Acta Neuropathol (2001) 102 : 385–392
DOI 10.1007/s004010100394

REGULAR PAPER

C. Bartoli · M. Civatte · J. F. Pellissier
D. Figarella-Branger

CCR2A and CCR2B, the two isoforms of the monocyte chemoattractant protein-1 receptor are up-regulated and expressed by different cell subsets in idiopathic inflammatory myopathies

Received: 10 January 2001 / Revised, accepted: 19 March 2001 / Published online: 18 August 2001

Abstract The idiopathic inflammatory myopathies (IIM), including dermatomyositis (DM), polymyositis (PM) and inclusion body myositis (IBM), are a group of autoimmune diseases characterized by chronic lymphocytic and macrophagic infiltration in muscle. The mechanism for recruitment of these cells probably involves chemokines. We have previously reported that monocyte chemoattractant protein-1 (MCP-1), a β chemokine, seems to play a major role in mononuclear cell recruitment especially in DM. Here we have investigated the distribution of the main MCP-1 receptors CCR2A and CCR2B in IIM by polymerase chain reaction (PCR), immunohistochemistry and in situ hybridization. We have shown by reverse transcription-PCR that both CCR2A and CCR2B were expressed at low level in normal muscle and that CCR2A was up-regulated in IIM (P=0.02) and was higher in PM and IBM than in DM (P=0.04). By immunohistochemistry and in situ hybridization we have observed that CCR2 isoforms were expressed by different cell subsets in both normal and IIM muscle. CCR2A was expressed in vessel walls and by some mononuclear cells, especially in cells involved in partial invasion in PM and IBM. CCR2B expression was observed in all satellite cells, in the muscular domain of neuromuscular junctions and in some regenerative fibers of IIM, but not in inflammatory exudates. In conclusion, the present study highlights the major role played by MCP-1 and its counter-receptor CCR2 in the pathophysiology of IIM, and shows that the CCR2 receptors are cell specific. The variation of the total amount of CCR2A and its local distribution according to the type of IIM might be a new path towards the understanding of the constitution of mononuclear infiltrates in IIM.

C. Bartoli · M. Civatte · J.F. Pellissier · D. Figarella-Branger (✉)
Laboratoire de Biopathologie Nerveuse et Musculaire, EA 3281,
Faculté de médecine, IBDM Marseille,
27 boulevard Jean Moulin, 13385 Marseille Cedex 05, France
e-mail: Dominique.Figarella-Branger@medecine.univ-mrs.fr,
Tel.: +33-4-91324443, Fax: +33-4-91254232

Keywords CCR2 · Regeneration · Dermatomyositis · Polymyositis · Inclusion body myositis

Introduction

The idiopathic inflammatory myopathies (IIM) including dermatomyositis (DM), polymyositis (PM) and inclusion body myositis (IBM) are a heterogeneous group of diseases morphologically characterized by chronic lymphocytic and macrophagic infiltration in muscles [4, 10].

In DM, perivascular inflammatory exudates, mainly composed of $CD4^+$ T lymphocytes, B lymphocytes and macrophages, are observed. The main immune effector response appears to be humoral and directed against the microvasculature. Membrane attack complex (MAC) deposits are observed within the intramuscular capillaries [24]. In PM and IBM, cytotoxic $CD8^+$ T cells and macrophages focally surround and invade non-necrotic muscle fibers expressing major histocompatibility complex class I [13, 16, 23].

Numerous studies have shown that cytokines play a major role in autoimmune diseases including IIM. We and others have shown strong proinflammatory cytokine expression, predominant Th1 immune response and up-regulation of cell adhesion molecule expression in IIM [12, 26, 27, 29]. In addition, strong expression of β-chemokines controlling at least monocytes and T cell migration has been reported in IIM [1]. Monocyte chemoattractant protein-1 (MCP-1), the major β-chemokine is highly expressed in IIM [9, 28]. Moreover, we have observed that mRNA expression was highest in DM and that local expression of MCP-1 was different in each IIM. Perivascular inflammatory cells highly expressed MCP-1 mRNA in DM, whereas it was strongly expressed by mononuclear cells partially invading non-necrotic muscle fibers in both PM and IBM. These results suggest that MCP-1 plays a major role in the local accumulation of discrete cell subsets in these diseases [28]. Because chemokines bind and activate specific transmembrane receptors expressed by target cells, it was relevant to search for MCP-1 receptor

(CCR2) expression in IIM. CCR2 exists as two isoforms CCR2A and CCR2B by alternative splicing of a single gene [7]. CCR2 is closely related to the MIP-1α/RANTES receptor, transduces signals in response to nanomolar concentrations of MCP-1 in a highly specific manner and appears to be the high-affinity MCP-1-specific receptor predicted by the pharmacological studies [7]. CCR2 also binds MCP-2, MCP-3 and MCP-4, but with a lower affinity [18, 20]. CCR2A and CCR2B differ by their C-terminal tails [7]. This probably represents a mechanism to increase the diversity of cellular responses to this important chemokine. To investigate the role of CCR2 in the immune response in human IIM, we have studied the expression of its two isoforms CCR2A and CCR2B by reverse transcription (RT)-PCR in a series of muscle biopsy specimens from patients suffering from IIM. Moreover, we report the cellular distribution of both CCR2A and CCR2B using immunohistochemistry and the focal tissue distribution of CCR2A mRNA by in situ hybridization.

Materials and methods

Human muscle specimens

Muscle biopsy specimens were obtained for diagnostic purposes. None of the patients had received corticosteroids or immunosuppressive therapy at the time of muscle biopsy. The diagnoses were based on conventional criteria. Eight cases were classified as DM (four men and four women, mean age 22 years, range 2–42 years), five cases as PM (three men and two women, mean age 48 years, range 22–74 years), and four cases as IBM (two men and two women, mean age 70 years, range 61–79 years). The mean duration of disease before diagnosis was 28 days for DM cases, 4 months for PM cases, and 5 years for IBM cases. All muscle samples were frozen in isopentane, cooled in liquid nitrogen and stored at –80°C until required. Muscle specimens from four patients biopsied for investigation of familial malignant hyperthermia susceptibility and showing negative contracture test results served as negative controls. Human tonsil was used as positive controls for CCR2A and CCR2B expression.

RNA extraction, PCR amplification and radioactive hybridization

Total cellular RNA was extracted from about ten 25-µm sections of each muscle specimen with the total quick RNA cell and tissues kit from Euromedex (Souffelweyersheim, France). RT was performed with 10 µl of total RNA preparation and maintained for 1.5 h at 37°C. The reaction was stopped by heat inactivation for 5 min at 95°C. The cDNA products were then amplified by an automated thermocycler (Robocycler, Coger, France). Control PCR amplifications for the house keeping gene glyceraldehyde 3-phosphate dehydrogenase (G3PDH) were performed for each sample to confirm cDNA integrity and ensure, after computer program analysis (NIH image 1.61), that equivalent amounts of cDNAs were utilized for PCR analysis. The G3PDH primers used for this reaction were 5´-CCTCAAAGGCATCCTGGGCTACAC-3´, as direct primer and 5´-CATGTGGGCCATGAGGTCCACCAC-3´as reverse primer, located respectively at positions 867–890 and 1053–1030 (GenBank GI 31644). CCR2A expression was tested using the direct primer 5´-CATTAGCCTATGTGCATGC-3´ and the reverse primer 5´-TTACTTCAGGGGAGCTTCTCC-3´, located, respectively at positions 1590–1608 and 1900–1881 (GenBank GI 472555); CCR2B expression was tested using the following primers: direct primer 5´-TGGACAAAGACAAAGGTG-3´, reverse primer 5´-AAGAAGCATCTGAACAATGG-3´ located respectively at positions 1604–1621 and 1880–1861 (GenBank GI 472557). Negative controls were performed by omitting RNA from cDNA synthesis and PCR amplification. The mixtures were subjected to 26 (G3PDH) and 35 (CCR2A and CCR2B) amplification cycles, each cycle consisting of 95°C for 10 s, 65°C (G3PDH) or 46°C (CCR2A) for 15 s and 72°C for 8 s (G3PDH) or 10 s (CCR2A and CCR2B). The 311-bp (CCR2A) and 277-bp (CCR2B) products were purified with the Geneclean kit (Bio 101, La Jolla, Calif.) subcloned into a pGEM-T vector (Promega, Madison Wis.), sequenced to verify specific amplification, and used as probes for RT-PCR Southern blots. PCR products were electrophoresed in 2% agarose gels (Nusieve, TEBU, Le Perray en Yvelines, France) and the bands were visualized with ethidium bromide staining. The size of the PCR products was determined using DNA molecular weight marker VI (Boehringer, Mannheim, Germany).

PCR products were transferred onto the Hybond-N nylon membrane (Amersham, Les Ullis, France) and fixed by UV cross-linking. Probe was [^{32}P]dCTP-labeled (Amersham) using the "NonaPrimer Kit II" (Appligene, Illkirch, France). Hybridizations were done at 65°C overnight in a 6× SSC (standard sodium citrate), 5× Denhardt, and 0.5% SDS solution. Washes were performed as follows: 30 min in 0.5× SSC-0.1% SDS and 30 min in 0.1× SSC-0.1% SDS. Signals were detected by exposing the membrane to X-ray-sensitive films (Eastman Kodak, Rochester, N.Y.); intensity analysis of the amplified bands was performed with an imager (Appligene, Illkirch, France) and the appropriate computer program (NIH image 1.61). Statistical analysis was performed using the Mann and Whitney test to compare subgroups of patients.

In situ hybridization

The "SureSite II in Hybridization System" kit (Novagen, Oxon, UK) was used for this experiment. For CCR2A, a template consisting of the 311-bp complementary DNA in the expression vector pGEM-T was used for riboprobe synthesis. pGEM-T vector was linearized with the restriction enzymes *Nde*I and *Nco*I (Biolabs, Montagny le Bretonneux, France) as recommended by the manufacturer and Sp6 and T7 RNA polymerases were used to synthesize sense and antisense riboprobes as determined by sequencing. Probes were labeled with [^{35}S]UTP (Amersham) and purified. They were electrophoresed on formaldehyde gel, transferred onto Hybond-N nylon membrane, and exposed to X-ray-sensitive films to monitor their integrity.

Several cryostat sections, 5 µm thick, were used for in situ hybridization. Slides were coated with 3-aminopropyl-triethoxy-silane, fixed in 4% paraformaldehyde for 20 min, treated with proteinase K (3 µg/ml) for 5 min, acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine, and rinsed in PBS. Slides were then submerged in prehybridization solutions. Hybridization was carried out overnight at 50°C in a moist chamber with 5×10^5 dpm per slide. After hybridization, the slides were washed according to manufacturer's instructions. Tissue sections were dipped in liquefied NTB-2 emulsion (Eastman Kodak) and exposed at 4°C in boxes containing desiccant for 4 weeks before being developed (Kodak D 19 developer), counterstained with Mayer's hematoxylin, and coverslipped. Sense probes were used in each experiment to stain adjacent sections under the same experimental conditions.

Immunohistochemistry

Immunohistochemistry was performed on serial 5-µm-thick frozen sections in all muscle specimens. The first one was stained with hematoxylin and eosin. The others were treated for anti-CCR2A (1/50^e) and CCR2B (1/20^e) (Santacruz, Calif.) or used as a negative control. For that, ten times the blocking peptide (pCCR2A or pCCR2B) was added to 4 µg/ml and 10 µg/ml of the diluted primary antibody, (anti-CCR2A or anti-CCR2B, respectively), and incubated overnight at room temperature. Then, instead of the primary antibody, the mixture of the blocking peptide and the primary antibody was applied to the section, which was then processed by conventional immunohistochemistry. In addition, im-

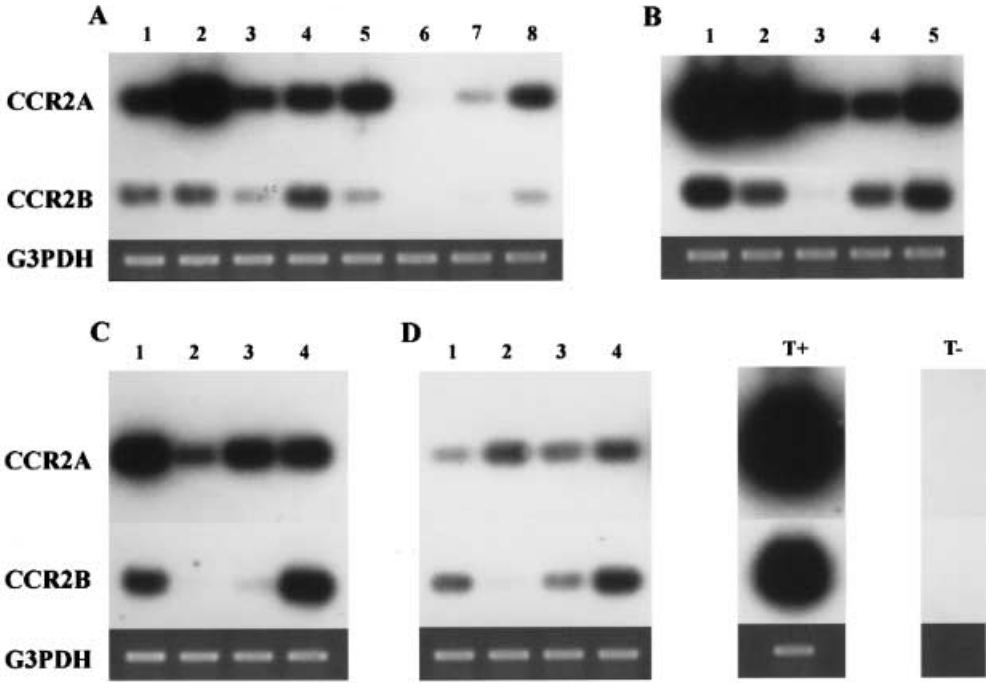

Fig. 1A–D Reverse transcription-PCR analysis of CCR2A and CCR2B expression in IIM. **A** DM, **B** PM, **C** IBM, and **D** normal muscles. G3PDH amplification shows homogenized cDNA amounts (agarose gel electrophoresis and ethidium bromide staining) (*CCR* CC chemokine receptor, *IIM* idiopathic inflammatory myopathies, *DM* dermatomyositis, *PM* polymyosititis, *IBM* inclusion body myositis, T^+ tonsil positive control, T^- negative control)

munohistochemical detection of proteins involved in the regeneration process was performed [17]; serial 5-µm-thick frozen sections were incubated with anti-polysialic acid-neural cell adhesion molecule (PSA-NCAM) antibody (kind gift of G. Rougon) and developmental myosin heavy chain antibody (Novocastra, Tebu, Le Perray en Yvelines, France).

Results

CCR2A and CCR2B mRNA expression in muscles and controls

CCR2A and CCR2B mRNA expression was studied in IIM and control muscles using RT-PCR (Fig. 1). CCR2A and CCR2B amplification products were observed in all samples tested at the predicted size (311 bp and 287 bp for CCR2A and CCR2B, respectively). However, the intensity of the signal varies from one case to another and is sometimes observed only after prolonged exposure of the membrane to X-ray-sensitive films.

The signal for CCR2B PCR products was almost the same whatever the subtype of IIM (DM, PM or IBM), and was not significantly higher in IIM versus control muscle (P=0.96). In contrast, the signal for CCR2A was higher in muscles from patients suffering from IIM versus (P= 0.02). Moreover, the intensity was higher in the PM/IBM group than in the DM group (P=0.04). The signal for both CCR2A and CCR2B was intense in tonsil.

Localization of CCR2A mRNA expression in muscles

This was done by in situ hybridization (Fig. 2). Using mRNA sense probe, no signal was observed. In contrast, the mRNA CCR2A antisense probe showed a signal in mononuclear inflammatory infiltrates in all IIM. Interestingly, in IIM the signal was stronger in the endomysial inflammatory exudates and especially in inflammatory cells involved in partial invasion in PM and IBM. A low signal was also observed in vascular smooth muscle cells in both normal muscle and IIM (data not shown). Endothelial cells, however, were negative (data not shown).

Localization of CCR2A and CCR2B protein products in muscles

In normal muscles, anti-CCR2A immunoreactivity was observed in all vessels except capillaries. The immunostaining was localized in smooth muscle fibers but not in endothelial cells. No immunostaining was observed after the preincubation of the primary antibody with the blocking peptide. In IIM, the same pattern of immunostaining was observed in blood vessels with anti-CCR2A (Fig. 3A). In addition, some mononuclear cells expressed CCR2A especially among the mononuclear cells involved in partial invasion in PM and IBM (Fig. 3B). Immunoreactive inflammatory cells were rarely seen in DM (Fig. 3A). In normal muscles, anti-CCR2B expression was observed in all satellite cells (Fig. 3C) and in the muscular domain of neuromuscular junctions (Fig. 4A, B). The immunostaining was abolished after preincubation of the antibody with the blocking peptide. In IIM some regenerative fibers were strongly and diffusely immunostained with anti-CCR2B antibody (Figs. 3C, 4D). The same expression pattern was observed in rhabdomyolytic muscles (data not shown).

On serial sections, it was obvious that CCR2B-immunostained fibers also expressed other regenerative markers such as developmental myosin heavy chain (Fig. 4E) and PSA-NCAM (Fig. 4F).

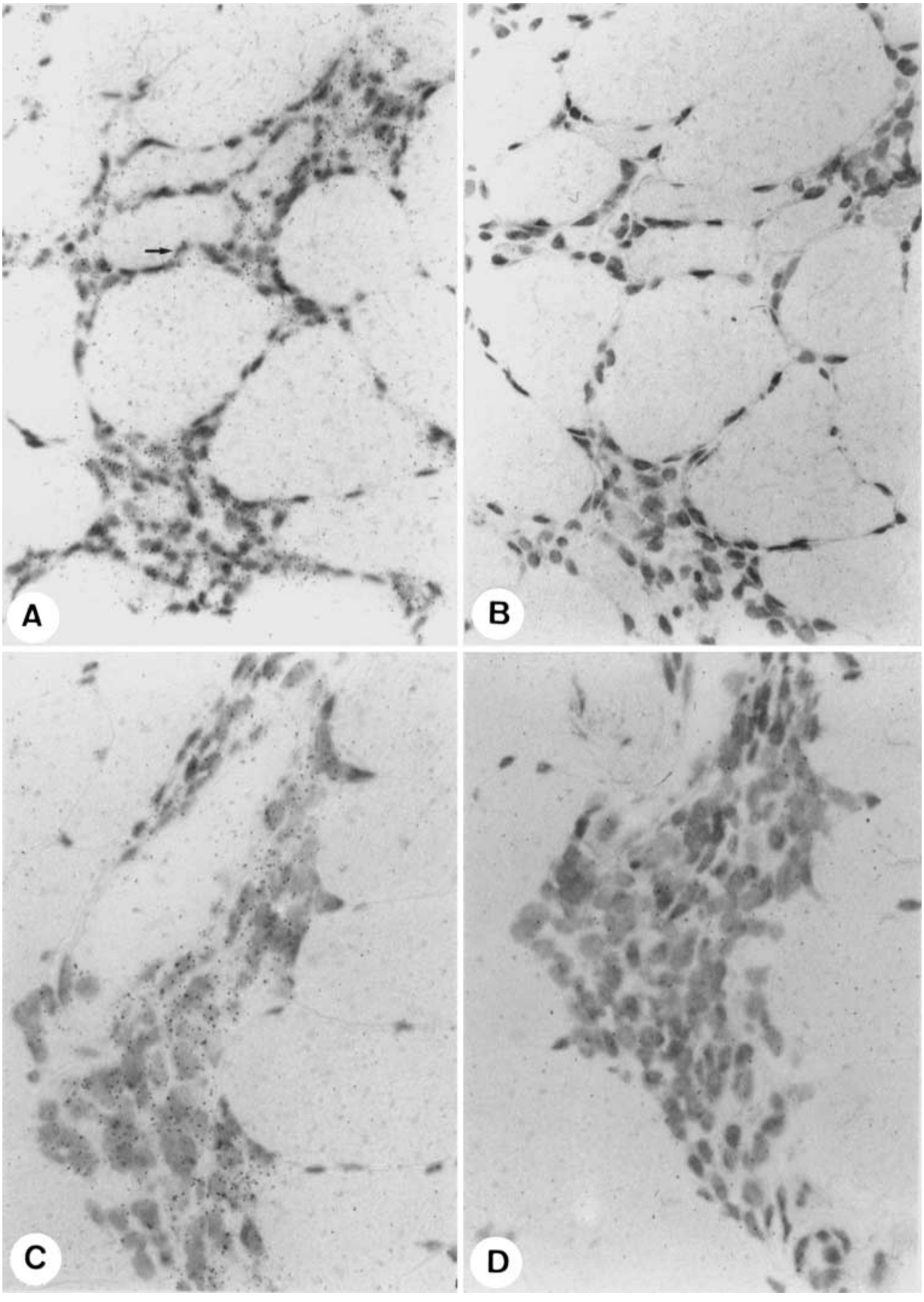

Fig. 2A–D Distribution of the CCR2A mRNA by in situ hybridization. Positive cells were mainly located in inflammatory infiltrates in PM (**A**) and DM (**C**). In PM, cells involved in partial invasion were strongly positive (**A**, *arrow*). Controls with the sense probe were negative (**B**, PM, and **D,** DM, serial sections with **A** and **C**, respectively). **A**, **B** ×300; **C**, **D** ×500

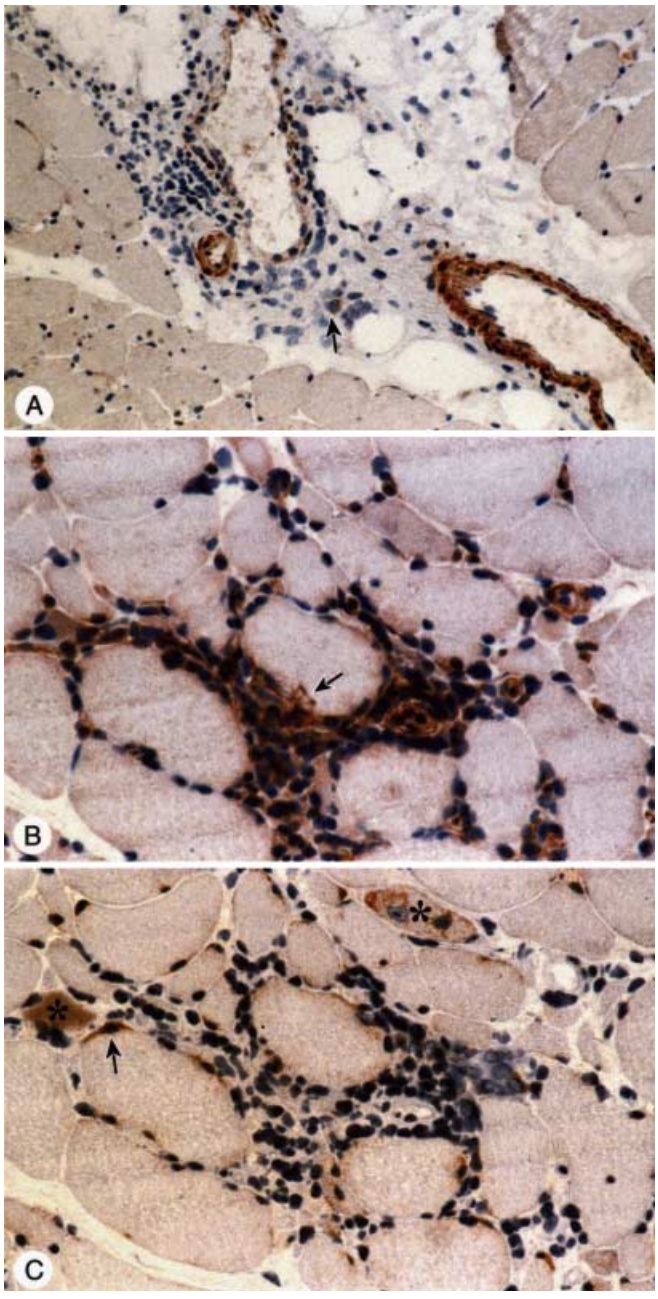

Fig. 3A–C Immunohistochemical localization of CCR2A and CCR2B in DM and PM. **A** CCR2A stained vessel walls and few mononuclear cells (*arrow*) in DM. **B** Numerous inflammatory cells partially invading non-necrotic muscle fibers strongly expressed CCR2A in PM (*arrow*). **C** These inflammatory cells do not express CCR2B. Note the CCR2B expression in a satellite cell (*arrow*) and in regenerative fibers (*asterisk*). **A** ×185; **B**, **C** ×250

In tonsil, very few mononuclear cells expressed CCR2B, whereas numerous cells expressed CCR2A (Fig. 4C).

Discussion

The results of recent studies on the molecules produced in inflammatory muscle tissue have provided new insights into the functional aspects of the chronic inflammation in IIM. Recently, cytokines have become targets for the development of a completely new concept of therapy in these diseases [6, 11]. Because of the significant role of monocyte/macrophage infiltration in IIM, MCP-1 receptors also represent a potentially important target for therapy. A prerequisite was the definition of CCR2 expression pattern in IIM. In this study we report that (1) CCR2 is expressed at low level in normal muscle, (2) CCR2 (A and B) is up-regulated in IIM, (3) in both normal and IIM muscles CCR2 isoforms (CCR2A and CCR2B) are expressed by different cell subsets, and finally (4) CCR2A is mainly expressed by mononuclear cells partially invading non-necrotic muscle fibers in both PM and IBM.

In normal and diseased muscles, CCR2A was observed in vascular smooth muscle cells, in keeping with the CCR2 expression previously reported by Hayes et al. [21]. We also report for the first time CCR2B expression in satellite cells and in the muscular domain of the neuromuscular junction. CCR2B was also observed in regenerative fibers and the immunoreactivity was abolished after preincubation of the CCR2B antibody with the CCR2B blocking peptide. CCR2B expression by these fibers was transient and disappeared before the down-regulation of the developmental myosin heavy chain [17]. Recently, Yamamoto et al. [36] described in the 5´ untranslated region of the CCR2 gene Oct-1 and C/EBP binding sequences essential for the transcriptional activation and the tissue-specific expression of CCR2. Moreover, several consensus E-boxes (-CANNTG-) [31] were present in this region. E-boxes are the molecular target for muscle regulatory factors (MRF) and, although additional flanking nucleotides also have a role in DNA target specificity, binding of the MRF to E-box elements located within the regulatory regions of most muscle-specific genes is often sufficient to activate muscle gene expression [19]. Additional studies need to be done to elucidate the factors responsible for CCR2A and CCR2B muscle-specific expression, and moreover, although the expression of CCR2A by the smooth muscle cells is associated with chemotaxis and proliferation [21], the specific role of CCR2B in the regeneration process is unknown at present. Previous studies have also reported other signaling molecules such as interleukin (IL)-1 or IL-6 in normal end plates and in the regeneration process [2, 22, 25]. Interestingly, IL-6 expression by regenerative fibers was up-regulated by local production of cytokines in IIM [3].

It is likely that the locally produced cytokines also regulate CCR2B expression by regenerative fibers. However, CCR2 regulation by cytokines is complex, and it has been demonstrated that the same cytokine (such as IL-1 or TNF-α) regulates CCR2 expression either negatively or positively in distinct cellular systems [14, 34].

In contrast to some previous reports [34], but in keeping with others [15], we failed to show any CCR2 expression in normal or inflamed endothelial cells.

RT-PCR analysis demonstrates CCR2A up-regulation in IIM. Quantification, however, reveals that CCR2B was not significantly up-regulated in IIM. Immunohistochem-

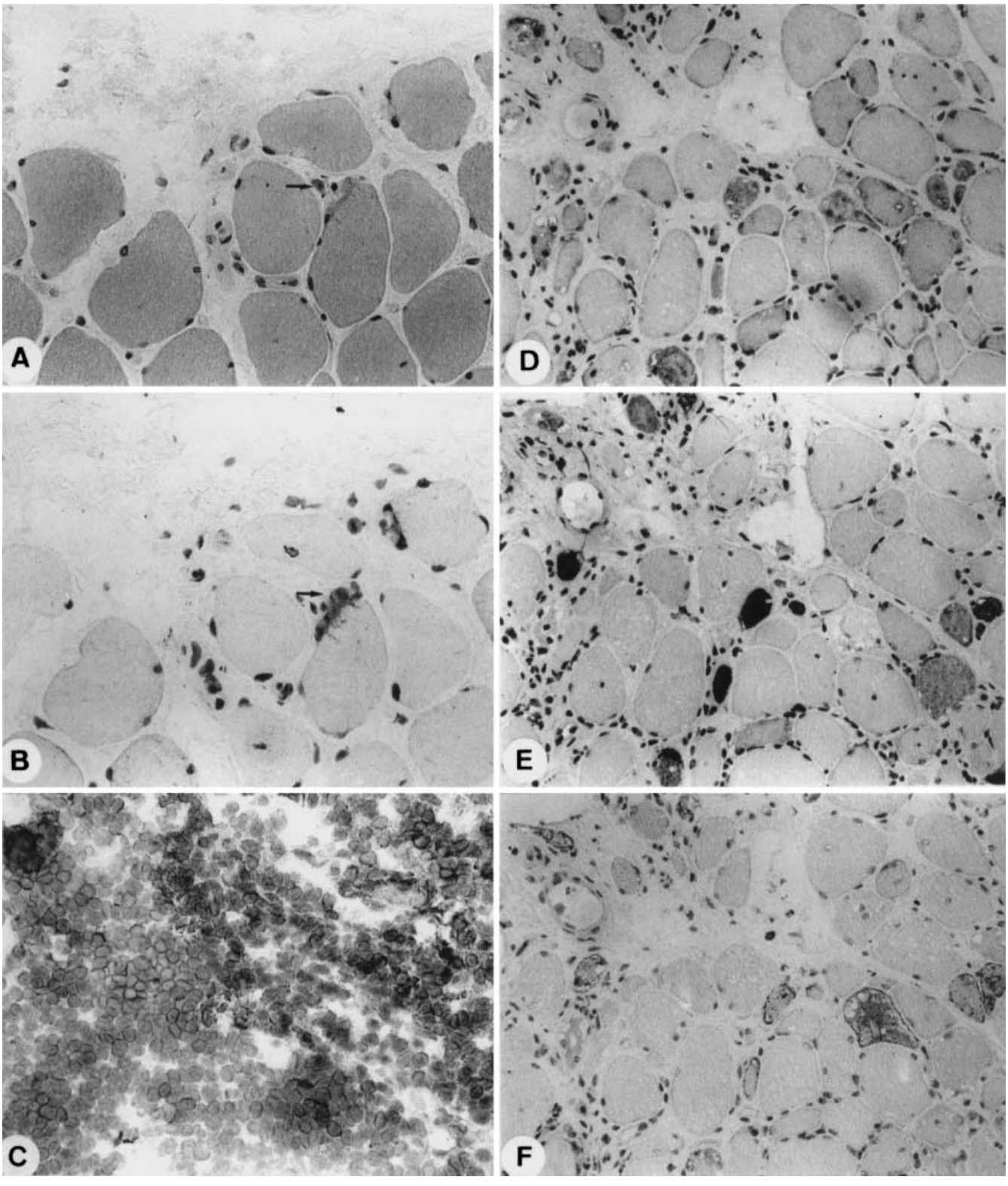

Fig. 4 **A**, **B** Hematoxylin eosin staining (**A**) and immunohistochemical localization of CCR2B (**B**) in normal muscle. The muscular domain of the neuromuscular junction is stained by anti-CCR2B (*arrow*). **C** Immunohistochemical localization of CCR2A in tonsil. Several mononuclear cells are stained. **D**–**F** Distribution of CCR2B and two different regeneration markers in IBM. Immunohistochemical localization of CCR2B (**D**), developmental myosin heavy chain (**E**) and PSA-NCAM (**F**). CCR2B and PSA-NCAM stained the same regenerative fibers (*PSA* polysialic acid, *NCAM* neural cell adhesion molecule). **A**, **B** ×300; **C**–**F** ×185

istry failed to demonstrate significant CCR2B accumulation in inflammatory cells. Although CCR2B is the predominant CCR2 isoform described in human monocytes, it is possible that this isoform is not preferentially expressed in other cells expressing CCR2, such as T lymphocytes [5]. In keeping with that, immunohistochemical detection of CCR2 in tonsil (lacking monocytes) demonstrates strong CCR2A immunoreactivity on mononuclear cells but very low CCR2B expression. However, in spite of CCR2B expression by regenerative fibers in IIM, RT-PCR failed to demonstrate significant CCR2B up-regulation in IIM. In contrast, CCR2A was the predominant isoform of the MCP-1 receptor expressed by mononuclear cells in IIM [35]. Although the biological significance of the existence of two CCR2 variants has not been elucidated, results of the present study suggest that in some cases a given cell may choose to express one type of receptor. There is precedent, however, for differential expression of chemokine receptors by different cell subsets [8, 33].

Semiquantitative RT-PCR analysis showed that CCR2A is higher in PM and IBM than in DM. Moreover, the local expression of CCR2A is different in DM and in PM and IBM. In DM, only a few perivascular inflammatory exudates express CCR2A mRNA by in situ hybridization, whereas the protein is almost absent. The perivascular inflammatory exudates in DM are mainly composed of $CD4^+$ lymphocytes, B lymphocytes and macrophages. The perivascular macrophages result from the recruitment of monocytes to inflammatory foci after specific endothelial interaction and migration under chemotactic gradient in which MCP-1 plays a major role. We have previously reported that perivascular macrophages in DM strongly upregulate MCP-1 mRNA [28]. This is in contrast with the low level of CCR2A in these cells. However, it has been recently demonstrated that differentiation of monocytes into macrophages is associated with a strong reduction of CCR2 expression at the plasma membrane as well as in the MCP-1 binding [35]. Moreover, monocyte differentiation also resulted in an increased secretion of MCP-1 that, at least in part, is responsible for the down-modulation of its receptor, CCR2. The disappearance of CCR2 in differentiated macrophages results in the unresponsiveness of these cells to MCP-1 providing an efficient regulatory system for controlling the extent of macrophage recruitment and activation, and explaining the perivascular location of macrophages exudates in DM.

In contrast, endomysial inflammatory cells, especially cells partially invading non-necrotic muscle fibers, strongly express CCR2 in both PM and IBM together with MCP-1 [28]. These cells are mainly $CD8^+$ T cells and macrophages, and it is likely that in both PM and IBM, $CD8^+$ cells are mainly responsible for CCR2A expression. $CCR2^+$ T cells also occurred in synovial effusions of patients with different forms of arthritis [30]. Moreover, it has previously been shown that, in the course of intracerebral viral infection, virus-activated $CD8^+$ T cells strongly express CCR2 [32].

In conclusion, the present study highlights the major role played by MCP-1 and its counter-receptor CCR2 in the pathophysiology of IIM. It shows that the CCR2 isoforms are cell specific, that CCR2B is expressed by satellite and regenerative fibers and that CCR2A is the major isoform expressed by mononuclear cells and strongly upregulated in IIM. Moreover, variations in the total amount of CCR2A production as well as in its local distribution are observed according to the type of IIM in keeping with their distinctive pathogenesis. These results are of interest and identify CCR2A as a possible new target for therapeutic intervention in IIM.

References

1. Adams EM, Kirkley J, Eidelman G, Dohlman J, Plotz PH (1997) The predominance of beta (CC) chemokine transcripts in idiopathic inflammatory muscle diseases. Proc Assoc Am Physicians 108:275–285
2. Authier FJ, Chazaud B, Mhiri C, Eliezer-Vanerot MC, Poron F, Barlovatz-Meimon G, Gherardi RK (1997) Interleukin-1 expression in normal motor endplates and muscle fibers showing neurogenic changes. Acta Neuropathol 94:272–279
3. Bartoccioni E, Michaelis D, Hohlfeld R (1994) Constitutive and cytokine-induced production of interleukin-6 by human myoblasts. Immunol Lett 42:135–138
4. Carpenter S, Karpati G (1992) The pathological diagnosis of specific inflammatory myopathies. Brain Pathol 2:13–19
5. Cau MW, Roth SJ, Luther E, Rose SS, Springer TA (1994) Monocyte chemoattractant protein 1 acts as a T-lymphocyte chemoattractant. Proc Natl Acad Sci USA 91:3652–3656
6. Charles P, Elliott MJ, Davis D, Potter A, Kalden JR, Antoni C, Breedveld FC, Smolen JS, Eberl G, deWoody K, Feldmann M, Maini RN (1999) Regulation of cytokines, cytokine inhibitors, and acute-phase proteins following anti-TNF alpha therapy in rheumatoid arthritis. J Immunol 163:1521–1528
7. Charo IF, Myers SJ, Herman A, Franci C, Connolly AJ, Coughlin SR (1994) Molecular cloning and functional expression of two monocyte chemoattractant protein 1 receptors reveals alternative splicing of the carboxyl-terminal tails. Proc Natl Acad Sci USA 91:2752–2756
8. Chuntharapai A, Lee J, Hebert CA, Kim KJ (1994) Monoclonal antibodies detect different distribution pattern of IL-8 receptor A and on human peripheral blood leukocytes. J Immunol 153: 5682–5688
9. Confalonieri P, Bernasconi P, Megna P, Galbiati S, Cornelio F, Mantegazza R (2000) Increased expression of β-chemokines in muscle of patients with inflammatory myopathies. J Neuropathol Exp Neurol 59:164–169
10. Dalakas M (1991) Polymyositis, dermatomyositis and inclusion-body myositis. N Engl J Med 325:1487–1498
11. Dalakas M (1998) Molecular immunology and genetics of inflammatory muscle diseases. Arch Neurol 55:1509–1512
12. De Bleecker JL, Engel AG (1994) Expression of cell adhesion molecules in inflammatory myopathies and Duchenne dystrophy. J Neuropathol Exp Neurol 53:369–376
13. Engel AG, Arahata K (1986) Mononuclear cells in myopathies. Quantitation of functionally distinct subsets, recognition of antigen-specific cell-mediated cytotoxicity in some diseases, and implications for the pathogenesis of the different inflammatory myopathies. Hum Pathol 117:704–721
14. Fantuzzi L, Borghi P, Ciolli V, Pavlakis G, Belardelli F, Gessani S (1999) Loss of CCR2 expression and functionnal response to monocyte chemotactic protein (MCP-1) during the differentiation of human monocytes: role of secreted MCP-1 in the regulation of the chemotactic response. Blood 94:875–883
15. Feil C, Augustin HG (1998) Endothelial cells differentially expressed functional CXC-chemokine receptor-4 (CXR4/fusin) under the control of autocrine activity and exogenous cytokines. Biochem Biophys Res Commun 247:38–45

16. Figarella-Branger D, Pellissier JF, Bianco N, Devictor B, Toga M (1990) Inflammatory and non-inflammatory inclusion body myositis. Characterization of the mononuclear cells and expression of the immunoreactive class I major histocompatibility complex product. Acta Neuropathol 79:528–536
17. Figarella-Branger D, Pellissier JF, Bianco N, Karpati G (1999) Sequence of expression of MyoD1and various cell surface and cytoskeletal proteins in regenerating mouse fibers following treatment with sodium dihydrogen phosphate. J Neurol Sci 170: 151–160
18. Franci C, Wong LM, Van Damme J, Proost P, Charo IF (1995) Monocyte chemoattractant protein-3, but not monocyte chemoattractant protein-2 is a functional ligand of the human monocyte chemoattractant protein-1 receptor. J Immunol 154:6511–6517
19. Funk WD, Wright WE (1992) Cyclic amplification and selection of targets for multicomponent complexes: myogenin interacts with factors recognizing binding sites for basic helix-loop-helix, nuclear factor 1, myocyte-specific enhancer-binding factor 2, and COMP1 factor. Proc Natl Acad Sci USA 89:9484–9488
20. Gong X, Gong W, Kuhn DB, Ben-Baruch A, Howard OMZ, Wang JM (1997) Monocyte chemotactic protein-2 (MCP-2) uses CCR1 and CCR2B as its functional receptors. J Biol Chem 272:11682–11685
21. Hayes IM, Jordan NJ, Towers S, Smith G, Paterson JR, Earnshaw JJ, Roach AG, Westwick J, Williams RJ (1998) Human vascular smooth muscle cells express receptors for CC Chemokines. Arterioscler Thromb Vasc Biol 18:397–403
22. Kami K, Morikawa Y, Sekimoto M, Senba E (2000) Gene expression of receptors for IL-6, LIF, and CNTF in regenerating skeletal muscles. J Histochem Cytochem 48:1203–1213
23. Karpati G, Pouliot Y, Carpenter S (1988) Expression of immunoreactive major histocompatibility complex products in human skeleteal muscle. Ann Neurol 23:64–72
24. Kissel JT, Mendell JR, Rammohen KW (1986) Microvascular deposition of complement membrane attack complex in dermatomyositis. N Engl J Med 314:329–334
25. Kurek JB, Nouri S, Kannourakis G, Murphy M, Austin L (1996) Leukemia inhibitor factor and interleukin-6 are produced by disease and regenerating skeletal muscle. Muscle Nerve 19:1291–12301
26. Lepidi H, Frances V, Figarella-Branger D, Bartoli C, Machado-Baeta A, Pellissier JF (1998) Local expression of cytokins in idiopathic inflammatory myopathies. Neuropathol Appl Neurobiol 24:73–79
27. Liprandi A, Figarella-Branger D, Daniel L, Lepidi H, Bartoli C, Pellissier JF (1999) Expression des molécules d'adhésion dans les myopathies inflammatoires idiopathiques. Ann Pathol 19:12–18
28. Liprandi A, Bartoli C, Figarella-Branger D, Pellissier JF, Lepidi H (1999) Local expression of monocyte chemoattractant protein-1 (MCP-1) in idiopathic inflammatory myopathies. Acta Neuropathol 97:642–648
29. Lundberg IE, Nyberg P (1998) New developments in the role of cytokines and chemokines in inflammatory myopathies. Curr Opin Rheumatol 10:521–529
30. Mack M, Bruhl H, Gruber R, Jaeger C, Cihak J, Eiter V, Plachy J, Stangassinger M, Uhlig K, Schattenkirchner M, Schlondorff D (1999) Predominance of mononuclear cells expressing the chemokine receptor CCR5 in synovial effusions of patients with different forms or arthritis. Arthritis Rheum 42:981–988
31. Murre C, McCaw PS, Baltimore D (1989) A new DNA binding and dimerization motif in immunoglobulin enhancer binding, daughterless, MyoD, and myc proteins. Cell 56:777–783
32. Nansen A, Marker O, Bartholdy C, Thomsen AR (2000) $CCR2^+$ and $CCR5^+$ $CD8^+$ T cells increase during viral infection and migrate to sites of infection. Eur J Immunol 30:1797–1806
33. Raychowdhury MK, Yukawa M; Collins LJ, McGrail SH, Kent KC, Ware JA (1994) Alternative splicing procedures of a divergent cytoplasmic tail in the human endothelial receptor. J Biol Chem 269:19256–19261
34. Weber KSC, Nelson PJ, Gröne HJ, Weber C (1999) Expression of CCR2 by endothelial cells implications for MCP-1 mediated wound injury repair and in vivo inflammatory activation of endothelium. Arterioscler Thromb Vasc Biol 19:2085–2093
35. Wong LM, Myers SJ, Tsou CL, Gosling J, Arai H, Charo IF (1997) Organization and differential expression of the human monocyte chemoattractant protein 1 receptor gene. Evidence for the role of the carboxyl-terminal tail in receptor trafficking. J Biol Chem 272:1038–1045
36. Yamamoto K, Takeshima H, Hamada K, Nakao M, Kino T, Nishi T, Kochi M, Kuratsu JI, Yoshimura T, Ushio Y (1999) Cloning and functional characterization of the 5'-flanking region of the human monocyte chemoattractant protein-1 receptor (CCR2) gene. J Biol Chem 274:4646–4654