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Immunolocalization of Interleukin-1 Receptors in the Sarcolemma and Nuclei of Skeletal Muscle in Patients With Idiopathic Inflammatory Myopathies

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Objective. **Interleukin-1 (IL-1) acts via its receptors to induce gene expression that mediates protein synthesis involved in inflammation. Increased expression of IL-1α and IL-1β in muscle tissue from patients with polymyositis and dermatomyositis has been demonstrated. It is not known whether the reciprocal IL-1 receptors are expressed in human muscle tissue. The purpose of this study was to investigate the expression of IL-1 receptors and their ligands in muscle tissue from patients with myositis and from healthy controls.**

Methods. **Muscle biopsy tissues from 10 patients with polymyositis or dermatomyositis and 7 healthy control subjects were investigated by immunohistochemistry using antibodies against IL-1 receptor type I (IL-1RI), IL-1RII, IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1Ra). Quantification was performed by computerized image analysis, and localization of expression was determined by double staining using immunofluorescence and confocal microscopy.**

Results. **In tissue samples from the patients, IL-1RI and IL-1RII were expressed in muscle fibers, inflammatory cells, and endothelial cells. Expression in muscle fibers was localized to the sarcolemma and nuclei. IL-1α was expressed in endothelial cells and inflammatory cells, whereas IL-1β and IL-1Ra were expressed only in inflammatory cells. Expression of the two IL-1 receptors and their ligands was significantly higher in patients than in controls. IL-1 receptor expression on muscle fibers was most pronounced in the vicinity of cells expressing IL-1α and IL-1β.**

Conclusion. **The increased expression of IL-1 receptor and the colocalization with reciprocal ligands in patients with myositis but not in healthy controls support the hypothesis of a crucial role of IL-1 in the pathogenesis of polymyositis and dermatomyositis.**

Polymyositis and dermatomyositis are chronic inflammatory muscle disorders characterized by proximal muscle weakness and decreased muscle endurance (1–4). The detailed mechanisms causing the impaired muscle function have not been fully clarified. Muscle fiber degeneration and necrosis, as well as inflammatory cell infiltrates, often have a patchy distribution, and the degree of histopathologic changes in muscle biopsy tissues does not fully correspond to the degree of reduced muscle function (5–11).

In muscle biopsy tissues from patients with polymyositis or dermatomyositis, an overexpression of certain proinflammatory cytokines, such as interleukin-1α (IL-1α), IL-1β, and tumor necrosis factor, has previously been demonstrated (7,9). The most consistently detected proinflammatory cytokines in myositis patients are IL-1α and IL-1β. Notably, these cytokines have been recorded in muscle tissue from patients with early, as well as late, chronic polymyositis and dermatomyositis who have reduced muscle function. Moreover, increased expression of these cytokines has been detected in muscle tissue from myositis patients as compared with muscle tissue from healthy individuals, even in the absence of inflammatory cell clusters. IL-1α was mainly expressed in endothelial cells and in scattered mono-

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nuclear inflammatory cells, and IL-1β was detected only in scattered mononuclear inflammatory cells.

IL-1 affects nearly every cell type, and it is a potent proinflammatory cytokine. From the results of in vitro experiments, it has been suggested that IL-1 has several negative effects on muscle fibers that could affect their function, including reduced production of insulin-like growth factor (12), a negative effect on protein metabolism (12), and prevention of muscle regeneration (13). The production and/or activity of IL-1α and IL-1β are highly regulated events. In addition to controlling gene expression, synthesis, and secretion, this regulation extends to surface receptors, soluble receptors, and a receptor antagonist.

There are 3 members of the IL-1 gene family (IL-1α, IL-1β, and IL-1 receptor antagonist [IL-1Ra]) and 2 IL-1 receptors (IL-1 receptor type I [IL-1RI] and IL-1RII). When IL-1 binds to IL-1RI, a complex is formed that binds to the IL-1 receptor accessory protein (IL-1RAcP). This results in a high-affinity binding and triggers IL-1 signal transduction. However, binding of IL-1 to IL-1RII does not transduce a signal, and therefore IL-1RII functions as a decoy receptor (14–16). The extracellular, or soluble, portions of IL-1RI (sIL-1RI) and sIL-1RII naturally circulate in health and disease, functioning as "buffers" and binding to IL-1α, IL-1β, and IL-1Ra. At present, the exact nature of the intracellular signals elicited by IL-1 is only partially understood.

Based on these observations, it has been proposed that IL-1 might affect muscle fiber function and thus contribute to muscle impairment by mechanisms other than muscle fiber damage (5,8,17). A direct molecular effect of IL-1 on muscle fibers presumably requires the expression of IL-1 receptors on the muscle fiber membrane. However, it is not known whether muscle fibers in humans express IL-1 receptors.

The results of in vitro experiments in which IL-1 receptors were localized in skeletal muscle cells raised the question of whether interactions between muscle cells and the surrounding local environment may be of significance for the clinical in vivo situation. The aim of this study was to investigate whether muscle fibers express IL-1RI and/or IL-1RII and whether there was a qualitative and/or quantitative difference in expression between muscle from healthy individuals and patients with polymyositis or dermatomyositis. Furthermore, we investigated whether there was colocalization of IL-1 receptor expression and the cytokines IL-1α, IL-1β, and IL-1Ra.

PATIENTS AND METHODS

Patients. Muscle biopsy samples from patients newly diagnosed as having active polymyositis (n = 8) and dermatomyositis (n = 2), according to the classification system of Bohan and Peter (1,2), were evaluated in this study. Six patients were women and 4 were men. The time from symptom onset to diagnosis varied from 2 months to 6 years. The median age at diagnosis was 60 years (range 44–72 years). Creatine kinase levels were elevated in all patients (median 30 μkat/liter; range 5.3 to >76.8 μkat/liter) (to convert μkat/liter to units/liter, multiply by 60). Other details on the clinical and muscle biopsy data in this group of patients have previously been reported (18).

As controls, muscle biopsy samples from 7 healthy individuals (4 women and 3 men) with a mean age of 41 years (range 28–49 years) were included. All patients and controls gave informed consent, and the Local Ethics Committee at Karolinska University Hospital at Solna approved the study.

Muscle biopsies. Muscle biopsies were performed on patients newly diagnosed as having active myositis. Samples were obtained using local anesthesia and a semiopen conchotome technique (19). Biopsy samples were taken from 2 different muscle groups, one from a proximal symptomatic muscle and the other from a nonsymptomatic muscle, based on the patients' subjective symptoms of muscle weakness or tenderness. In most cases, both samples were taken before immunosuppressive treatment was begun, but in some cases (patients 3, 7, and 9), treatment had to be started before biopsy because of clinical considerations (Table 1).

All muscle samples were assessed for histopathologic changes by an experienced neuropathologist at the Division of Pathology, Karolinska University Hospital at Huddinge; the neuropathologist had no knowledge of the patient's medical history. Muscle biopsy samples were evaluated using conventional histopathology techniques and immunohistochemical stainings of serial sections to identify the presence of inflammatory infiltrates, degenerating and regenerating fibers, nonnecrotic fibers invaded by mononuclear inflammatory cells, muscle fiber atrophy, and central nuclei. The first and last section of each series of consecutive sections was stained with Mayer's hematoxylin and eosin to confirm that the histopathology remained unchanged in the consecutive series of sections from the same biopsy sample. These sections were also used for estimation of the number of inflammatory infiltrates.

Immunohistochemistry and immunofluorescence studies. The skeletal muscle biopsy specimens were frozen in precooled isopentane, embedded in OCT compound, and stored at −70°C until sectioning was performed. The staining procedures have been described previously (20). Tissues from 2 different muscles per patient and 1 muscle per healthy control were used for immunohistochemistry. As positive controls, human peripheral blood mononuclear cells (PBMCs) were stimulated for 0, 4, 6, and 23 hours with lipopolysaccharide (LPS) (catalog no. L6529; Sigma, St. Louis, MO) or with phorbol myristate acetate (PMA; Sigma) plus ionomycin (Calbiochem Novabiochem, San Diego, CA) and human tonsil tissues were used.

Recombinant human sIL-1RI antigen was used to neutralize the IL-1RI antibody in order to distinguish the specificity of the IL-1RI antibody. The blocking procedure for the IL-1RI antibody was performed in the same way as for the

Table 1. Percentage of IL-1 receptors and their ligands in different cellular compartments of muscle biopsy tissues from myositis patients and healthy controls, as determined by conventional microscopy*

	Treatment at biopsy 1/2 (daily dose)	IL-1RI, biopsy 1/2			IL-1RII, biopsy 1/2			IL-1α, biopsy 1/2		IL-1β, biopsy 1/2, inflammatory cells	IL-1Ra, biopsy 1/2, inflammatory cells	CD31, biopsy 1/2	
		Muscle nuclei	Sarcolemma	Inflammatory cells	Muscle nuclei	Sarcolemma	Inflammatory cells	Capillary ECs	Inflammatory cells			Total no. of capillary ECs	Total no. of larger vessel ECs
Patient, diagnosis													
1, definite PM	0/0	2+/2+	1+/2+	4+/4+	3+/2+	2+/2+	4+/4+	1+/2+	4+/4+	3+/4+	3+/4+	3+/5+	2+/2+
2, definite PM	0/0	2+/3+	2+/2+	3+/4+	4+/4+	2+/2+	4+/5+	2+/1+	1+/1+	2+/3+	3+/4+	4+/4+	3+/2+
3, definite PM	Pred. (40 mg) + AZA (100 mg)/0	2+/3+	1+/3+	4+/4+	2+/3+	2+/2+	4+/5+	2+/2+	2+/2+	4+/5+	4+/5+	3+/4+	3+/4+
4, definite PM	0/0	2+/2+	2+/2+	3+/3+	3+/3+	3+/3+	4+/4+	5+/5+	2+/2+	5+/5+	3+/3+	3+/5+	4+/5+
5, definite PM	0/0	1+/1+	1+/1+	2+/1+	1+/2+	2+/1+	3+/2+	2+/1+	1+/1+	2+/2+	1+/1+	3+/4+	3+/3+
6, probable PM	0/0	1+/1+	2+/1+	2+/1+	3+/3+	2+/1+	3+/3+	2+/1+	1+/1+	3+/2+	3+/2+	3+/3+	3+/3+
7, probable PM	Pred. (5 mg)/ pred. (5 mg)	1+/3+	1+/1+	4+/4+	1+/3+	1+/2+	4+/4+	1+/1+	3+/3+	4+/4+	3+/4+	3+/4+	1+/2+
8, probable PM	0/0	3+/2+	2+/2+	4+/2+	4+/4+	2+/2+	4+/3+	1+/1+	1+/1+	4+/4+	3+/3+	5+/4+	5+/4+
9, definite DM	Pred. (15 mg)/ pred. (15 mg)	1+/1+	1+/1+	2+/2+	2+/2+	2+/2+	3+/4+	3+/3+	1+/2+	4+/3+	2+/2+	4+/3+	3+/3+
10, probable DM	0/0	1+/1+	1+/1+	1+/1+	2+/2+	2+/2+	2+/2+	1+/1+	1+/1+	2+/2+	1+/2+	3+/5+	2+/2+
Controls													
1	0	1+	1+	–	2+	1+	–	1+	–	–	–	4+	3+
2	0	1+	1+	–	2+	1+	–	–	–	–	–	3+	3+
3	0	2+	1+	–	2+	1+	–	1+	–	–	–	4+	2+
4	0	2+	1+	–	2+	1+	–	–	–	–	–	4+	3+
5	0	1+	2+	–	2+	2+	–	1+	–	–	–	4+	3+
6	0	1+	1+	–	1+	1+	–	–	–	–	–	5+	3+
7	0	1+	1+	–	1+	1+	–	1+	–	–	–	4+	2+

* Two biopsy samples (biopsy 1/biopsy 2) from each myositis patient and 1 biopsy sample from each healthy control subject were evaluated. For conventional microscopic evaluation, positive cells, cytokines, and endothelial cells (ECs) were counted on whole tissue in the respective sections; thus, the results represent positive expression/mm^2. Interleukin-1 receptor type I (IL-1RI), IL-1RII, IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1Ra) were scored as follows: – = 0%, 1+ = 1–10%, 2+ = 11–25%, 3+ = 26–50%, 4+ = 51–75%, and 5+ = ≥76% capillaries/mm^2. CD31 in capillary ECs was scored as follows: – = 0, 1+ = 1–50, 2+ = 51–100, 3+ = 101–200, 4+ = 201–300, and 5+ = ≥301. CD31 in larger vessel ECs was scored as follows: – = 0, 1+ = 1–5, 2+ = 6–10, 3+ = 11–15, 4+ = 16–20, and 5+ = ≥21 larger vessels/mm^2. PM = polymyositis, pred. = prednisolone; AZA = azathioprine; DM = dermatomyositis.

Table 2. Total percentage of IL-1 receptors and their ligands in muscle biopsy tissues from myositis patients and healthy controls, as determined by computerized image analysis*

	Treatment at biopsy 1/2 (daily dose)	Total IL-1RI, biopsy 1/2	Total IL-1RII, biopsy 1/2	Total IL-1α, biopsy 1/2	Total IL-1β, biopsy 1/2	Total IL-1Ra, biopsy 1/2	Total CD31 in capillaries, biopsy 1/2	Total CD31 in larger vessels, biopsy 1/2
Patient, diagnosis								
1, definite PM	0/0	2+/5+	5+/5+	3+/3+	3+/3+	3+/3+	4+/4+	3+/3+
2, definite PM	0/0	5+/5+	5+/5+	4+/4+	3+/3+	3+/4+	3+/5+	3+/4+
3, definite PM	Pred. (40 mg) + AZA (100 mg)/0	5+/5+	5+/5+	3+/4+	2+/3+	3+/3+	5+/5+	4+/4+
4, definite PM	0/0	5+/5+	6+/5+	3+/3+	5+/4+	3+/2+	5+/5+	4+/4+
5, definite PM	0/0	3+/3+	3+/3+	3+/3+	3+/3+	2+/2+	4+/5+	3+/3+
6, probable PM	0/0	4+/3+	5+/5+	4+/3+	4+/3+	3+/2+	4+/5+	3+/3+
7, probable PM	Pred. (5 mg)/ pred. (5 mg)	5+/5+	5+/5+	5+/3+	4+/3+	3+/3+	4+/4+	3+/3+
8, probable PM	0/0	5+/4+	5+/5+	3+/3+	4+/3+	4+/3+	5+/5+	4+/4+
9, definite DM	Pred. (15 mg)/ pred. (15 mg)	3+/4+	4+/5+	4+/5+	4+/4+	3+/3+	5+/4+	4+/4+
10, probable DM	0/0	3+/3+	5+/5+	2+/2+	3+/2+	1+/1+	4+/5+	3+/3+
Controls								
1	0	1+	3+	1+	–	–	5+	3+
2	0	2+	3+	1+	–	–	4+	3+
3	0	2+	3+	2+	–	–	5+	3+
4	0	2+	2+	1+	–	–	4+	3+
5	0	1+	2+	2+	–	–	4+	4+
6	0	1+	1+	1+	–	–	5+	4+
7	0	1+	2+	2+	–	–	5+	3+

* Two biopsy samples (biopsy 1/biopsy 2) from each myositis patient and 1 biopsy sample from each healthy control subject were evaluated. For computerized image analysis, the area of positive immunostaining was expressed as a percentage of the total counterstained tissue area examined at 250× magnification. Interleukin-1 receptor type I (IL-1RI), IL-1RII, IL-1α, IL-1β, IL-1 receptor antagonist (IL-1Ra), and CD31 were scored as follows: – = 0%, 1+ = 0.001–0.05%, 2+ = >0.05–0.1%, 3+ = >0.1–0.5%, 4+ = >0.5–1%, 5+ = >1–5%, and 6+ = >5% positive expression/mm^2. PM = polymyositis, pred. = prednisolone; AZA = azathioprine; DM = dermatomyositis.

immunohistochemical staining, except that sections were preincubated with a 28× excess of recombinant antibody together with the IL-1RI antibody. The mixture of recombinant antibody and IL-1RI antibody was incubated overnight at 4°C. After 24 hours, 0.1% saponin and 1% normal horse serum were added to the mixture before it was applied to each section.

In parallel, double-immunofluorescence labeling was performed to colocalize IL-1RI and IL-1RII expression in muscle fiber membranes and nuclei. For intranuclear staining, LPS-stimulated and PMA plus ionomycin–stimulated (0, 4, 6, and 23 hours) human PBMCs were used as positive control cells. To detect IL-1RI and IL-1RII expression, we used the same antibodies as were used for the immunohistochemical staining (primary antibodies). Antibodies against laminin were used to localize the muscle fiber membrane, and BOBO-3 staining (which stains nucleic acids) was used to localize cell nuclei. Antibody concentrations were optimized for double-immunofluorescence staining. The staining procedures have been described in detail previously (21).

Antibodies and fluorophores. Primary antibodies were anti–IL-1RI (clone 35730), anti–IL-1RII (clone 32437), and anti–IL-1Ra (clone AF-280), which were purchased from R&D Systems (Abingdon, UK), anti–IL-1α (clone 1277-89-7) and anti–IL-1β (clones 2D8 combined with 1437-96-5), which were from Immunokontakt (Bioggo, Switzerland), anti-CD31 (clone EN4, human CD31, to detect endothelial cells), which was from Sanbio (BioZac; Uden, The Netherlands), anti–laminin 1 (code no. Z 0097), which was from Dakopatts (Glostrup, Denmark), and BOBO-3, a dimeric cyanin nuclear acid stain (B-3586), which was from Molecular Probes (Leiden, The Netherlands). Control mouse IgG1 and IgG2a were purchased from Dakopatts.

Secondary antibodies were biotinylated horse anti-mouse IgG and biotinylated goat anti-rabbit IgG, which were purchased from Vector (Burlingame CA). Biotinylated donkey anti-sheep/goat antibody was supplied by The Binding Site (Birmingham, UK).

Recombinant human sIL-1RI (code no. 269-1R) was obtained from R&D Systems.

Fluorophores were rhodamine red X–conjugated streptavidin (code no. 016-290-084), which was purchased from Jackson ImmunoResearch (Baltimore, MD), and Alexa Fluor 488–conjugated streptavidin (code no. S-11223), which was from Molecular Probes (Eugene, OR).

Quantification of staining results. The light microscope used for analyses of muscle tissue sections has been described in detail elsewhere (20). The sections were assessed by conventional microscopy on 2 different occasions by 2 independent observers (CG and SS) who were blinded to the identity of the specimens. The results from the 2 observers were concordant. Whole tissue sections were analyzed. The percentage of muscle fibers with positively stained membrane and the percentage of positively stained muscle fiber nuclei were calculated separately. Two biopsy samples were evaluated for each patient (Tables 1 and 2).

For conventional microscopic evaluation of IL-1RI and IL-1RII (percentages of muscle fibers with positively

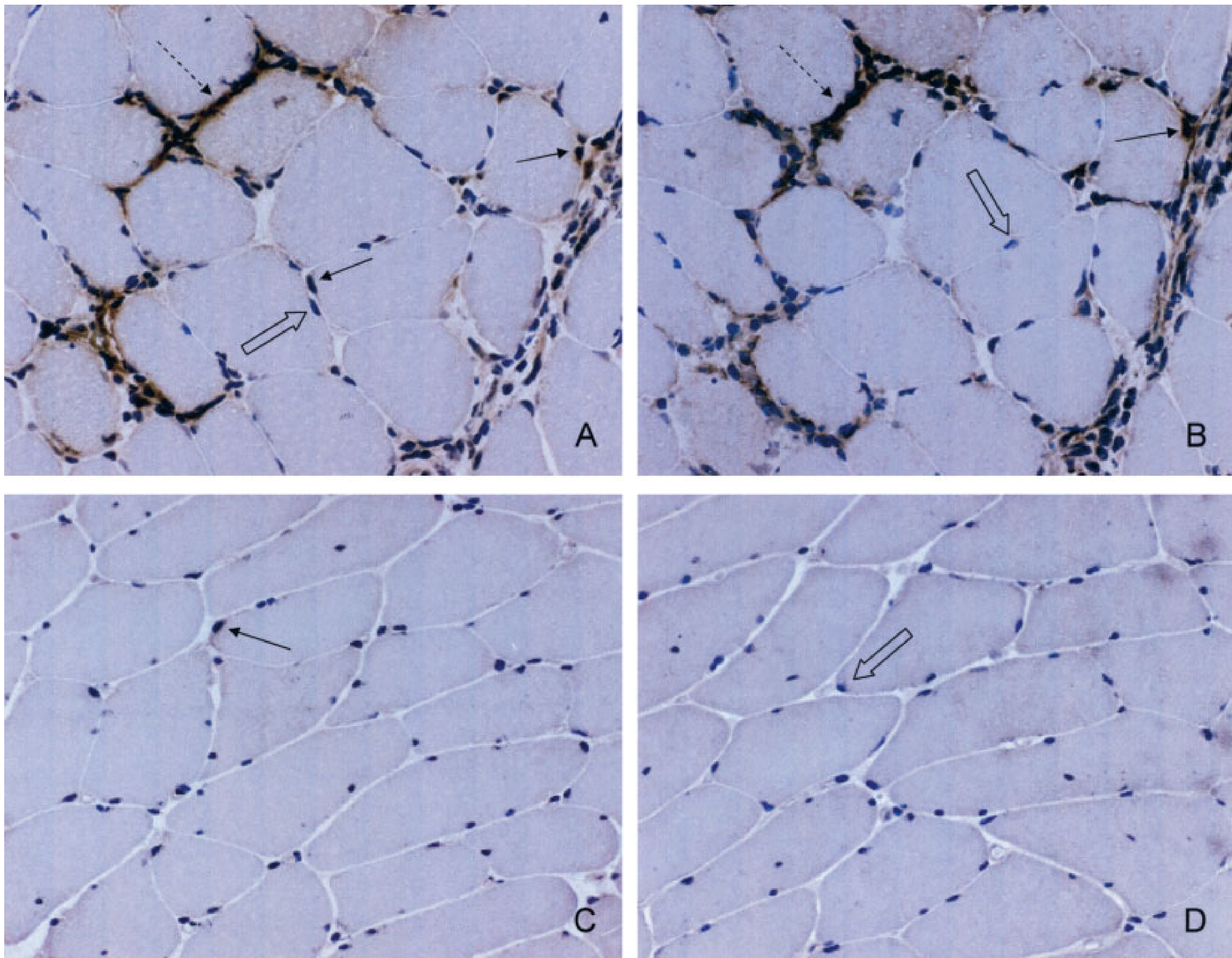

Figure 1. Staining for interleukin-1 receptor type I (IL-1RI) and IL-1RII in muscle cell nuclei and mononuclear inflammatory cells, by immunohistochemistry. Representative areas of consecutive muscle tissue sections from a polymyositis patient (**A** and **B**) and a healthy control subject (**C** and **D**) are shown. **A,** Staining for IL-1RI (brown). **Solid arrows** indicate muscle cell nuclei expressing IL-1RI, **broken arrow** indicates a mononuclear cell infiltrate expressing IL-1RI, and **open arrow** indicates a muscle cell nucleus negative for IL-1RI. **B,** Staining for IL-1RII (brown). **Solid arrow** indicates muscle cell nuclei expressing IL-1RII, **broken arrow** indicates a mononuclear cell infiltrate expressing IL-1RII, and **open arrow** indicates a muscle cell nucleus negative for IL-1RI. **C,** Staining for IL-1RI (brown). **Solid arrow** indicates a muscle cell nucleus expressing IL-1RI. **D,** Staining for IL-1RII (brown). **Open arrow** indicates negative expression of IL-1RII in the same muscle cell nucleus shown in **C**. (Original magnification × 25.)

stained sarcolemma, fiber nuclei, and inflammatory cells), IL-1α (percentages of positively stained inflammatory cells and endothelial cells in capillaries), and for IL-1β and IL-1Ra (percentages of positively stained inflammatory cells), the following scoring system was used: – = 0%, 1+ = 1–10%, 2+ = 11–25%, 3+ = 26–50%, 4+ = 51–75%, and 5+ = ≥76% positive expression/mm^2. For conventional microscopic evaluation of the number of CD31-positive endothelial cells in capillaries, the following scoring system was used: – = 0, 1+ = 1–50, 2+ = 51–100, 3+ = 101–200, 4+ = 201–300, and 5+ = ≥301 capillaries/mm^2. For conventional microscopic evaluation of the number of CD31-positive endothelial cells in larger vessels, the following scoring system was used: – = 0, 1+ = 1–5, 2+ = 6–10, 3+ = 11–15, 4+ = 16–20, and 5+ = ≥21 larger vessels/mm^2.

Quantification of staining was also assessed by digital image analysis, and the area of positive immunostaining was expressed as a percentage of the total counterstained tissue area examined at a magnification of 250×. For computerized image analysis of IL-1RI, IL-1RII, IL-1α, IL-1β, IL-1Ra, and CD31, the following scoring system was used: – = 0%, 1+ = 0.001–0.05%, 2+ = 0.05–0.1%, 3+ = 0.1–0.5%, 4+ = 0.5–1%, 5+ = 1–5%, and 6+ = >5% positive expression/mm^2.

Laser confocal microscopy. The laser confocal system consisted of a Bio-Rad MRC 1024 unit (Bio-Rad, Richmond,

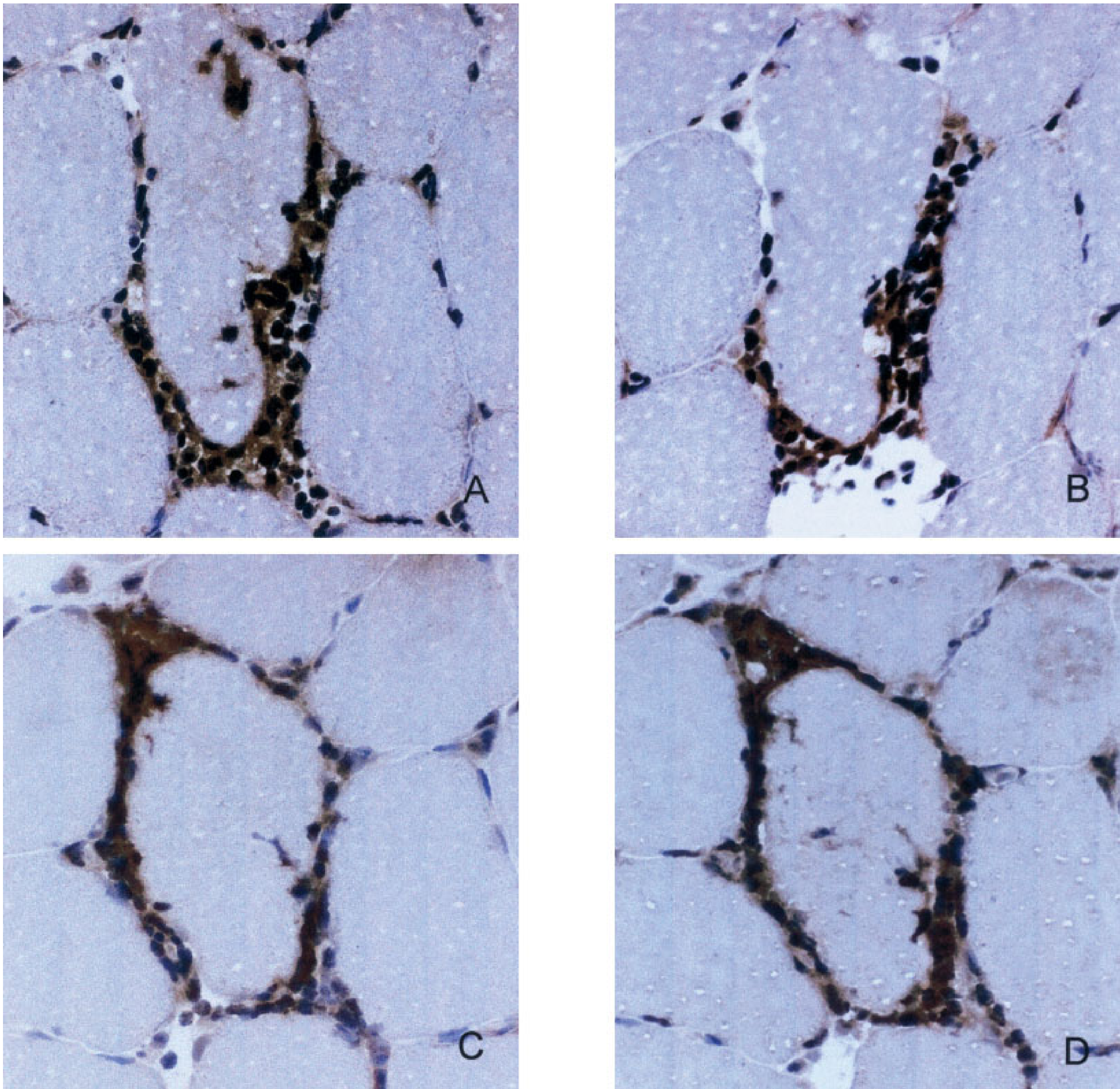

Figure 2. Staining for interleukin-1β (IL-1β), IL-1 receptor antagonist (IL-1Ra), IL-1 receptor type I (IL-1RI), and IL-1RII in muscle tissue by immunohistochemistry, demonstrating colocalization. Representative areas of consecutive muscle tissue sections (there are 3 sections between that shown in **C** and that shown in **D**) from a polymyositis patient are shown. **A,** Staining for IL-1β (brown). **B,** Staining for IL-1Ra (brown). **C,** Staining for IL-1RI (brown). **D,** Staining for IL-1RII (brown). (Original magnification $\times$ 25.)

CA) attached to a Nikon Diaphot 200 microscope (Nikon, Tokyo, Japan), using a Nikon Plan Apo 40× oil immersion lens objective (numerical aperture 1.3). Alexa Fluor 488 (IL-1R1 and IL-1RII stained structures) was excited with light at 488 nm of wavelength, and the emitted light was collected through a narrow band-pass filter at 515 nm. Rhodamine red X (laminin) was excited at 568 nm of wavelength, and the emitted light was collected through a long-pass filter at 585 nm. Images were converted from the Bio-Rad propriety file format to .tif format photomontages using Confocal Assistant (version 4.02) and ImageJ (NIH Image, National Institutes of Health, Bethesda, MD; online at: http://rsb.info.nih.gov/ij/).

Statistical analysis. Statistical analyses were performed using SPSS software version 11.5 for Windows (SPSS, Chicago, IL), and data are presented as the mean ± SD. The nonparametric Wilcoxon's signed rank test was used to compare different markers expressed in paired muscle samples, and adjustment for multiple comparisons was made by Bon-

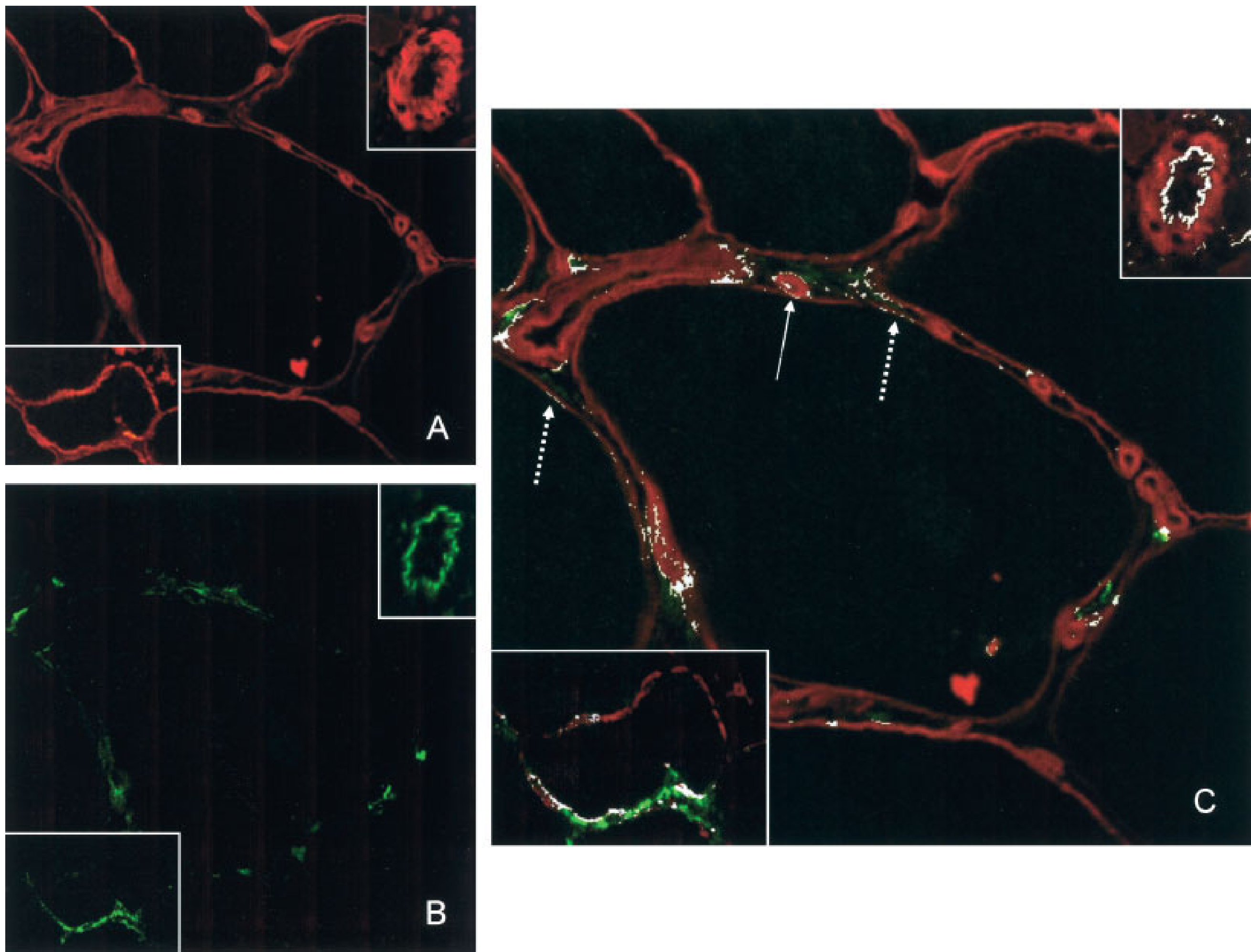

Figure 3. Double staining for interleukin-1 receptor type I (IL-1RI) and laminin in muscle tissue by immunofluorescence, demonstrating colocalization to the sarcolemma. Representative areas of muscle membrane and endothelial cell membrane in muscle tissue sections from a polymyositis patient are shown. **A,** Cell membrane showing laminin staining (red). **Inset** at the upper right shows laminin staining in an arteriole. **Inset** at the lower left shows laminin staining in a muscle fiber. **B,** Muscle membrane showing IL-1RI expression (green). **Inset** at the upper right shows IL-1RI staining in an arteriole. **Inset** at the lower left shows IL-1RI staining in a muscle fiber. **C,** Colocalization of laminin and IL-1RI expression (white). **Solid white arrow** indicates capillary endothelial cells expressing IL-1RI in the basal lamina. **Broken white arrows** indicate IL-1RI expression in the sarcolemma. **Inset** at the upper right shows IL-1RI staining in the intima of an arteriole. **Inset** at the lower left shows IL-1RI staining in the sarcolemma. (Original magnification × 40.)

ferroni correction. *P* values less than or equal to 0.05 were considered statistically significant. Comparisons between patients and healthy controls were done with the Mann-Whitney U test, and correlation analyses were done with Spearman's correlation.

RESULTS

Histopathology findings. In all 10 patients, inflammatory cell infiltrates were seen in muscle biopsy samples, and in all patients with polymyositis, endomysial infiltrates and non-necrotic fibers invaded by mononuclear inflammatory cells were present in at least 1 biopsy sample. The first and last section of each series of sections were evaluated and confirmed that the histopathologic features of the biopsy tissues remained unchanged in the consecutive sections. No histologic abnormalities were seen in the muscle biopsy samples from the 7 healthy control subjects.

IL-1RI and IL-1RII expression in muscle tissue from polymyositis and dermatomyositis patients. In all patients, the 2 IL-1 receptors were localized to the sarcolemma of scattered muscle fibers, showing a patchy staining pattern, as well as to scattered nuclei of muscle fibers (Table 1 and Figures 1 and 2). Expres-

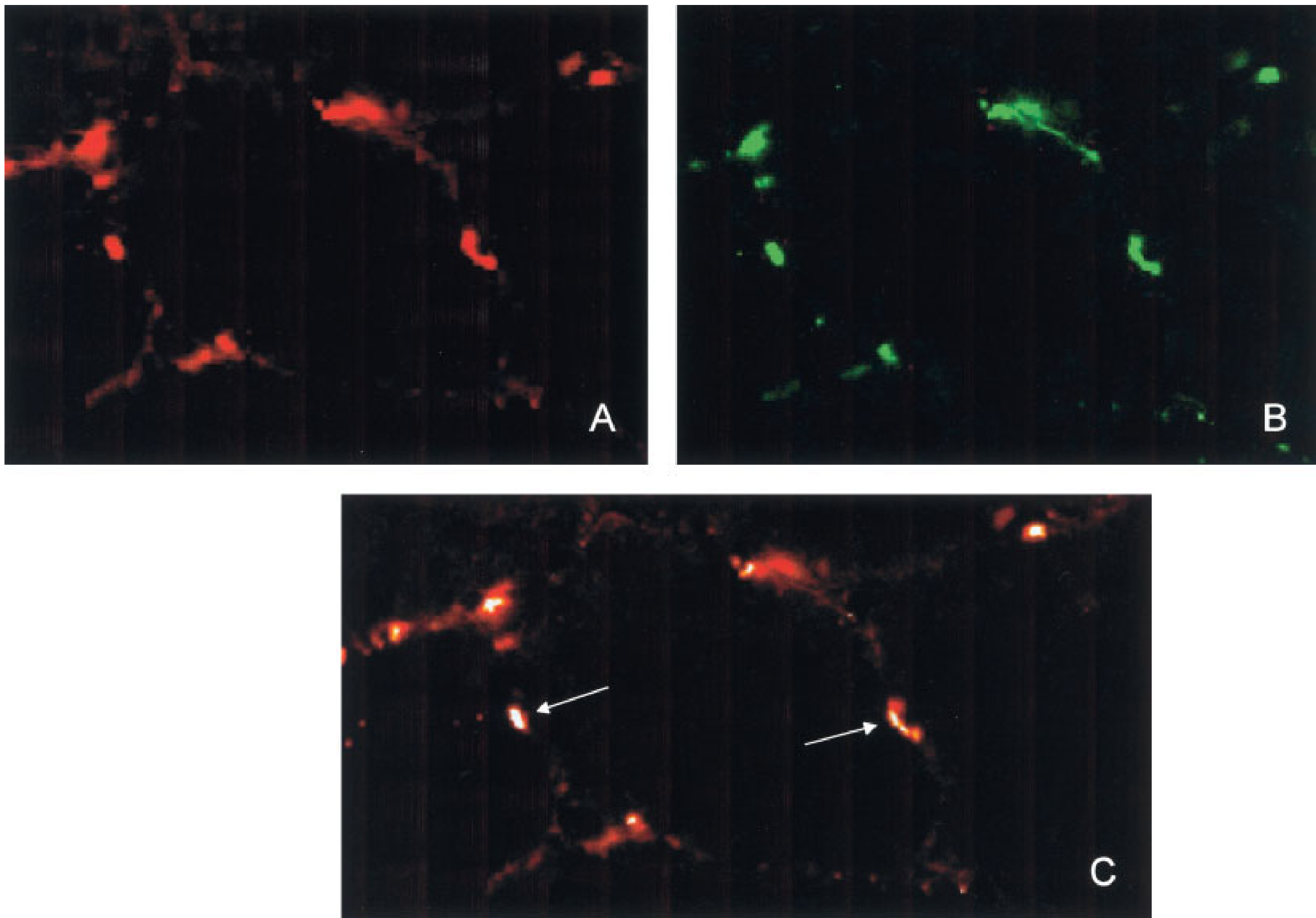

Figure 4. Double staining for interleukin-1 receptor type I (IL-1RI) and BOBO-3 in muscle tissue by immunofluorescence, demonstrating colocalization to the nucleus of muscle cells. Representative areas of muscle tissue sections from a polymyositis patient are shown. **A,** Muscle cell nucleus showing BOBO-3 staining (red). **B,** Muscle cell nucleus showing IL-1RI staining (green). **C,** Colocalization of BOBO-3 and IL-1RI expression (white). **Solid white arrows** indicate IL-1RI expression in the muscle cell nucleus. (Original magnification $\times$ 100.)

sion in the sarcolemma and nuclei was significantly increased in the patients as compared with the healthy controls ($P < 0.05$ and $P < 0.05$, respectively) (Table 1). The sarcolemma staining and the nuclear localization of the receptor were confirmed by double staining and confocal microscopy of muscle tissues (Figures 3 and 4). All patients also expressed IL-1RI and IL-1RII in occasional endothelial cells in blood vessels, both in larger vessels such as arterioles and venules, but more often in capillaries of the endomysium and the perimysium (Figure 3). In addition, both of the IL-1 receptors were also expressed by mononuclear inflammatory cells localized around and within non-necrotic muscle fibers as well as in large mononuclear cell infiltrates in the nuclei and in the cytosol (Table 1 and Figures 1 and 2). IL-1RI was expressed in the same compartments (sarcolemma, nuclei, and inflammatory cells) as IL-1RII, but to a lesser degree (Tables 1 and 2 and Figure 5).

Significantly higher total expression of IL-1RI and IL-1RII was seen in polymyositis and dermatomyositis patients as compared with healthy controls ($P < 0.05$ and $P < 0.05$, respectively) (Figure 5). There was no difference between polymyositis and dermatomyositis patients or between treated and untreated patients, and there was no difference between disease durations. The expression of IL-1RI was totally blocked by the corresponding peptide (data not shown). No statistically significant difference in the expression of IL-1RI and IL-1RII was found between the 2 samples that were taken from the different muscle groups ($P > 0.95$ and $P > 0.20$).

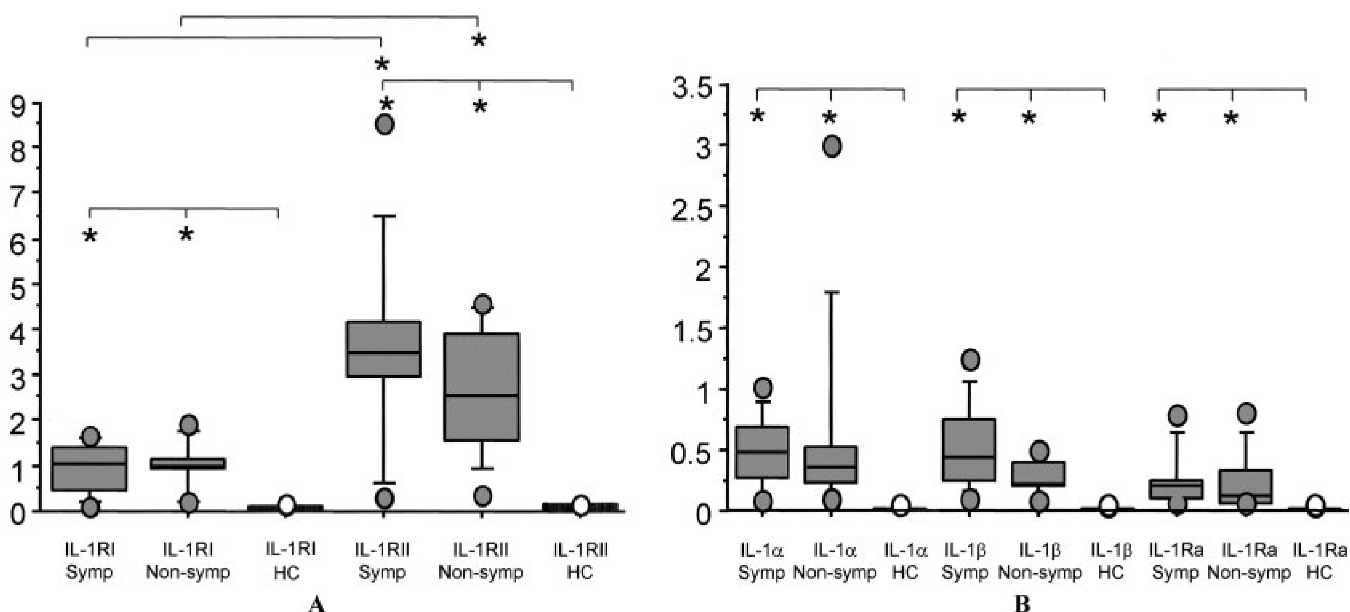

Figure 5. Quantification of muscle tissue immunostaining by digital image analysis. Muscle tissue was obtained from healthy controls (HC) and from symptomatic (Symp) and nonsymptomatic (Non-symp) muscles from patients with polymyositis and dermatomyositis. Sections were immunostained for interleukin-1 receptor type I (IL-1RI), IL-1RII, IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1Ra). **A,** Expression of IL-1RI and IL-1RII was significantly lower in the healthy controls than in the patients, both in symptomatic and in nonsymptomatic muscle tissue. In the patients, IL-1RI expression was significantly lower than IL-1RII expression. **B,** Expression of IL-1α, IL-1β, and IL-1Ra was significantly lower in the healthy controls than in the patients, both in symptomatic and in nonsymptomatic muscle tissue. The area of positive immunostaining (y-axis) was calculated as a percentage of the total counterstained tissue area examined at 250× magnification. Data are shown as box plots. Each box represents the 25th to 75th percentiles. Lines outside the boxes represent the 10th and the 90th percentiles. Lines inside the boxes represent the median. Circles indicate outliers. Shaded symbols represent patients; open symbols represent healthy subjects. $* = P < 0.05$.

IL-1RI and IL-1RII expression in muscle tissue from healthy controls. In muscle tissue from healthy controls, only scattered staining of IL-1RI and IL-1RII was seen. The observed expression was mainly localized to endothelial cells in larger vessels and to capillaries in the endomysium and the perimysium, but only sparse expression was seen in the sarcolemma and nuclei of the muscle fibers (Table 1 and Figure 1). There was no significant difference between IL-1RI and IL-1RII expression in healthy controls (Table 2 and Figure 5). The sarcolemma and nuclear association was confirmed by double staining and confocal microscopy.

IL-1RI and IL-1RII expression in unstimulated and stimulated PBMCs. Both IL-1RI and IL-1RII were expressed in the nucleus of stimulated PBMCs (Figure 6). A lower number of cells expressed the 2 receptors in unstimulated PBMCs. The number of cells expressing the receptors increased with increasing duration of stimulation (0, 4, 6, and 23 hours), but there was no difference between IL-1RI and IL-1RII expression. The morphology suggested that the positive cells were composed of a mixture of monocytes, granulocytes, and lymphocytes. The nuclear expression was confirmed by confocal microscopy applying double staining of the respective receptor with staining for nucleic acid (Figure 7).

IL-1α, IL-1β, IL-1Ra, and CD31 expression in muscle tissue from polymyositis and dermatomyositis patients and healthy controls. In all patients, IL-1α expression was mainly localized to endothelial cells of capillaries and larger vessels and to mononuclear inflammatory cells. Positive staining was also seen in the smooth muscle cells of arteries and arterioles. IL-1α–positive mononuclear cells were located within and around non-necrotic muscle fibers and were also present in large cellular infiltrates (Table 1). In healthy controls, IL-1α was expressed only in a few endothelial cells of capillaries and/or larger blood vessels. Total IL-1α expression, as assessed by computerized image analysis, was significantly increased in myositis patients as compared with healthy controls ($P < 0.05$) (Tables 1 and 2 and Figure 5). Total IL-1α expression was expressed to an equal degree in both tissue samples from the same individual.

IL-1β had a slightly different staining pattern compared with that of IL-1α. IL-1β was mainly ex-

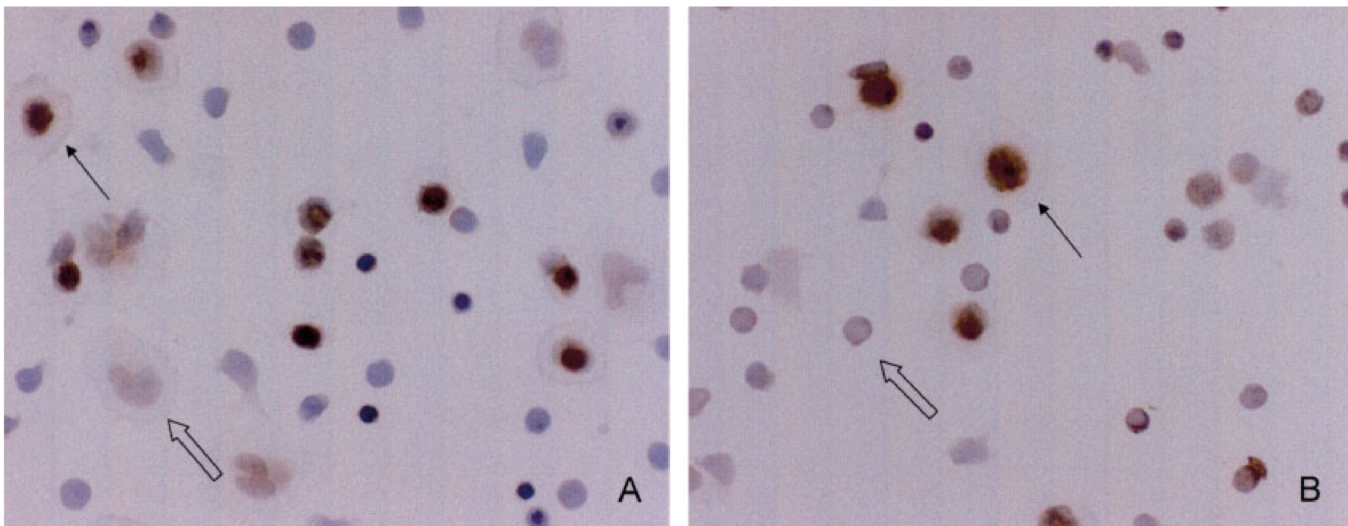

Figure 6. Staining for interleukin-1 receptor type I (IL-1RI) and IL-1RII in nuclei of human peripheral blood monocytes (PBMCs) by immunocytochemistry. PBMCs were stimulated with phorbol myristate acetate plus ionomycin for 23 hours prior to analysis. Representative findings in PBMCs are shown. **A,** Several cells show nuclear staining for IL-1RI (brown). **Solid arrow** indicates a PBMC expressing IL-1RI in the nucleus. **Open arrow** indicates a PBMC negative for IL-1RI. **B,** Several cells show nuclear staining for IL-1RII (brown). **Solid arrow** indicates a PBMC expressing IL-1RII in the nucleus. **Open arrow** indicates a PBMC negative for IL-1RII. (Original magnification × 40.)

pressed by macrophages or fibroblast-like cells (Table 1 and Figure 2). This was found in all patients. IL-1β–expressing cells were mainly located in cellular infiltrates in the endomysium, but were present to a lesser extent and with a more patchy distribution in mononuclear cells and, in some cases, in mononuclear cells surrounding non-necrotic muscle fibers. IL-1β was not expressed in muscle tissues from healthy controls (Tables 1 and 2).

The pattern of IL-1Ra expression was similar to that of IL-1β. It was mainly expressed in macrophages or fibroblast-like mononuclear inflammatory cells. IL-1Ra was mainly localized to cellular infiltrates in the endomysium, but with a more scattered distribution, in cells in all patients (Figure 2). IL-1Ra was not expressed in healthy controls (Tables 1 and 2).

To identify blood vessels, we used anti-CD31 antibody, which mainly stains endothelial cells. Capillaries in all patients appeared wider than those in the healthy controls, with thick endothelial cells resembling high endothelial venules (22). There was no difference between the 2 biopsy samples from the same patients. There was no statistically significant difference in the number of capillaries or larger vessels in healthy controls as compared with those in the patients (Tables 1 and 2).

Colocalization of the IL-1 receptors and IL-1α, IL-1β, and IL-1Ra in muscle tissue from polymyositis and dermatomyositis patients. When consecutive muscle biopsy sections were compared, IL-1RI and IL-1RII staining was colocalized with expression of IL-1α, IL-1β, and IL-1Ra (Figure 2). This colocalization was essentially observed in macrophages or fibroblast-like mononuclear cells, with nuclear and cytoplasmic staining in cellular infiltrates. In muscle specimens without cellular infiltrates, IL-1 receptor expression showed a patchy distribution in scattered mononuclear cells, and the receptors were mainly colocalized with cells expressing IL-1β and IL-1Ra. In addition, IL-1RI and IL-1RII were expressed in muscle cell sarcolemma, mainly in close contact with mononuclear cell infiltrates, where expression of IL-1α, IL-1β, and/or IL-1Ra was seen. Nuclear expression of the IL-1 receptors in muscle cells was concentrated in fibers in the vicinity of mononuclear cell infiltrates, but scattered muscle cell nuclei also expressed IL-1 receptors without close contact with mononuclear cell infiltrates. No expression of IL-1α, IL-1β, or IL-1Ra was seen in the nuclei of muscle cells. IL-1 receptors were only occasionally expressed in endothelial cells, some of which were also positive for IL-1α.

DISCUSSION

To our knowledge, this is the first study to demonstrate the localization of IL-1RI and IL-1RII to the sarcolemma and nuclei of human muscle fibers. Staining for IL-1 receptors was more frequently observed in muscle fibers of patients with idiopathic inflammatory myopathies as compared with healthy controls. As expected, the IL-1 receptors were also

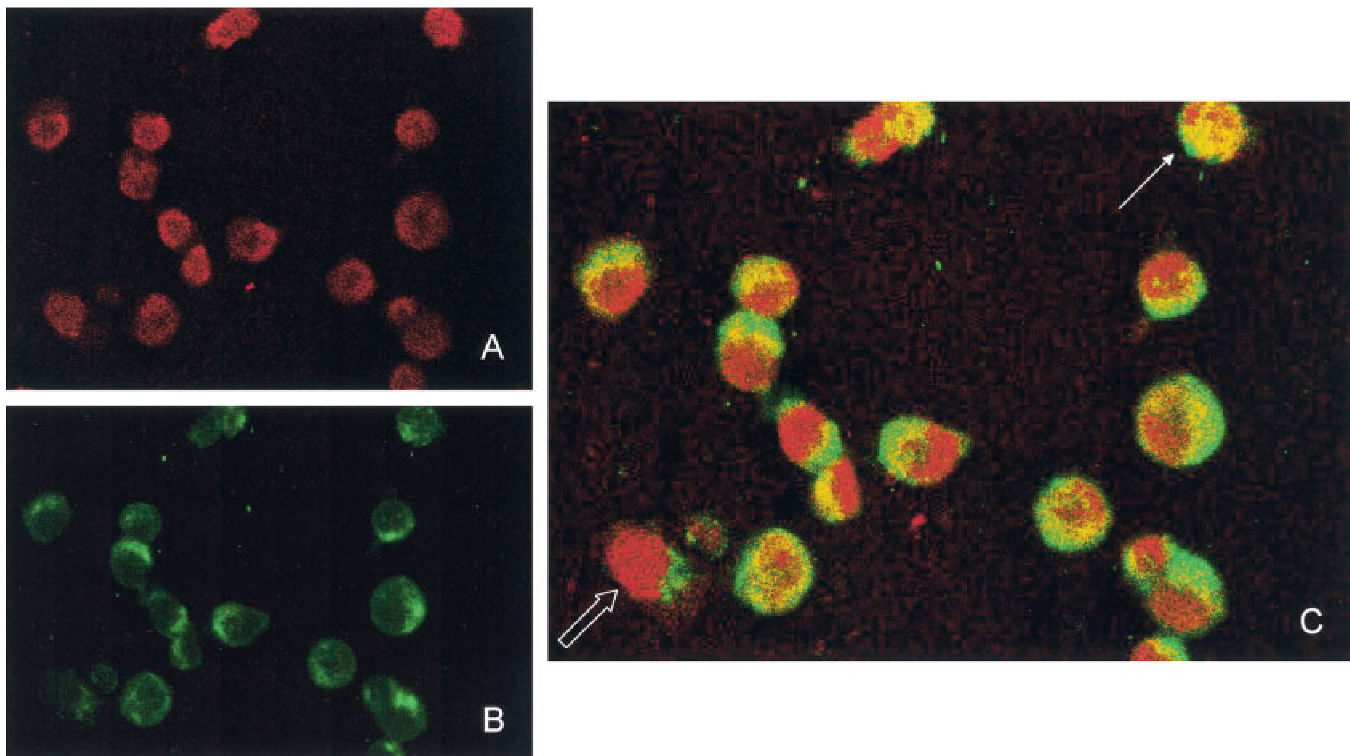

Figure 7. Double staining for interleukin-1 receptor type I (IL-1RI) and BOBO-3 in nuclei of human peripheral blood monocytes (PBMCs) by immunofluorescence, demonstrating colocalization to the nucleus. Representative findings in PBMCs are shown. **A,** PBMC nuclei showing BOBO-3 staining (red). **B,** PBMC nuclei showing IL-1RI staining (green). **C,** Colocalization of BOBO-3 and IL-1RI expression (yellow). **Solid white arrow** indicates IL-1RI expression in a PBMC nucleus. **Open white arrow** indicates a PBMC nucleus negative for IL-1RI. (Original magnification × 100.)

expressed in endothelial cells and mononuclear inflammatory cells, but were unexpectedly found in the nuclei of mononuclear inflammatory cells and PBMCs. The observed colocalization of IL-1 receptors in the sarcolemma of muscle fibers and in cells expressing IL-1α, IL-1β, and IL-1Ra, as well as the increased expression of the receptors in the patients as compared with the healthy controls, supports our hypothesis of an important role of the IL-1 pathway in the pathogenesis of polymyositis and dermatomyositis.

The immunohistochemistry technique alone does not always distinguish staining in different cellular structures, but with the use of immunohistochemistry together with immunofluorescence and confocal microscopy at high magnifications, we were able to resolve this issue. IL-1 receptor expression was localized in human muscle tissues by immunohistochemical analysis in conjunction with immunofluorescence analysis. These techniques, in combination with double staining and confocal microscopy, allowed the localization of protein expression to cellular structures, such as the sarcolemma and nuclei.

Since inflammatory changes within affected muscles of polymyositis or dermatomyositis patients are often focally distributed, there is always the possibility of missing a site of inflammation when obtaining a small biopsy and not getting a representative tissue sample for examination. To minimize this risk of sampling error, we analyzed biopsy samples from 2 different muscles, and both samples demonstrated similar results. The sites of biopsy samples were selected according to each patient's subjective impression of muscle weakness; samples from symptomatic sites were taken from proximal muscles, and samples from nonsymptomatic sites were taken mainly from distal muscles, which is the classic distribution of muscle weakness in these disorders. Notably, the histopathologic changes did not differ between the 2 biopsy samples (18). In addition, we compared histopathologic changes in the first and the last sections of each biopsy sample and found no detectable differences.

These patients were in the same stage of myositis, with newly diagnosed, active disease, and in most cases there were pronounced inflammatory cell infiltrates in

the tissues. Thus, these investigations cannot be considered to be directly representative of IL-1RI and IL-1RII expression in the early or late chronic stages of disease, nor representative of expression in other myopathies. A separate study would need to be performed in order to make such assessments.

The specificity of antibody binding was confirmed by blocking experiments. The results showed that IL-1RI expression was totally abolished by blocking with the IL-1RI peptide.

For quantification of the staining results, we used both conventional microscopic evaluation and computerized digital image analysis, by which the total area of positive immunostaining with different markers per tissue section was calculated as a percentage of the total counterstained tissue area (examined at a magnification of 250×). Since IL-1 receptors are known to be expressed in endothelial cells, a difference in the total IL-1 receptor expression could be dependent upon a difference in the number of blood vessels per tissue section. We could not detect any difference in the number of capillaries or larger vessels between samples from the patients and the healthy controls or between the 2 different muscle biopsy samples from the patients. These findings exclude the possibility that the detected difference in IL-1 receptor expression between patients and controls was the result of only an increased number of capillaries.

IL-1α, IL-1β, and intracellular IL-1Ra (icIL-1Ra) lack secretory leader peptides and therefore are secreted through a pathway that bypasses the classic exocytotic route. ProIL-1α remains in the cytosol after translation, and proIL-1β colocalizes with procaspase 1 in secretory lysosomes. They are secreted together with lysosomal enzymes following exocytosis of these organelles (23). In contrast, secreted IL-1Ra (sIL-1Ra) possesses a leader sequence, and upon stimulation with LPS, human blood monocytes initially express the gene for sIL-1Ra (24), and sIL-1Ra can be visualized in the Golgi apparatus (25). On the other hand, icIL-1Ra, which lacks a leader peptide, diffusely stains in the cytosol and remains intracellular (25). There is no appreciable accumulation of IL-1α in any cytosolic organelle, and it is usually not found in the circulation or in inflammatory body fluids, where IL-1β and IL-1Ra can be seen. In some previous studies, it was demonstrated that IL-1α/IL-1 receptor complexes can bind to nuclear DNA (26). It has been proposed that icIL-1Ra, which is constitutively produced in certain cells, may block the binding of IL-1α to nuclear DNA (24,27). ProIL-1α has been shown to be an intracrine proinflammatory activator of transcription (28). IL-1 can move from the cell membrane to the cytoplasm, where it can be found in proximity to nuclei or within lysosomes in T lymphocytes and fibroblasts (29), and IL-1α as a complex with its receptor has also been shown to express DNA binding activity (30). Thus, intracellular functions of IL-1α might play an unforeseen role in the pathogenesis of inflammation.

There are 2 receptors that can bind IL-1: IL-1RI and IL-1RII. IL-1RI affects the biologic response to IL-1, whereas IL-1RII acts as a decoy receptor and does not transduce a biologic signal (14–16). IL-1RI is constitutively expressed at low levels, which is consistent with the low numbers of surface receptors on most cells. IL-1 signal transduction has been observed in cells expressing fewer than 10 IL-1RI per cell (16) but, more often, transduction occurs in cells expressing ~100 IL-1RI per cell, and a biologic response occurs when only 2–3% of IL-1RI receptors are occupied (31,32). In some cells, IL-1 can down-regulate the number of its own surface receptors (31,33). By binding to IL-1, the type II receptor can shunt the ligand away from IL-1RI (15,34). In addition, IL-1RII may bind the coreceptor IL-1RAcP, thus removing the coreceptor from signaling IL-1RI (35,36). Therefore, increased expression of IL-1RII (both soluble and bound) may be a mechanism by which to reduce the biologic effects of IL-1.

Muscle tissues from myositis patients showed a significantly increased expression of IL-1RII as compared with tissues from healthy individuals. The results of our study suggest that this could reflect a possible way to block the effects of the increased IL-1 in muscle tissue in myositis patients. IL-1RII preferably and almost irreversibly binds to IL-1β. Soluble IL-1RII likely serves as a constitutive antagonist of IL-1β. There are ways to diminish the effect of IL-1 other than increasing the levels of soluble and bound IL-1RII, such as up-regulation of soluble and possibly intracellular levels of IL-1Ra, and up-regulation of sIL-1RI. IL-1, prostaglandin E_2, and IL-4 are some of the factors that are capable of increasing the surface expression of IL-1RII (37), and they could all be increased during inflammation. It is also known that corticosteroids suppress IL-1 gene expression and secretion and increase IL-1RII expression (38,39). Corticosteroids can also induce the expression of IκB proteins and inhibit the activation of NF-κB by IL-1 (40,41). In our study, 3 of the 10 patients were treated with prednisolone during a short period before the muscle biopsies were performed. However, there was no difference in histopathologic features between treated and untreated patients, nor was there any differ-

ence in the expression of IL-1α, IL-1β, IL-1Ra, and the IL-1 receptors.

In conclusion, using immunostaining and laser confocal microscopy, we demonstrated the expression of both receptors for IL-1 in sarcolemma and nuclei from human muscle tissue samples. The expression of both receptors was increased in patients with polymyositis and dermatomyositis as compared with healthy individuals. The IL-1 receptor expression in muscle fibers was associated with increased expression of the corresponding cytokines IL-1α, IL-1β, and IL-1Ra. This colocalization of receptors and their ligands supports our hypothesis that IL-1 could play an important role in the disease mechanisms of myositis. This could be mediated through direct effects on muscle fibers and hence affect muscle fiber metabolism and function.

AUTHOR CONTRIBUTIONS

Dr. Grundtman had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Drs. Grundtman and Lundberg.

Acquisition of data. Drs. Grundtman, Salomonsson, Dorph, Bruton, and Lundberg.

Analysis and interpretation of data. Drs. Grundtman, Andersson, and Lundberg.

Manuscript preparation. Drs. Grundtman, Salomonsson, Andersson, and Lundberg.

Statistical analysis. Dr. Grundtman.

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