tuscaloosa, AL).

Vascular smooth muscle cell cultures

Murine aortas were harvested and placed in glass petri dishes containing dissection media (HEPES-buffered saline). After removal of extraneous tissue, the aortas were incubated for 30 minutes at 37°C in an enzyme solution composed of HEPES buffer containing 2mg/ml BSA, 1 mg/ml collagenase CLS, 0.375 mg/ml soybean trypsin inhibitor, and 0.125 mg/ml elastase type III (Worthington Biochemical Corporation, Lakewood, NJ). The adventitia was then removed, and the denuded aortas were minced into small pieces, and placed in fresh enzyme solution for 1.5-2 hours with slight agitation. After dissociation, cells were pelleted by centrifugation, washed 3× with DMEM (high glucose) media containing 10% FBS, and plated in T-25 flasks that are maintained at 37°C and 5% CO_2. Cultured cells were used

after 3 passages. For Western blot analysis, cells were scraped into ice-cold 8M urea and lysates were analyzed as described below.

Transwell assay

CTLL-2 cells, 1×10^5/ml, were cultured in RPMI 1640 media with 1% FBS. Transwell inserts (Nunc, Rochester, NY), each containing one 2 × 2 mm (8 μg) piece of iliac artery, were used to provide a physical separation between the cells and the tissue. The CTLL-2 cells were first plated in the wells and the tissue was placed in the transwell insert. Anti-heparanase antibodies, PI-88, or neutralizing anti-IL-2 antibodies were added to the culture media as indicated in the figure legend (Figure 4c). Proliferative responses were assessed as previously described (Miller et al., 2008). Briefly, the CTLL-2 cells were cultured for 96 h at 37°C, and [^{3}H] thymidine, 1 μCi/μl, was added during the last 8 hours of the culture period. Wells were then harvested using a cell harvester (Skatron Instruments, Lier, Norway), and the samples counted in a scintillation counter.

Heparanase activity assay

Heparanase activity was tested in culture supernatants from CTLL-2 cells. PI-88 or anti-heparanase antibodies were pre-incubated with select samples for 1 h at 24° C as indicated in the figure legend. [^{3}H]heparin conjugated to agarose beads (10,000 cpm/reaction; generously provided by Dr. Jeffrey Platt) was resuspended in the sample to be tested, incubated at 37°C for 2 hours, then centrifuged. [^{3}H]heparin cleaved by heparanase was then detected in the supernatant by scintillation counting. CAG myeloma cells, transfected with heparanase, were used as a source of heparanase as a positive control.[47]

Dye-labeled IL-2

Murine IL-2 (R & D Systems) was labeled with an activated infrared dye (800CW, LI-COR Biosciences, Lincoln, NE) per the manufacturer's instructions. Briefly, activated infra-red dye was added to the IL-2 at a molar ratio of 1:1. Following a 2 hour incubation at 24°C, unconjugated dye was removed from the preparation using a mini de-salting column. The concentration of the dye-conjugated IL-2 was then determined by Bradford assay (Sigma). To confirm that the IL-2 was covalently conjugated to the 800CW dye, 0.5 μg of dye-IL-2 was separated by SDS-PAGE, transferred to nitrocellulose, and analyzed on an Odyssey infra-red scanner.

Morphometry on aortic tissue sections

The space between aortic wall laminae was measured using Adobe Photoshop 7, and was expressed as a percentage of the total area of the laminae plus the interlaminar spaces.

Statistical analysis

The majority of the data is expressed as a mean ± S.D. Please see Supplemental Methods for statistical analysis of morphometric data.

Additional methodology

Please see Supplemental Methods for methods pertaining to Western blot analysis, histology, and reverse transcriptase PCR.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Lan RY, Selmi C, Gershwin ME. The regulatory, inflammatory, and T cell programming roles of interleukin-2 (IL-2). J. Autoimmun. 2008; 31:7–12. [PubMed: 18442895]
2. Setoguchi R, Hori S, Takahashi T, Sakaguchi S. Homeostatic maintenance of natural Foxp3(+) CD25(+) CD4(+) regulatory T cells by interleukin (IL)-2 and induction of autoimmune disease by IL-2 neutralization. J. Exp. Med. 2005; 201:723–35. [PubMed: 15753206]
3. Wrenshall LE, Platt JL. Regulation of T cell homeostasis by heparan sulfate-bound IL-2. J. Immunol. 1999; 163:3793–800. [PubMed: 10490977]
4. Miller JD, Stevens ET, Smith DR, Wight TN, Wrenshall LE. Perlecan: a major IL-2-binding proteoglycan in murine spleen. Immunol. Cell Biol. 2008; 86:192–9. [PubMed: 18040286]
5. Melrose J, Roughley P, Knox S, Smith S, Lord M, Whitelock J. The structure, location, and function of perlecan, a prominent pericellular proteoglycan of fetal, postnatal, and mature hyaline cartilages. J. Biol. Chem. 2006; 281:36905–14. [PubMed: 16984910]
6. Kinsella MG, Tran PK, Weiser-Evans MC, Reidy M, Majack RA, Wight TN. Changes in perlecan expression during vascular injury: role in the inhibition of smooth muscle cell proliferation in the late lesion. Arterioscler. Thromb. Vasc. Biol. 2003; 23:608–14. [PubMed: 12615671]
7. Whitelock JM, Graham LD, Melrose J, Murdoch AD, Iozzo RV, Underwood PA. Human perlecan immunopurified from different endothelial cell sources has different adhesive properties for vascular cells. Matrix Biol. 1999; 18:163–78. [PubMed: 10372557]
8. Xu Y, Slayter HS. Immunocytochemical localization of endogenous anti-thrombin III in the vasculature of rat tissues reveals locations of anticoagulantly active heparan sulfate proteoglycans. J. Histochem. Cytochem. 1994; 42:1365–76. [PubMed: 7930519]
9. Laguri C, Arenzana-Seisdedos F, Lortat-Jacob H. Relationships between glycosaminoglycan and receptor binding sites in chemokines-the CXCL12 example. Carbohydr. Res. 2008; 343:2018–23. [PubMed: 18334249]
10. Kinsella MG, Irvin C, Reidy MA, Wight TN. Removal of heparan sulfate by heparinase treatment inhibits FGF-2-dependent smooth muscle cell proliferation in injured rat carotid arteries. Atherosclerosis. 2004; 175:51–7. [PubMed: 15186946]
11. Iozzo RV. Basement membrane proteoglycans: from cellar to ceiling. Nat. Rev. Mol. Cell Biol. 2005; 6:646–56. [PubMed: 16064139]
12. Wu B, Crampton SP, Hughes CC. Wnt signaling induces matrix metalloproteinase expression and regulates T cell transmigration. Immunity. 2007; 26:227–39. [PubMed: 17306568]
13. Parish CR. The role of heparan sulphate in inflammation. Nat. Rev. Immunol. 2006; 6:633–43. [PubMed: 16917509]
14. Vreys V, David G. Mammalian heparanase: what is the message? J. Cell. Mol. Med. 2007; 11:427–52. [PubMed: 17635638]

15. Taipale J, Keski-Oja K. Growth factors in the extracellular matrix. FASEB J. 1997; 11:51–9. [PubMed: 9034166]
16. Freeman C, Parish CR. Human platelet heparanase: purification, characterization and catalytic activity. Biochem. J. 1998; 330:1341–50. [PubMed: 9494105]
17. Joyce JA, Freeman C, Meyer-Morse N, Parish CR, Hanahan D. A functional heparan sulfate mimetic implicates both heparanase and heparan sulfate in tumor angiogenesis and invasion in a mouse model of multistage cancer. Oncogene. 2005; 24:4037–51. [PubMed: 15806157]
18. Podolin PL, Wilusz MB, Cubbon RM, Pajvani U, Lord CJ, Todd JA, Peterson LB, Wicker LS, Lyons PA. Differential glycosylation of interleukin 2, the molecular basis for the NOD Idd3 type 1 diabetes gene? Cytokine. 2000; 12:477–82. [PubMed: 10857762]
19. Saparov A, Wagner FH, Zheng R, Oliver JR, Maeda H, Hockett RD, Weaver CT. Interleukin-2 expression by a subpopulation of primary T cells is linked to enhanced memory/effector function. Immunity. 1999; 11:217–80.
20. Shafat I, Vlodavsky I, Ilan N. Characterization of mechanisms involved in secretion of active heparanase. J. Biol. Chem. 2006; 281:23804–11. [PubMed: 16790442]
21. Dempsey LA, Plummer TB, Coombes SL, Platt JL. Heparanase expression in invasive trophoblasts and acute vascular damage. Glycobiology. 2000; 10:467–75. [PubMed: 10764835]
22. Wrenshall LE, Platt JL, Stevens ET, Wight TN, Miller JD. Propagation and control of T cell responses by heparan sulfate-bound IL-2. J. Immunol. 2003; 170:5470–4. [PubMed: 12759423]
23. Bae J, Park D, Lee YS, Young D. Interleukin-2 promotes angiogenesis by activation of Akt and increase of ROS. J. Microbiol. Biotechnol. 2008; 18:377–82. [PubMed: 18309287]
24. Plaisance S, Alileche A, Han D, Rubinstein E, Sahraoui Y, Jasmin C, Azzarone B. How interleukin-2 can affect human fibroblasts behavior. Pathol. Res. Pract. 1994; 190:942–9. [PubMed: 7899144]
25. Tesfamariam B, DeFelice AF. Endothelial injury in the initiation and progression of vascular disorders. Vascul. Pharmacol. 2007; 46:229–37. [PubMed: 17218160]
26. Itano AA, McSorley SJ, Reinhardt RL, Ehst BD, Ingulli E, Rudensky AY, Jenkins MK. Distinct dendritic cell populations sequentially present antigen to CD4 T cells and stimulate different aspects of cell-mediated immunity. Immunity. 2003; 19:47–57. [PubMed: 12871638]
27. Li XC, Demirci G, Ferrari-Lacraz S, Groves C, Coyle A, Malek TR, Strom TB. IL-15 and IL-2: a matter of life and death for T cells in vivo. Nat. Med. 2001; 7:114–8. [PubMed: 11135625]
28. Li J, Gyorffy S, Lee S, Kwok CS. Effect of recombinant human interleukin 2 on neutrophil adherence to endothelial cells in vitro. Inflammation. 1996; 20:361–72. [PubMed: 8872500]
29. Kradin RL, Xia W, Pike M, Byers HR, Pinto C. Interleukin-2 promotes the motility of dendritic cells and their accumulation in lung and skin. Pathobiology. 1996; 64:180–6. [PubMed: 9031326]
30. Bosco MC, Espinoza-Delgado I, Rowe TK, Malabarba MG, Longo DL, Varesio L. Functional role for the myeloid differentiation antigen CD14 in the activation of human monocytes by IL-2. J. Immunol. 1997; 159:2922–31. [PubMed: 9300716]
31. Lentsch AB, Edwards MJ, Miller FN. Interleukin-2 induces increased platelet-endothelium interactions: a potential mechanism of toxicity. J. Lab. Clin. Med. 1996; 128:75–82. [PubMed: 8759938]
32. Pankonin G, Reipert B, Ager A. Interactions between interleukin-2-activated lymphocytes and vascular endothelium: binding to and migration across specialized and non-specialized endothelia. Immunology. 1992; 77:51–60. [PubMed: 1398764]
33. Aronson FR, Libby P, Brandon EP, Janicka MW, Mier JW. IL-2 rapidly induces natural killer cell adhesion to human endothelial cells. A potential mechanism for endothelial injury. J. Immunol. 1988; 141:158–63. [PubMed: 3259966]
34. Jumani A, Hala K, Tahir S, Al-Ghamdi G, Al-Flaiw A, Hejaili F, Qureshi J, Raza H, Ghalib M, Khader AA. Causes of acute thrombotic microangiopathy in patients receiving kidney transplantation. Exp. Clin. Transplant. 2004; 2:268–72. [PubMed: 15859940]
35. Gingis-Velitski S, Zetser A, Kaplan V, Ben-Zaken O, Cohen E, Levy-Adam F, Bashenko Y, Flugelman MY, Vlodavsky I, Ilan N. Heparanase uptake is mediated by cell membrane heparan sulfate proteoglycans. J. Biol. Chem. 2004; 279:44084–92. [PubMed: 15292202]

36. Li JP. Heparin, heparan sulfate and heparanase in cancer: remedy for metastasis? Anticancer Agents Med. Chem. 2008; 8:64–76. [PubMed: 18220506]

37. Myler HA, West JL. Heparanase and platelet factor-4 induce smooth muscle cell proliferation and migration via bFGF release from the ECM. J. Biochem. 2002; 131:913–22. [PubMed: 12038989]

38. Taipale J, Lohi J, Saarinen J, Kovanen PT, Keski-Oja J. Human mast cell chymase and leukocyte elastase release latent transforming growth factor-beta 1 from the extracellular matrix of cultured human epithelial and endothelial cells. J. Biol. Chem. 1995; 270:4689–96. [PubMed: 7876240]

39. Partenheimer A, Schwarz K, Wrocklage C, Kölsch E, Kresse H. Proteoglycan form of colony-stimulating factor-1 (proteoglycan-100). Stimulation of activity by glycosaminoglycan removal and proteolytic processing. J. Immunol. 1995; 155:5557–65. [PubMed: 7499838]

40. Miyamoto S, Nakamura M, Yano K, Ishii G, Hasebe T, Endoh Y, Sangai T, Maeda H, Shi-Chuang Z, Chiba T, Ochiai A. Matrix metalloproteinase-7 triggers the matricrine action of insulin-like growth factor-II via proteinase activity on insulin-like growth factor binding protein 2 in the extracellular matrix. Cancer Sci. 2007; 98:685–91. [PubMed: 17359288]

41. Galkina E, Kadl A, Sanders J, Varughese D, Sarembock IJ, Ley K. Lymphocyte recruitment into the aortic wall before and during development of atherosclerosis is partially L-selectin dependent. J. Exp. Med. 2006; 203:1273–82. [PubMed: 16682495]

42. Duechting A, Tschöpe C, Kaiser H, Lamkemeyer T, Tanaka N, Aberle S, Lang F, Torresi J, Kandolf R, Bock CT. Human parvovirus B19 NS1 protein modulates inflammatory signaling by activation of STAT3/PIAS3 in human endothelial cells. J. Virol. 2008; 82:7942–52. [PubMed: 18550668]

43. Takahashi Y, Fujioka Y, Takahashi T, Domoto K, Takahashi A, Taniguchi T, Ishikawa Y, Yokoyama M. Chylomicron remnants regulate early growth response factor-1 in vascular smooth muscle cells. Life Sci. 2005; 77:670–82. [PubMed: 15921998]

44. Lotze MT, Matory YL, Ettinghausen SE, Rayner AA, Sharrow SO, Seipp CA, Custer MC, Rosenberg SA. In vivo administration of purified human interleukin 2. II. Half life, immunologic effects, and expansion of peripheral lymphoid cells in vivo with recombinant IL 2. J Immunol. 1985; 135:2865–75. [PubMed: 2993418]

45. Downie GH, Ryan US, Hayes BA, Friedman M. Interleukin-2 directly increases albumin permeability of bovine and human vascular endothelium in vitro. Am. J. Respir. Cell Mol. Biol. 1992; 7:58–65. [PubMed: 1627337]

46. Nabata T, Fukuo K, Morimoto S, Kitano S, Momose N, Hirotani A, Nakahashi T, Nishibe A, Hata S, Niinobu T, Suhara T, Shimizu M, Ohkuma H, Sakurai S, Nishimaki H, Ogihara T. Interleukin-2 modulates the responsiveness to angiotensin II in cultured vascular smooth muscle cells. Atherosclerosis. 1997; 133:23–30. [PubMed: 9258403]

47. Yang Y, Macleod V, Miao HQ, Theus A, Zhan F, Shaughnessy JD Jr, Sawyer J, Li JP, Zcharia E, Vlodavsky I, Sanderson RD. Heparanase enhances syndecan-1 shedding: a novel mechanism for stimulation of tumor growth and metastasis. J. Biol. Chem. 2007; 282:13326–33. [PubMed: 17347152]

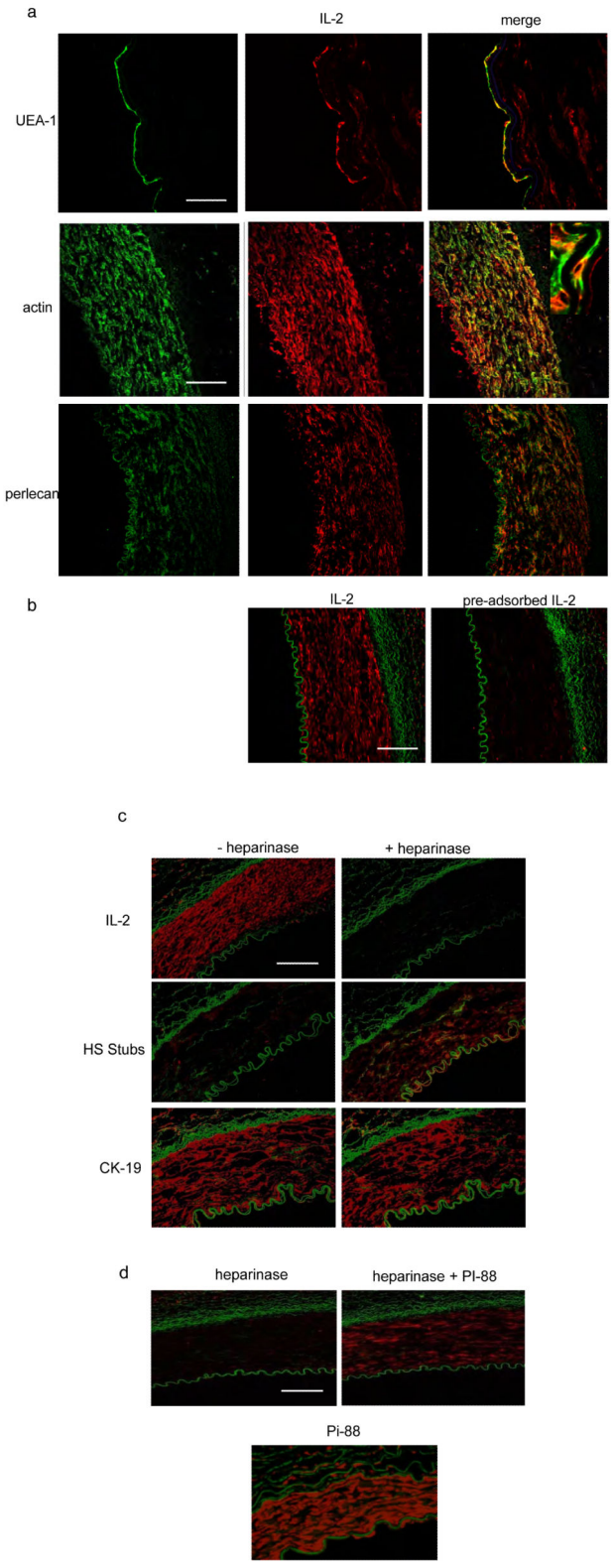

Figure 1. IL-2 is present in human blood vessel walls, and its detection is eliminated by heparinase digestion

(a) Sections of human iliac artery were labeled with antibodies recognizing IL-2 (TRITC) and, in FITC, either antibodies recognizing actin (smooth muscle cells), the lectin UEA (endothelial cells), or anti-perlecan antibodies, then assessed by confocal microscopy. Areas of co-localization are evident in yellow. Secondary controls were negative (not shown). Confocal images were generated using a Zeiss 510 Meta confocal microscope utilizing AIM software. (b) Sections of human iliac artery were stained with an anti-human IL-2 antibody (TRITC) in the presence ("pre-adsorbed IL-2") or absence ("IL-2") of pre-incubation with a 5M excess of human IL-2. In Figures b-d, the green color originates from autofluorescence at 488 nm of elastin fibers within the internal and external elastic laminae. (c) Sections of human iliac artery were incubated for 2 h at 37°C with heparinase I, or buffer alone, and then stained, in TRITC, with antibodies recognizing IL-2, HS stubs (sugar moieties exposed after digestion of HSPGs with heparinase), or cytokeratin 19 (CK-19). All stains were performed in TRITC. (d) Heparinase I was pre-incubated for 1h with or without 150 ng PI-88, then incubated for 2 h at 37°C as above. Control sections received PI-88 alone ("PI-88") or buffer alone (see c, "- heparinase") under identical conditions. Sections were then stained for IL-2 (TRITC). Tissue sections were mounted in Permount and visualized using a Nikon Eclipse 80i microscope. Images were captured using a Cool Snap ES camera and processed using Metamorph image acquisition software. Results shown are representative of 20 (a), 5 (b), 8 (c), and 3 (d) separate specimens. Scale bars, 25 μm (a), 50 μm (b – d).

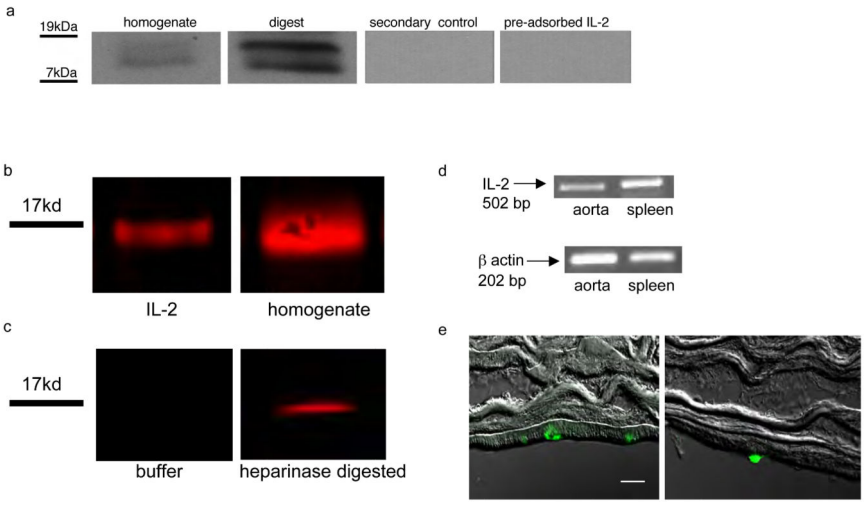

Figure 2. IL-2 in vessels is acquired via the bloodstream and local production, retained by heparan sulfate, and released by heparinase digestion

(a) Six 2 × 2 mm pieces of human iliac artery were incubated at 37°C with 2 U heparinase I for 18 h. Soluble and insoluble materials were separated by centrifugation, and insoluble tissue was homogenized in 4M urea. The resulting homogenate (100 μg) and soluble material (digest; 50 μg) were then analyzed by Western blot for the presence of IL-2. A higher amount of homogenate versus digestate was analyzed to facilitate visualization of the IL-2 band. The results shown are representative of 20 experiments. Secondary antibodies alone, and preabsorption of the primary antibody with a 5M excess of IL-2, were negative. (b) Balb/c mice were given 1 μg infrared-IL-2 daily by intraperitoneal injection for 8 doses, and sacrificed 3 days following the last dose. Tissues were processed as described for Western blot analysis, and analyzed on an Odyssey infra-red scanner. Results shown are representative of 10 experiments. (c) Balb/c mice were given a single dose of 1.5 μg infrared-IL-2, and sacrificed 2.5 days later. Five 1 mm long pieces of murine aorta were incubated at 37°C with 2 U heparinase I or heparinase buffer for 18 h, and the released material was separated by SDS-PAGE and analyzed on an Odyssey infra-red scanner. Results shown are representative of 2 experiments. IL-2 was labeled with infrared dye as described in Materials and Methods. (d) Murine aortas were harvested, thoroughly flushed, and the adventitia was removed. Total RNA was extracted and assayed for the presence of IL-2 mRNA by reverse transcriptase PCR. A small piece of murine spleen was similarly processed for comparison. Expression of β actin was used as a loading control. The results shown are representative of 3 experiments. (e) Transgenic Balb/c mice expressing an IL-2 promoter/GFP reporter were given 10 μg anti-CD3 by intraperitoneal injection. Tissues were harvested 16 h later and processed for staining with anti-GFP antibodies to enhance the GFP signal. Results shown are representative of 2 separate experiments. Scale bar, 10 μm.

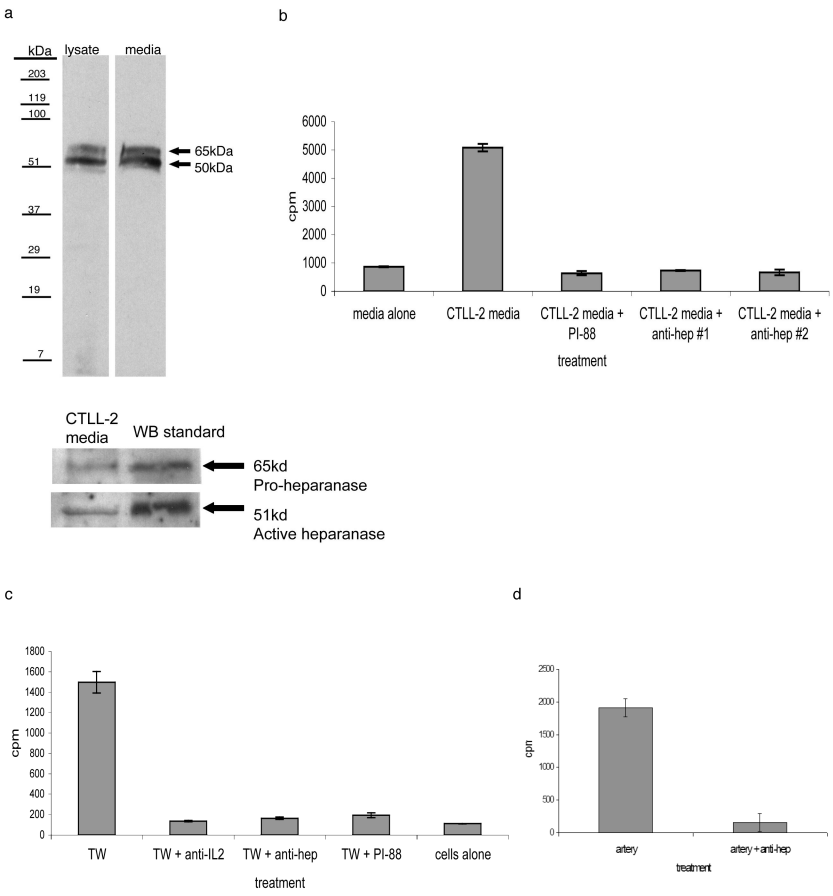

Figure 3. CTLL-2 cells release active heparanase, and when co-cultured with pieces of human artery, proliferate in an IL-2 and heparanase-dependent fashion

(a) In the upper gel, media and cell lysates of CTLL-2 cells were assessed by Western blot analysis for the presence of both the inactive (65 kD) and active (50 kD) forms of heparanase using an anti-heparanase antibody (anti-hep; Platt antibody). In the lower gel, CTLL-2 media was first passed through a heparin-sepharose column. Bound proteins were eluted with 2M NaCl and then assessed for the presence of heparanase as described above. The eluted material was compared with a purified heparanase standard. (b) Media from CTLL-2 cultures were assessed for heparanase activity as described in Materials and Methods. Select aliquots were pre-incubated for 1 h prior to the assay with 250 ng PI-88, or 10 μg each of 2 distinct anti-heparanase antibodies. The “media alone” control represents RPMI 1640 media plus 5% FBS without CTLL-2 cells. The results shown are the means ± SD of duplicate wells, and representative of 5 separate experiments.

(c) CTLL-2 cells were cultured with one 2 x 2 mm piece of human iliac artery/well in the presence/absence of 0.3 μg/ml anti-heparanase antibodies (anti-hep), 3 ng/ml PI-88, or 0.15 μg/ml goat anti-human IL-2 antibodies. Physical separation between the tissue and CTLL-2 cells was achieved by using a transwell (TW) insert. (d) CTLL-2 cells were cultured as in (c), in the presence or absence of anti-heparanase antibodies, without the transwell insert. The results shown are the means ± SD of triplicate wells, and representative of 14 (c) and 8 (d) separate experiments.

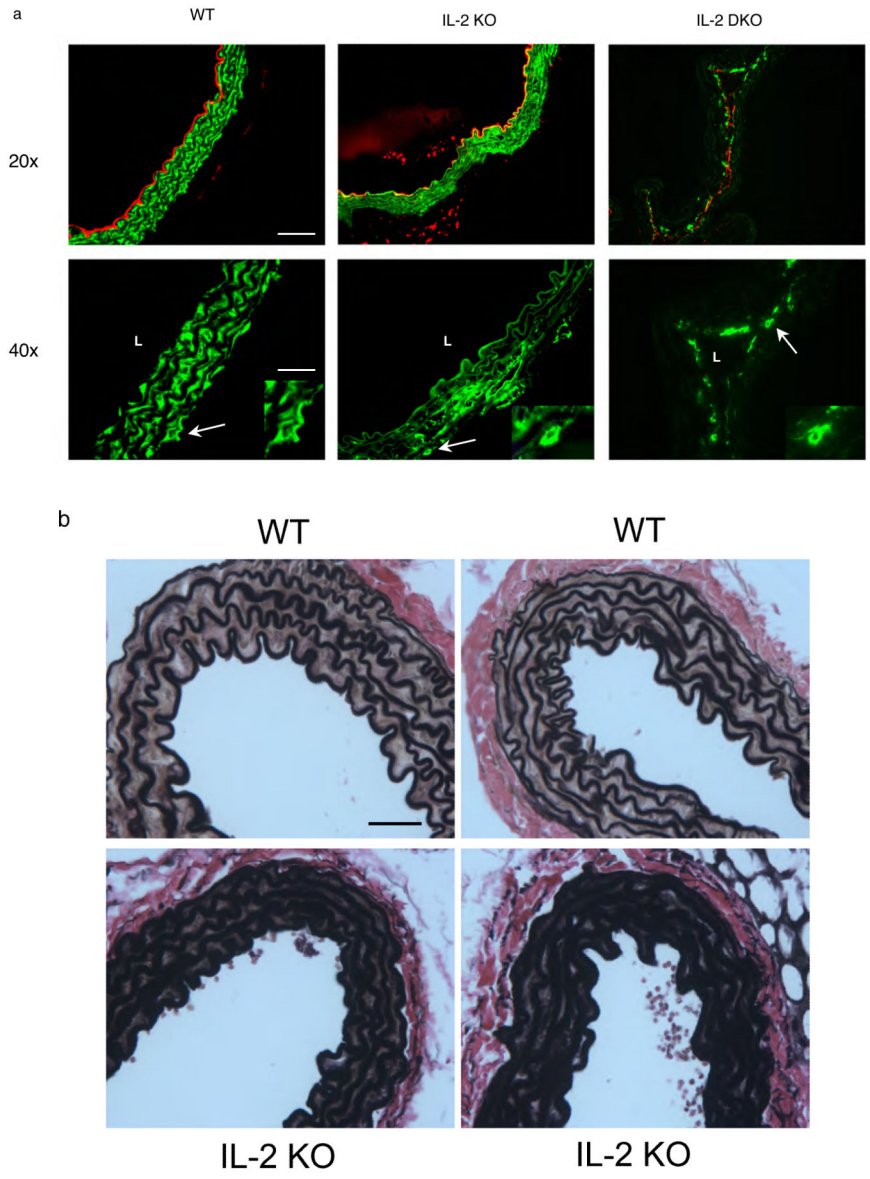

Figure 4. The aortas of IL-2 deficient mice exhibit several abnormalities

(a) Sections of murine aortas were double labeled with smooth muscle cell actin (FITC) and, to identify endothelial cells, anti-CD31 (TRITC). Aortas from 24 week-old DO11.10/IL-$2^{+/+}$ (WT) mice were compared with aortas from 27 week-old DO11.10/IL-$2^{-/-}$ mice (IL-2 KO), and from DO11.10/$RAG^{-/-}$/IL-$2^{-/-}$ (DKO). The vascular lumen is identified with an L. A digital zoom image of the smooth muscle cell identified by an arrow is shown as an insert. Because the extremely abnormal IL-2 KO aortas were ectatic and fragile, the lumens often collapsed on themselves when harvested (see DKO) and the endothelial layer was prone to lifting away from the smooth muscle cells. The results shown are representative of approximately 5 IL-2 KO and 2 IL-2 DKO mice of ages similar to those above. Tissue sections were imaged as described in Figure 1B. Scale bars, 50 μm (20×), 25 μm (40×). (b) Pieces of murine aortas from WT and IL-2 KO mice were preserved in 4% formalin and embedded in paraffin blocks. Five μm sections were cut, deparaffinated, and then stained

with Verhoeff van Gieson stain. Two different WT and IL-2 KO aortas of comparable ages are shown (WT 36 and 48 weeks old; IL-2 KO 34 and 35 weeks old). Tissue sections were imaged as described in Figure 1B. The percentage of interlaminar space [interlaminar area / laminae + interlaminar area) × 100] was significantly greater in IL-2 WT mice ($p < 0.0001$). Scale bar, 25 μm.

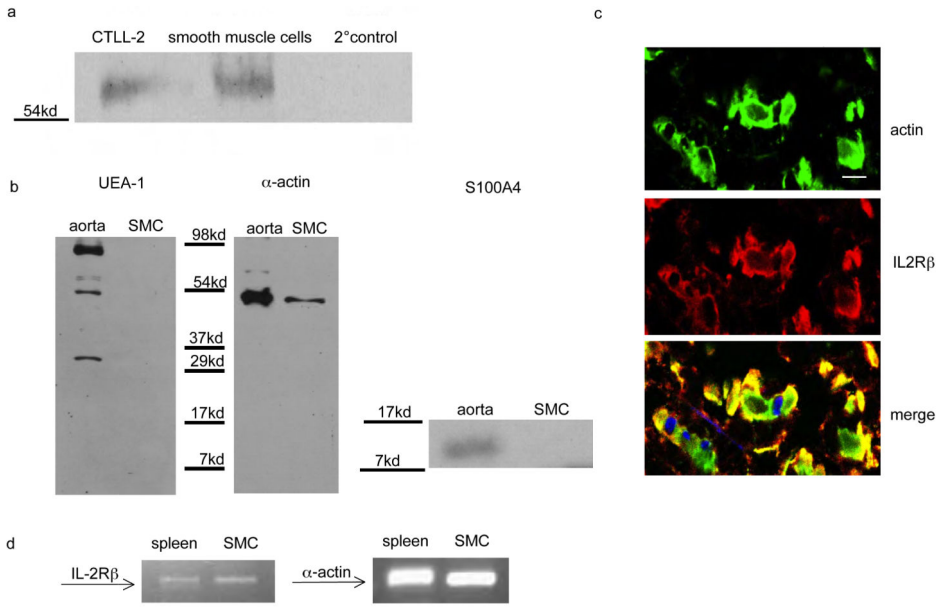

Figure 5. Vascular smooth muscle cells express IL-2 receptor β

(a) Lysates of murine vascular smooth muscle cells or CTLL-2 cells (positive control) were prepared for Western blot analysis as described in Figure 3 and probed with antibodies against murine IL-2 receptor β. The results shown are representative of 3 experiments. (b) The purity of the smooth muscle cell cultures was confirmed by Western blot analysis of cell lysates, in which α smooth muscle cell actin (43 kD) was present, but contaminating endothelial cells (UEA-1) and fibroblasts (S100A4, 12kD) were not detected. A tissue preparation of murine aorta is shown as a positive control. (c) Sections of human iliac artery were labeled with antibodies recognizing IL-2Rβ (TRITC) and α smooth muscle cell actin (FITC) and examined by confocal microscopy as described in Figure 1. Nuclei in the merged image are identified with DAPI. Scale bar, 10 μm. (d) Total RNA was extracted from cultured human vascular smooth muscle cells or a small piece of human spleen and assayed for the presence of IL-2 mRNA by reverse transcriptase PCR. Expression of β actin was used as a loading control. The results shown are representative of 3 experiments.