[Frontiers in Bioscience 13, 2548-2577, January 1, 2008]

Chemokines in idiopathic inflammatory myopathies

Boel De Paepe, Kim K. Creus, Jan L. De Bleecker

Department of Neurology, Ghent University Hospital, Ghent, Belgium

TABLE OF CONTENTS

1. ABSTRACT

The idiopathic inflammatory myopathies (IIM) represent a heterogeneous group of acquired muscle diseases. The three best-studied subgroups: dermatomyositis (DM), polymyositis (PM), and sporadic inclusion body myositis (IBM), differ considerably both clinically and pathophysiologically. DM is a chiefly humoral endotheliopathy often associated with characteristic skin manifestations. In PM and IBM nonnecrotic muscle fibers are invaded by auto-aggressive cytotoxic T-cells and macrophages. IBM presents with additional structural abnormalities of myofibers, including rimmed vacuoles and depositions of ectopic proteins. Data accumulates implicating the chemokines in the immunopathogenesis of the different IIM. This review bundles current knowledge of the chemokine and chemokine receptor expression in the skeletal muscle of DM, PM and IBM patients. The IIM are characterized by a general increase of specific chemokines, while the chemokine distribution reflects the two different immune responses represented within these muscle diseases: (I) The endotheliopathy of DM is characterized by increased levels of CXCL12 and CCL2 in the affected blood vessels, (II) In the myocytotoxic immune response of PM and IBM, active invasion of nonnecrotic myofibers by inflammatory cells is associated with CXCL10 and CCL2 upregulation. The ever accumulating data illustrates the important role of the chemokine system in IIM, indicating the therapeutic potential of interfering agents. This raises hopes for future treatments for DM and PM with fewer side effects, and the possible establishment of a therapy suited for IBM, a myopathy which has proven unresponsive to all available immuno-modulating interventions up till now.

2. IDIOPATHIC INFLAMMATORY MYOPATHIES

The idiopathic inflammatory myopathies (IIM) are a group of acquired inflammatory muscle diseases of which the three main disease entities are: dermatomyositis (DM), polymyositis (PM) and sporadic inclusion body myositis (IBM). DM, PM and IBM can be differentiated by a combination of clinical features, muscle histology, and response to immunosuppressive therapy "Table 1". The cause of the IIM remains unknown, but an autoimmune pathogenesis is suspected.

2.1. Dermatomyositis

DM affects children and adults. It most often develops subacutely, but symptoms may also appear abruptly or insidiously. DM patients complain of myalgias and muscle weakness of proximal muscle groups. The skin manifestations that accompany or precede muscle weakness are distinct. They include a heliotrope rash of the face, neck and chest (1). DM can present with systemic symptoms which include joint, pulmonary, cardiac, and gastrointestinal manifestations. DM can also occur in an overlap syndrome with a systemic collagen vascular disease (2) or in association with malignancy (3).

DM is a complement-mediated microangiopathy. Blood vessel damage, mostly at perifascicular and perimysial sites, is initiated by deposition of the membrane attack complex, the end-product of the complement cascade (4). Tissue ischemia results in atrophy of the perifascicular myofibers and inflammatory cells accumulate at the perimysium. These leukocytic aggregates consist mostly of CD4 positive T-cells and B-cells, and contain smaller amounts of dendritic cells and macrophages. High relative

Table 1. Clinical and myopathological characteristics of idiopathic inflammatory myopathies

	DM	PM	IBM
Clinical presentation			
Age of onset	all ages	adult	over 50
Sex preference	female	female	male
Family history	no	no	rare
Weakness	proximal>distal	proximal>distal	proximal=distal
Rash	yes	no	no
Serum creatine kinase	↑↑↑	↑↑↑	normal or ↑
Association with cancer	yes (15%)	maybe	no
Response to glucocorticoids	good	good	refractory
Muscle fiber damage			
Myopathic changes	mostly perifascicular	mostly endomysial	mostly endomysial
Rimmed vacuoles	-	-	++
Amyloid-like fibrils	-	-	+++
Blood vessel damage			
Endotheliopathy	+++	+	+
Complement deposition	+++	-	-
Inflammation			
Most frequent site	perimysial, perivascular	endomysial	endomysial
Invasion of non-necrotic fibers	-	+++	+++
CD4+ T-cells	+++	++	++
CD8+ T-cells	-	++	++
B-cells	+++	-	-
Macrophages	+	++	++
Dendritic cells	plasmacytoid>myeloid	plasmacytoid<myeloid	plasmacytoid<myeloid

The three main idiopathic inflammatory myopathies are: dermatomyositis (DM), polymyositis (PM) and sporadic inclusion body myositis (IBM). Pathological features and effector cells are scored very abundant (+++) abundant (++), moderate (+), to absent (-).

amounts of dendritic cells of the plasmacytoid lineage, illustrate the important role of the innate immune system in DM (5).

2.2. Polymyositis

Most PM patients are 18 years or older. They develop subacute symmetric proximal weakness of arms and legs, and suffer from myalgias that can last for weeks or months. PM mimics other myopathies, and remains a diagnosis of exclusion. Usually it occurs in association with systemic autoimmune disorders or viral infections (6). As a separate disease entity, PM is very rare. Re-diagnosis of treatment-resistant PM to IBM has been repeatedly recognized (7-8), and re-examination has revealed that a subset of patients later develop IBM features (9).

In PM, muscle tissues are affected by an antigen-directed myocytotoxic immune reaction mediated by CD8 positive T-cells. Cytotoxic T-cells and macrophages surround and invade nonnecrotic muscle fibers. Myofibers with normal appearance express major histocompatibility complex class I (MHCI) antigens (10) and co-stimulatory molecules (11), and on the sarcolemma of fibers facing autoaggressive infiltrates intercellular adhesion molecule-1 (ICAM-1) is induced (12). By expressing these immuno-active molecules on their surface, myofibers act as active participants in the immune reactions of PM.

2.3. Sporadic inclusion body myositis

IBM is the most common IIM among patients older than 50. Patients suffer from progressive distal and proximal myopathy. Muscle wasting and weakness are usually most pronounced in knee extensors, and hip and finger flexors. The disease is usually slowly progressive and has a more benign nature, but can eventually lead to severe disability (13).

Immunopathologic features are distinct from DM, but the differential diagnosis with PM may require additional electron microscopic or immuno-pathological studies. A fundamental difference with PM is that in IBM the autoimmune inflammatory changes coexist with degenerative changes. In conjunction with invasion of intact myofibers by auto-aggressive infiltrates, vacuolated myofibers appear that contain deposits of degeneration-associated molecules. These fibers accumulate ectopic proteins such as beta-amyloid (14), ubiquitin (15) and phosphorylated tau (16), but rarely are the target of endomysial cytotoxic T-cells and macrophages. It is unknown whether the degeneration and inflammation pathomechanisms of IBM are linked or not. Recently, it has been demonstrated that beta-amyloid is targeted for lysosomal degradation via macro-autophagy (17). A viral etiology has been speculated, and evidence accumulates that implicates retroviruses as initiators of the disease. IBM can be seen in association with human immunodeficiency virus (HIV) and human T-lymphocyte virus type 1 (HTLV1) infection (18). Clonally expanded HIV-specific CD8 positive T-cells invade myofibers in HIV-infected IBM patients (19). Although the inflammatory changes are the most prevalent myopathologic abnormality (20), no immunosuppressive or immunomodulating therapy seems to be effective for IBM.

3. CHEMOKINE LEVELS IN THE IDIOPATHIC INFLAMMATORY MYOPATHIES

Based on comparative patient versus control studies, chemokines can be divided into two functional categories: (I) constitutively expressed chemokines that regulate lymphatic system architecture and homeostasis, and (II) inducible chemokines that are expressed during tissue repair and inflammation. Inappropriate and prolonged expression of inducible chemokines leads to the infiltration of healthy tissues by leukocytes and the development of autoimmunity (21).

Table 2. Chemokine and chemokine receptor transcripts in skeletal muscle biopsies

	NM	DM	PM	IBM	Ref
alpha-family					
CXCL9	low (n=18)	ND	ND	strongly elevated (n=19)*	22
CXCL10	low (n=18)	ND	ND	elevated (n=19)*	22
Beta-family					
CCR1	268±344 (n=4)	6259±3219 (n=6)*	5784±2999 (n=5)*	1788±1612 (n=5)	23
CCR2A	present in (n=4)	elevated (n=8)*	strongly elevated (n=5)*	elevated (n=4)*	24
CCR2B	present in 3/4	present in 6/8	present in 4/5	present in 2/4	24
CCR5	179±212 (n=4)	3938±4490 (n=6)	4077±1673 (n=5)*	3186±1308 (n=5)*	23
CCR7	ND#	ND	present (n=3)	present (n=1)	25
CCL2	low (n=4)	strongly elevated (n=8)	elevated (n=5)	slightly elevated (n=4)	26
	absent (n=3)	present in (n=3)	present (n=3)	present (n=3)	27
CCL3	absent (n=3)	present in (n=7)	present in 6/7	ND	28
	absent (n=3)	present in (n=3)	present (n=3)	present (n=3)	27
	1±1	4903±3460 (n=6)*	8597±12273 (n=5)*	2844±1214 (n=5)*	23
CCL4	absent (n=3)	present in 3/7	present in 3/7	ND	28
	264±527 (n=4)	3449±4150 (n=6)*	3644±2588 (n=5)*	2704±2667 (n=5)	23
CCL5	absent (n=3)	present in 4/7	present in 4/7	ND	28
	274±335 (n=4)	1810±1667 (n=6)	1248±1991 (n=5)	2379±3017 (n=5)	23
CCL19	ND#	ND	present (n=3)	absent (n=1)	25
CCL21	ND#	ND	present (n=3)	absent (n=1)	25

mRNA densities were evaluated through RT-PCR. Where available, arbitrary quantitative units are given. Abbreviations: normal skeletal muscle (NM), dermatomyositis (DM), polymyositis (PM), sporadic inclusion body myositis (IBM), reference (Ref), not determined (ND).*Studies in which a significant increase in the different IIM compared to NM was shown; #Studies where only disease controls were included.

Table 3. Chemokine and chemokine receptor protein levels in skeletal muscle biopsies

	NM	DM	PM	IBM	Reference
alpha-family					
CXCR1	0 (n=3)	0 (n=1)	0 (n=1)	0 (n=1)	29
CXCR2	474±107 (n=5)	2433 (n=1)	2818±2207 (n=5)*	4019 (n=1)	29
CXCR3	183±26 (n=4)	1027±338 (n=4)*	1888±614 (n=4)*	1170 (n=1)	29
CXCR4	237±59 (n=3)	2059 (n=1)	1696 (n=1)	1210 (n=1)	30
CXCL9	0 (n=3)	0 (n=1)	0 (n=1)	0 (n=1)	29
CXCL10	128±26 (n=4)	7549±1510 (n=4)*	7246±5016 (n=4)*	924 (n=1)	29
CXCL11	370 (n=1)	350 (n=1)	390 (n=1)	470 (n=1)	29
CXCL12a	623±85 (n=3)	1373 (n=1)	970 (n=1)	1418 (n=1)	30
CXCL12b	541±101 (n=3)	544 (n=1)	820 (n=1)	912 (n=1)	30
beta-family					
CCR1	3603 (n=1)	150 (n=1)	382±239 (n=4)*	214 (n=1)	31
CCR2A	0 (n=4)	370±49 (n=4)*	538±1232 (n=4)*	520 (n=1)	31
CCR2B	156±122 (n=4)	344±78 (n=4)	472±258 (n=4)	1776 (n=1)	31
CCR3	750 (n=1)	288 (n=1)	1367 (n=1)	178 (n=1)	31
CCR4	862±442 (n=4)	1779 (n=1)	1458±328 (n=4)	4920 (n=1)	31
CCR5	215±62 (n=4)	598 (n=1)	1179±203 (n=4)*	557 (n=1)	31
CCR8	346±179 (n=4)	ND	274±146 (n=4)	ND	32

Protein densities were quantified through Western blotting; Abbreviations: normal skeletal muscle (NM), dermatomyositis (DM), polymyositis (PM), sporadic inclusion body myositis (IBM), number of samples analyzed (n), not determined (ND), significantly altered levels compared to NM (*).

Several studies have been published comparing the levels of chemokine and chemokine receptor transcript "Table 2" (22-28) and protein "Table 3" (29-32) in muscle biopsies of IIM patients with normal muscle. The results show that the expression of some chemokines and chemokine receptors is absent from controls but is induced in IIM patients, whereas others are constitutively expressed but markedly upregulated in IIM muscle. Large variations in expression levels are observed within and between different studies. These differences probably originate from variations during disease progression, and the variable severity of inflammation in individual samples and patients.

Autoimmune diseases mediated by auto-aggressive T-cells are usually controlled by helper T-cells type 1 (Th1)-driven immune reactions (33). Based on their main immunologic features, PM and IBM are considered Th1-mediated. In DM a polarization favoring Th1 is also presumed, as the inflammatory infiltrates of all three IIM equally express tumor necrosis factor-alpha (TNF-alpha) (34) and interleukin-18 (IL-18) (35). An increase of the Th1-associated chemokine receptors CXCR3 and CCR5 is observed in IIM muscle, which is accompanied by significantly higher levels of their ligands CXCL9, CXCL10, CCL3 and CCL4. In contrast, their Th2-associated counterparts CCR4 and CCR8 are unaltered in patients. This further supports the Th1-mediated immunopathogenesis of the three different IIM.

4. LOCALIZATION OF CHEMOKINES IN THE IDIOPATHIC INFLAMMATORY MYOPATHIES

The quantitative data presented in Tables 2 and 3 has been obtained through analysis of total skeletal muscle biopsies, and thus reflect the general chemokine levels of the mixture of tissue constituents. The distribution of these factors between myofibers, blood vessels and leukocytes will be discussed hereunder.

Table 4. Blood vessel chemokine and chemokine receptor expression in the idiopathic inflammatory myopathies

	NM	DM	PM	IBM	Reference
alpha-family					
CXCR1	-	-	-	-	29
CXCR2	-	-	-	-	29
CXCR3	-	-	-	-	29
	+	ND	ND	+	22
CXCR4	++	++	++	++	29
CXCL1	+	++	+	+	29
CXCL2	+	++	+	+	29
CXCL3	+	++	+	+	29
CXCL8	±	±	±	±	39
CXCL9	-	-	-	-	29
	-	ND	ND	-	22
CXCL10	++	++	++	++	29
	-	ND	ND	-	22
CXCL11	±	±	±	±	29
	-	ND	ND	-	22
CXCL12α	-	-	-	-	30
CXCL12β	++	+++	++	++	30
beta-family					
CCR1	-	-	-	++	23
	+	+	+	+	31
CCR2A	+	+++	+++	+++	31
	++	++	++	++	24
CCR2B	±	±	±	±	31
	-	-	-	-	24
CCR3	+	+	+	+	31
CCR4	+	+	+	+	31
CCR5	+	+	+	+	31
CCR7	-	-	-	ND	38
	ND	-	-	-	25
CCR8	+	+	+	+	32
CCL2	-	+	+	+	26
	±	++	+	+	39
	ND	++	+	ND	35
CCL4	±	+++	+++	+++	23
CCL5	-	-	-	-	39
	-	-	-	-	23
CCL7	-	-	-	-	39
CCL19	ND	+	++	+	25
CCL21	ND	-	±	-	25
	ND	-	ND	ND	38

Chemokine and chemokine receptor protein expression as determined by immunohistochemical and immunofluorescent staining of muscle sections. For the IIM, the immunoreactivity of blood vessels in the vicinity of infiltrates are indicated. Few positive vessels (±); moderate (+), strong (++) to very strong (+++) staining. Altered expression in inflammatory myopathy is underlined. Abbreviations: normal skeletal muscle (NM), dermatomyositis (DM), polymyositis (PM), sporadic inclusion body myositis (IBM).

4.1. Intramuscular blood vessels

Blood vessels play an active role in targeting inflammatory cells to tissue sites. Leukocytic extravasation from the blood stream is a regulated multi-step process involving a series of coordinated interactions between leukocytes and the endothelial cells (36). These cellular interactions are both broad spectrum and highly specific in nature. In addition to cytokines and cell surface adhesion molecules, endothelial chemokine receptors allow more selective leukocyte recruitment and are capable of generating a local haptotactic chemokine gradient (37).

Several immunohistochemical studies have investigated chemokine expression in the intramuscular blood vessels of controls and IIM patients (22-26,29-32,35,38-39) "Table 4". A broad range of chemokine receptors is constitutively expressed by the endothelium and the mural elements of blood vessels. These factors presumably ensure normal immune surveillance of skeletal muscle (40), and organize endothelial cell migration and proliferation, events that are crucial for tissue response to injury and wound healing (41). In DM, the endothelium of endomysial blood vessels is the primary antigenic target. The earliest immunopathogenic event is the deposition of complement, which triggers vascular necrosis, perivascular inflammation and subsequent ischemia and myofiber destruction (42). The endotheliopathic immune response of DM is characterized by increased endothelial CXCL1, CXCL2, CXCL3, CXCL12beta, CCL2 and CCL4. In PM and IBM, focal upregulation of endothelial CCR2A and CCL4 in endomysial blood vessels (31) may aid to recruit the inflammatory aggregates, and drive the formation of endomysial inflammatory foci.

4.2. Muscle fibers

Muscle fibers are immuno-active structures that participate in the processes of leukocyte recruitment and activation (43). Myocytes can act as facultative antigen presenting cells, and express functional classical MHC, non-classical MHC, and adhesion and co-stimulatory molecules (44). This active involvement of muscle fibers in immune reactions is also illustrated by the intense focal staining for ICAM-1 on the sarcolemma of PM and IBM muscle fibers facing inflammatory cells (12-45). Normal

Table 5. Muscle fiber expression of chemokines and receptors in idiopathic inflammatory myopathies

	NM	DM	PM	IBM	Reference
alpha-family					
CXCR1	-	-	-	-	29
CXCR2	MN+	MN+	MN+	MN+	29
CXCR3	-	-	-	-	29
	-	ND	ND	MN+	22
CXCR4	-	-	-	-	29
CXCL1	-	-	-	-	29
CXCL2	-	-	-	-	29
CXCL3	-	-	-	-	29
CXCL8	-	-	-	-	39
	-	-	-	ND	47
CXCL9	-	-	-	-	29
	-	ND	ND	NNIF+	22
CXCL10	-	-	-	-	29
	-	ND	ND	-	22
CXCL11	-	-	-	-	29
	-	ND	ND	-	22
CXCL12α	T1F++,T2F+	T1F++,T2F+,RF++	T1F++,T2F+,RF++	T1F++,T2F+,RF++	30
CXCL12β	-	SNF+1/6,RF++	SNF+5/8,RF++	SNF+2/8,RF++,VF++	30
beta-family					
CCR1	-	-	-	-	31
	-	ND	NNIF±	NNIF±	23
CCR2A	-	-	-	-	31
	-	-	-	-	24
CCR2B	-	-	-	-	31
	SC+	SC+,RF+	SC+,RF±	SC+RF±	24
CCR3	-	-	-	-	31
CCR4	-	MNRF++	MNRF++	MNRF++	31
CCR5	-	-	-	-	31
	-	ND	NNIF±	NNIF±	23
	-	-	-	ND	38
CCR7	-	AF++,RF++	-	ND	38
	ND	-	-	-	25
CCR8	-	-	-	-	32
CCL2	-	-	-	-	31
	-	-	-	-	26
	-	SNF±	SNF±	ND	47
	-	-	-	-	27
CCL3	-	-	-	-	23
	-	-	-	-	27
CCL4	-	-	-	-	23
	-	-	-	-	32
CCL5	-	-	-	-	39
	-	-	-	-	23
CCL7	-	-	-	-	39
CCL19	ND	SNF±,AF±	SNF+	-	25
CCL21	ND	-	SNF±	-	25

Chemokine and chemokine receptor protein expression as determined by immunohistochemical and immunofluorescent staining of muscle sections, scored negative (-), varying staining intensities (±), moderate (+) to strong staining (++). Abbreviations: normal skeletal muscle (NM), dermatomyositis (DM), polymyositis (PM), sporadic inclusion body myositis (IBM), not determined or specified (ND), myonuclei of normal fibers (MN), myonuclei of regenerating fibers (MNRF), sarcoplasm of type 1 fibers (T1F), sarcoplasm of type 2 fibers (T2F), sarcoplasm of nonnecrotic invaded muscle fibers (NNIF), sarcoplasm of regenerating muscle fibers (RF), sarcolemma of normal fibers (SNF), sarcoplasm of vacuolated muscle fibers (VF), sarcoplasm of atrophic fibers (AF), satellite cells (SC).

muscle fibers are ICAM-1 negative. Possibly, ICAM-1 serves to bind lymphocyte function associated antigen-1 (LFA-1) which is present on cytotoxic T-cells. This adhesion molecule-integrin interaction may result in the continuation of the cytotoxic immune effector response of PM and IBM, possibly even in the absence of the eliciting antigen(s).

In vitro studies have shown the chemokine producing potential of myoblasts (46), but the chemokine array of human skeletal muscle fibers as determined in immunohistochemical studies is relatively limited (22-25, 27,29-32,38-39,47) "Table 5". The sparcely detected chemokines and chemokine receptors in IIM fibers are mostly regarded as disease unspecific, as comparative studies have shown that they are equally present in muscle tissues from patients with other neuromuscular disorders characterized by inflammation, atrophy and active regeneration.

So far, no sarcolemmal expression of chemokine receptors has been reported in human muscle biopsies. Some chemokine receptors are detected in the sarcoplasm either dispersed or around myonuclei. The functional significance of non-membrane bound chemokine receptors is unclear. It has been postulated that subcellular delocalisation of receptors ensures the dynamic regulation of the chemotactic response and chemokine desensitisation. For CCR5, ligand-mediated receptor phosphorylation, internalisation, accumulation in perinuclear regions and recycling to the cell surface has been observed (48).

Table 6. Chemokine repertoire of inflammatory cells in the idiopathic inflammatory myopathies

	DM			*PM/IBM*		
	CD4	**CD20**	**CD68**	**CD4**	**CD8**	**CD68**
alpha-family						
CXCL1	- (29)	- (29)	- (29)	- (29)	± (29)	± (29)
CXCL2	- (29)	- (29)	- (29)	- (29)	± (29)	± (29)
CXCL3	- (29)	- (29)	- (29)	- (29)	- (29)	± (29)
CXCL8	- (39)	- (39)	- (39)	- (39)	- (39)	- (39)
CXCL9	± (29)	- (29)	± (29)	- (22,29)	+ (22) ± (29)	- (22) ± (29)
CXCL10	++ (29)	- (29)	+ (29)	- (22) ++ (29)	- (22) ++ (29)	- (22) ++ (29)
CXCL11	- (29)	- (29)	- (29)	- (29)	- (29)	- (29)
CXCL12alpha	++ (30)	+ (30)	++ (30)	++ (30)	- (30)	++ (30)
CXCL12beta	± (30)	± (30)	± (30)	± (30)	± (30)	± (30)
beta-family						
CCL2	± (39)	- (39)	- (39)	± (39)	++ (39)	± (39)
CCL4	- (23)	- (23)	+ (23)	- (23)	- (23)	+ (23)
CCL5	- (23) - (39)	- (23,39)	+ (23) - (39)	- (23) - (39)	- (23,39)	+ (23) - (39)
CCL7	- (39)	- (39)	- (39)	- (39)	- (39)	± (39)
CCL17	- (39)	- (39)	- (39)	- (39)	- (39)	- (39)

Chemokine protein expression as determined using immunohistochemical or immunofluorescent double staining in muscle sections; inflammatory cells were scored all negative (-), mostly negative (±), mostly positive (+), all positive (++). Abbreviations: normal skeletal muscle (NM), dermatomyositis (DM), polymyositis (PM), sporadic inclusion body myositis (IBM), cluster of differentiation (CD), alpha-chemokine (CXCL), beta-chemokine (CCL). References are placed between brackets.

4.3. Inflammatory cells

Studies linking chemokine expression with the phenotype of the inflammatory cells are fairly limited (22-23,29-30,39). Nonetheless, considerable heterogeneity is observed "Table 6". In IBM, infiltrating T-cells are described as either CXCL9+ CXCL10- (22), or CXCL9 low CXCL10 high (29). Noteworthy is that CXCL9 and CXCL10 messengers are both significantly increased in IBM samples (22), and that in DM patients the blood levels of CXCL10 are elevated while CXCL9 levels are comparable to controls (49). The distribution of CCL5 is reported absent (39) or present in low numbers of scattered endomysial CD4+ T-cells (23). The expression of chemokine receptors on the intramuscular T-cells, B-cells and macrophages is schematized in "Figure 1**"** and "Figure 2**"**. Reported CCR1 expression on macrophages ranges from a rare observation (31) to more widespread (23). Also, conflicting data have been published regarding the distribution of the B-isoform of CCR2. Similar distribution of CCR2A is described by two groups, but one report describes CCR2B as absent from IIM inflammatory cells (24), while the other finds CCR2B to be the more abundant of the two receptors (31). The latter is more in line with the relative importance currently attributed to the two isoforms, and the distribution pattern of human circulating monocytes which have been typed CCR2A low CCR2B high (50). The heterogeneity in the reported chemokine profile may well be the result of inter-patient variability or antibody-related methodological aspects. Also, the level of chemokine expression may vary between different muscle sites and the different stages of the disease.

5. PERSPECTIVES FOR THERAPY: BLOCKING CHEMOKINES AS A NOVEL APPROACH

Conventional therapy administered to DM and PM patients consists of oral corticosteroids. However, important side effects or clinically insufficient improvement may occur, necessitating the addition of other immunosuppressive drugs, such as methotrexate and azathioprine, to the therapeutic plan. IBM remains unresponsive to all available immuno-modulating interventions up till now. Alternative treatment options for IIM are currently being explored. The efficacy of intravenous immunoglobulins has been established for DM (51). Other alternative therapeutic agents include monoclonal antibodies targeting B-cells and complement for DM, and adhesion molecules and TNF-alpha for DM/PM (reviewed in 52). The accumulating evidence that the chemotactic cytokines are important mediators of human inflammatory diseases is overwhelming, and warrants the exploration of chemokine blockers as a novel therapeutic strategy. Reports of clinical trials with chemokine blockers are beginning to surface. The benefits of an oral CCR1 antagonist have been described for rheumatoid arthritis patients (53), and a first murine study has been published that shows the benefits of CX3CL1 inhibition for experimental autoimmune myositis (54).

The picture emerges of two different chemokine-driven disease systems governing the IIM. Based on the data available at present, we propose two working hypothesis of how chemokines may regulate inflammation in DM on the one hand "Figure 1", and in PM/IBM on the other hand "Figure 2":

(I) In DM, deposition of complement on blood vessels initiates endothelial damage. Endothelial production of CXCL12 could, in conjunction with receptor bound CCL2, form a haptotactic gradient for capturing a subset of circulating leukocytes at perimysial target sites. This allows selective leukocyte penetration and proliferation of inflammatory cells, and the formation of aggregates of CD4+ T-cells, B-cells and macrophages in the perimysium. The infiltrates produce CXCL12, which could further sustain the inflammatory response. In addition, the activated CD4+ T-cells and macrophages express CCL4, which could enhance the recruitment and activation of inflammatory cells expressing the responsive receptor CCR5. Following intramuscular penetration, the expression of CCR5 is downregulated (31) which may serve as a homing signal for inflammatory cells, aiding the build up of

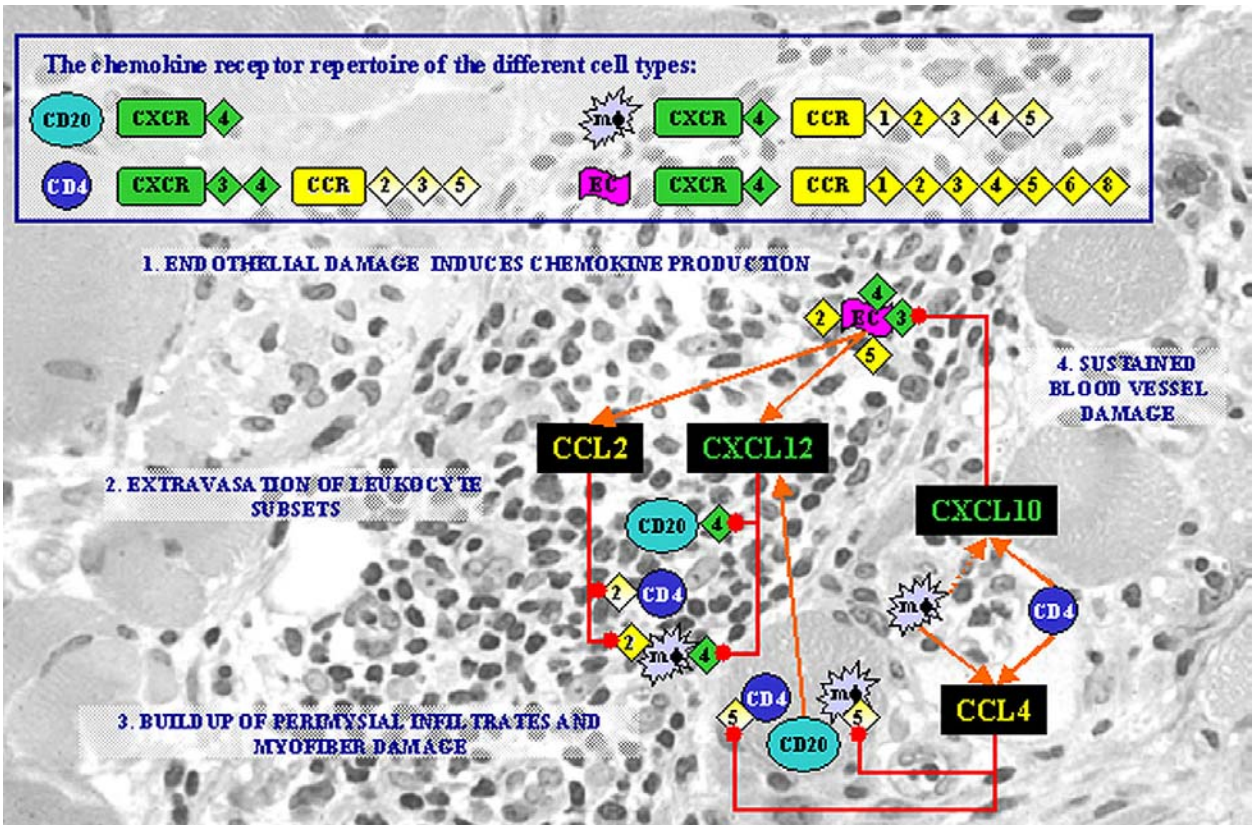

Figure 1. Principal chemokine-driven processes in the immunopathogenesis of dermatomyositis. This hypothetical disease model is based on the data published in references 22, 23, 29-31, 39. Deposition of complement on blood vessels initiates endothelial damage and the production of CXCL12 which, in conjunction with receptor bound CCL2, forms a haptotactic gradient for capturing a subset of circulating leukocytes at perimysial target sites. Leukocytes penetrate the muscle tissue and proliferate, leading to an accumulation of CD4+ T-cells, B-cells and macrophages in the perimysium. The infiltrates produce CXCL12, which sustains the inflammatory response. In addition, the activated CD4+ T-cells and macrophages express CCL4, which leads to auto-stimulation of CCR5+ cells. Perimysial CD4+ T-cells and macrophages are stimulated to express CXCL10, a chemokine that displays angiostatic activities. CXCL10 prevents the reconstitution of damaged blood vessels, further enhancing the endotheliopathy. Abbreviations: alpha-chemokine receptor (CXCR), beta-chemokine receptor (CCR), endothelial cell (EC), macrophage (mϕ), CD4+ T-cell (CD4), CD20+ B-cell (CD20). Color legend: chemokine expression (orange), chemokine – receptor interactions (red), alpha-chemokine family (green), beta-chemokine family (yellow), color intensity of receptors reflects their relative abundance.

perimysial inflammatory foci. CD4+ T-cells and macrophages express CXCL10. CXCL10 is an angiostatic chemokine, and could prevent the reconstitution of damaged blood vessels, further enhancing the endotheliopathy.

(II) In PM and IBM the myofibers are the primary target of the immune response. Local expression of CCL2 on blood vessels could selectively recruit circulating antigen-primed inflammatory cells to the muscle tissue. Their immobilization allows their extravasation, infiltration and proliferation, and leads to the formation of an endomysial infiltrate that contains large numbers of activated CD4+ and CD8+ T-cells, and macrophages. These cells respond by producing high quantities of CXCL10, CXCL12 and CCL2. Stimulation of the responsive receptors CXCR3, CXCR4 and CCR2 could facilitate the active invasion of nonnecrotic muscle fibers by macrophages and CD8+ T-cells, and stimulate the CD4+ T-cells to sustain the inflammatory response.

From the studies available at present, the receptors CXCR3, CXCR4, CCR2 and their ligands have come forward as most amenable targets to be explored for future IIM therapy. The evidence for a key role for CCL2 in particular (26-27,35,39), puts this chemokine forward as the first choice for targeted therapy, although CCL2 gene polymorphisms could not be linked with IIM susceptibility (55).

Several specificities of the system complicate the development of a chemokine-based therapeutic strategy. Chemokines and chemokine receptors are involved in normal skeletal muscle function, and therapy should neutralize pathogenic chemokines specifically leaving others, with activities advantageous for normal immune

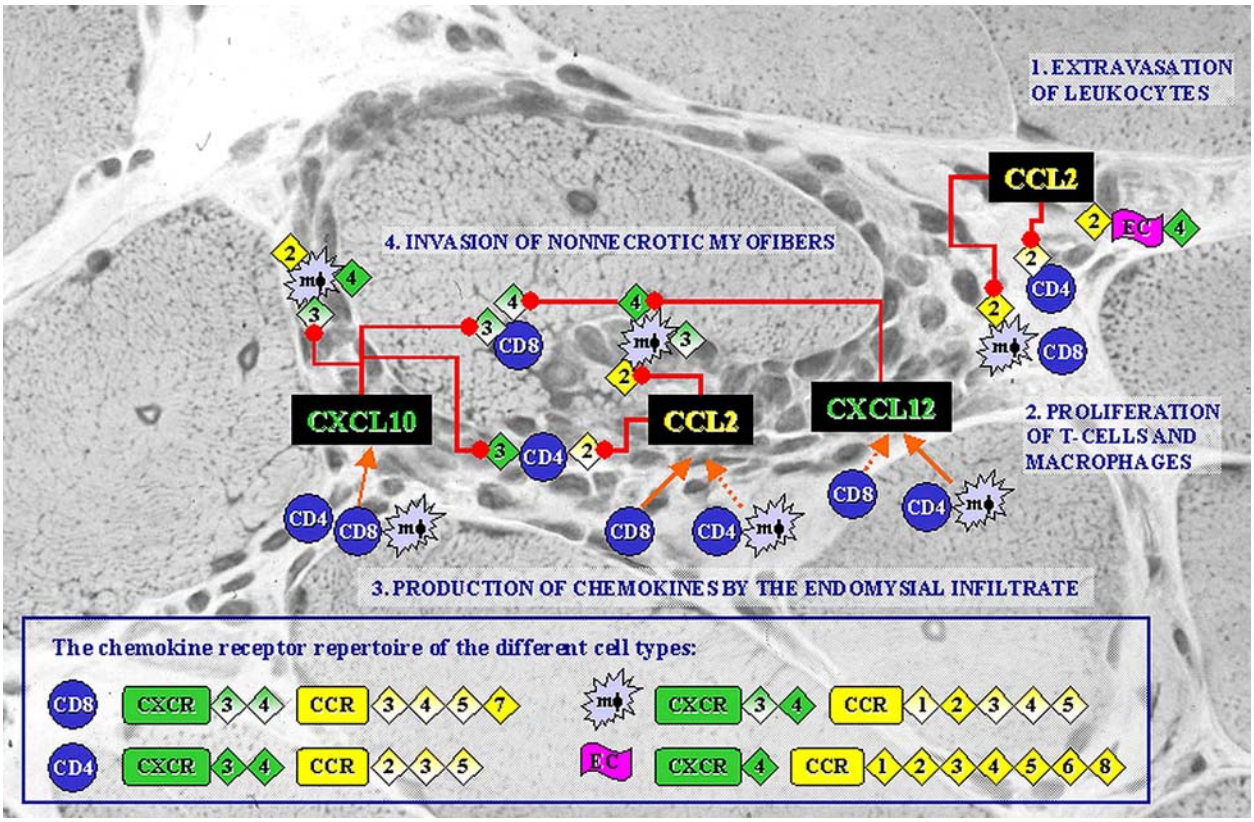

Figure 2. Principal chemokine-driven processes in the immunopathogenesis of polymyositis and sporadic inclusion body myositis. This hypothetical disease model is based on the data published in references 22-25,29-31, 39. Adhesion molecules and chemokine receptors on the endothelium of intramuscular blood vessels, immobilize subsets of circulating leukocytes. Local expression of CCL2 targets inflammatory cells to endomysial tissue sites, allowing their extravasation, infiltration and proliferation, which leads to the formation of an endomysial infiltrate that contains large numbers of activated CD4+ and CD8+ T-cells, and macrophages. These cells respond by producing high quantities of CXCL10, CXCL12 and CCL2. Stimulation of the responsive receptors CXCR3, CXCR4 and CCR2 allows active invasion of non-necrotic muscle fibers by macrophages and CD8+ T-cells, and stimulation of CD4+ T-cells sustains inflammatory reactions. Abbreviations: alpha-chemokine receptor (CXCR), beta-chemokine receptor (CCR), endothelial cell (EC), macrophage (mϕ), CD4+ T-cell (CD4), CD8+ T-cell (CD8). Color legend: chemokine expression (orange), chemokine – receptor interactions (red), alpha-chemokine family (green), beta-chemokine family (yellow), color intensity of receptors reflects their relative abundance.

surveillance and disease recovery, unharmed. The chemokine system represents a complex framework of factors with interrelated activities, and appears difficult to inhibit selectively and individually. A single chemokine can bind to different receptors and vice versa. The whole of these mechanisms operates to increase the selectivity of cell recruitment. Nonetheless, interfering with an essential link in the chain might result in a total inhibition of the inflammatory processes. More in-depth knowledge of the chemokine system in normal muscle physiology, and its specificities in IIM, are essential requirements for successful chemokine-based therapy in the future.

6. REFERENCES

1. J.P. Callen: Dermatomyositis. *Lancet* 355, 53-57 (2002)

2. T. Mimori T: Scleroderma-polymyositis overlap syndrome: clinical and serologic aspects. *Int J Dermatol* 26, 419-425 (1987)

3. J.P. Callen: Relation between dermatomyositis and polymyositis and cancer. *Lancet* 357, 85-86 (2001)

4. J.T. Kissel, J.R. Mendell, K.W. Rammohen: Microvascular deposition of complement membrane attack complex in dermatomyositis. *N Engl J Med* 314, 329-334 (1986)

5. S.A. Greenberg, J.L. Pinkus, G.S. Pinkus, T. Burleson, D. Sanoudou, R. Tawil, R.J. Barohn, D.S. Saperstain, H.R. Briemberg, M. Ericsson, P. Park, A.A. Amato: Interferon-alpha/beta-mediated innate immune mechanisms in dermatomyositis. *Ann Neurol* 57: 664-678 (2005)

6. M.C. Dalakas, R. Hohlfeld: Polymyositis and dermatomyositis. *Lancet* 362, 971-982 (2003)

7. A.A. Amato, G.S. Gronseth, C.E. Jackson, G.I. Wolfe, J.S. Katz, W.W. Bryan, R.J. Barohn: Inclusion body

myositis: clinical and pathological boundaries. *Ann Neurol* 40, 581-586 (1996)

8. M.F.G. van der Meulen, J.E. Hoogendijk, G.H. Jansen, H. Veldman, J.H.J. Wokke: Absence of characteristic features in two patients with inclusion body myositis. *J Neurol Neurosurg Psychiatry* 64, 396-398 (1998)

9. I.M. Bronner, W.H. Linssen, M.F. van der Meulen, J.E. Hoogendijk, M. de Visser: Polymyositis: an ongoing discussion about a disease entity. *Arch Neurol* 61, 132-135 (2004)

10. A.M. Emslie-Smith, K. Arahata, A.G. Engel: Major histocompatibility complex class I antigen expression, immunolocalization of interferon subtypes, and T cell-mediated cytotoxicity in myopathies. *Hum Pathol* 20**,** 224-231 (1989)

11. K. Murata, M.C. Dalakas: Expression of the costimulatory molecule BB-1, the ligands CTLA-4 and CD28, and their mRNA in inflammatory myopathies. *Am J Pathol* 155, 453-460 (1999)

12. J.L. De Bleecker, A.G. Engel: Expression of cell adhesion molecules in inflammatory myopathies and Duchenne dystrophy. *J Neuropathol Exp Neurol* 53, 369-376 (1994)

13. S. Carpenter: Inclusion body myositis, a review. *J Neuropathol Exp Neurol* 55, 1105-1114 (1996)

14. V. Askanas, W.K. Engel: Inclusion-body myositis: newest concepts of pathogenesis and relation to aging and Alzheimer disease. *J Neuropathol Exp Neurol* 60, 1-14 (2001)

15. J. Lowe, H. McDermott, K. Loeb, M. Landon, A.L. Haas, J. Mayer: Immunohistochemical localization of ubiquitin cross-reactive protein in human tissues. *J pathol* 177, 163-169 (1995)

16. C.A. Maurage, T. Bussiere, N. Sergeant, A. Ghesteem, D. Figarella-Branger, M.M. Ruchoux, J.F. Pellissier, A. Delacourte: Tau aggregates are abnormally phosphorylated in inclusion body myositis and have an immunoelectrophoretic profile distinct from other tauopathies. *Neuropath Appl Neurobiol* 30, 624-634 (2004)

17. J.D. Lunemann, J. Schmidt, D. Schmid, K. Barthel, A. Wrede, M.C. Dalakas, C. Munz: Beta-amyloid is a substrate of autophagy in sporadic inclusion body myositis. *Ann Neurol* 61, 476-483 (2007)

18. E.J. Cupler, M. Leon-Monzon, J. Miller, C. Semino-Mora, T.L. Anderson, M.C. Dalakas: Inclusion body myositis in HIV-I and HTLV-I infected patients. *Brain* 119, 1887-1893 (1996)

19. M.C. Dalakas, G. Rakocevic, A. Shatunov, L. Goldfarb, R. Raju, M. Salajegheh: Inclusion body myositis with human immunodeficiency virus infection: four cases with clonal expansion of viral-specific T cells. *Ann Neurol* 61, 466-475 (2007)

20. J.N. Pruitt, C.J. Showalter, A.G. Engel: Sporadic inclusion body myositis: counts of different types of abnormal fibers. *Ann Neurol* 39, 139-143 (1996)

21. M. Baggiolini: Chemokines in pathology and medicine. *J Intern Med* 250, 91-104 (2001)

22. R. Raju, O. Vasconcelos, R. Granger, M.C. Dalakas: Expression of IFN-gamma-inducible chemokines in inclusion body myositis. *J Neuroimmunol* 141, 125-131 (2003)

23. M. Civatte, C. Bartoli, N. Schleinitz, B. Chetaill, J.F. Pellisier, D. Figarella-Branger: Expression of the beta-chemokines CCL3, CCL4, CCL5 and their receptors in idiopathic inflammatory myopathies. *Neuropath Appl Neurobiol* 31, 70-79

24. 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. *Acta Neuropathol* 102, 385-392 (2001)

25. M. Tateyama, K. Fujihara, T. Misu, J. Feng, Y. Onodera, Y. Itoyama: Expression of CCR7 an its ligands CCL19/CCL21 in muscles of polymyositis. *J Neurol Sci* 249, 158-165 (2006)

26. A. Liprandi, C. Bartoli, D. Figarella-Branger, J.F. Pellisier, H. Lepidi: Local expression of monocyte chemoattractant protein-1 (MCP-1) in idiopathic inflammatory myopathies. *Acta Neuropathol* 97, 642-648 (1999)

27. P. Confalonieri, P. Bernasconi, P. Megna, S. Galbiati, F. Cornelio, R. Mantegazza: Increased expression of beta-chemokines in muscle of patients with inflammatory myopathies. *J Neuropathol Exp Neurol* 59, 164-169 (2000)

28. E.M. Adams, J. Kirkley, G. Eidelman, J. Dohlman, P.H. Plotz: The predominance of beta (CC) chemokine transcripts in idiopathic inflammatory muscle diseases. *Proc Assoc Am Physicians* 109, 275-285

29. B. De Paepe, K. De Keyzer, J.J. Martin, J.L. De Bleecker: Alpha-chemokine receptors CXCR1-3 and their ligands in idiopathic inflammatory myopathies. *Acta Neuropathol* 109, 576-582 (2005)

30. B. De Paepe, J.M. Schröder, J.J. Martin, G.Z. Racz, J.L. De Bleecker: Localization of the alpha-chemokine SDF-1 and its receptor CXCR4 in idiopathic inflammatory myopathies. *Neuromusc Disord* 14, 265-273 (2004)

31. B. De Paepe, J.L. De Bleecker: Beta-chemokine receptor expression in idiopathic inflammatory myopathies. *Muscle Nerve* 31, 621-627 (2005)

32. B. De Paepe, K.K. Creus, J.L. De Bleecker: Chemokine profile of different inflammatory myopathies reflects humoral versus cytotoxic immune responses. *Ann NY Acad Sci* 1109, 441-453 (2007)

33. G. Kroemer, F. Hirsch, A. Gonzales-Garcia, C. Martinez: Differential involvement of Th1 and Th2 cytokines in autoimmune diseases. *Autoimmunity* 24, 25-33 (1996)

34. J.L. De Bleecker, V.I. Meire, W. Declercq, E.H. Van Aken: Immunolocalisation of tumor necrosis factor-alpha and its receptors in inflammatory myopathies. *Neuromusc Disord* 9, 239-246 (1999)

35. M. Tucci, C. Quatraro, F. Dammacco, F. Silvestris: Interleukin-18 overexpression as a hallmark of the activity of autoimmune inflammatory myopathies. *Clin Exp Immunol* 146, 21-31 (2006)

36. E.C. Butcher, W. Picker: Lymphocyte homing and homeostasis. *Science* 272, 60-66 (1996)

37. D.D. Patel, W. Koopman, T. Imai, L.P. Whichard, O. Yoshie, M.S. Krangel: Chemokines have diverse abilities to form solid phase gradients. *Clin Immunol* 99, 43-52 (2001)

38. C. Minetti, M. Gattorno, S. Repetto, A. Gregorio, M. Pedemonte, S. Assereto, F. Zara, C. Bruno, A. Martini: Chemokine receptor CCR7 is expressed in muscle fibers in juvenile dermatomyositis. *Biochem Biophys Res Commun* 333, 540-543 (2005)

39. J.L. De Bleecker, B. De Paepe, I.E. Vanwalleghem, J.M. Schröder: Differential expression of chemokines in inflammatory myopathies. *Neurology* 58, 1779-1785 (2002)

40. S.K. Gupta, P.G. Lysko, K. Pillarisetti, E. Ohlstein, J.H. Stadel: Chemokine receptors in human endothelial cells: functional expression of CXCR4 and its transcriptional regulation by inflammatory cytokines. *J Biol Chem* 273, 4282-4287 (1998)

41. E. Engelhardt, A. Toksoy, M. Goebeler, S. Debus, E.B. Brocker, R. Gillitzer: Chemokines IL-8, GROalpha, MCP-1, and Mig are sequentially and differentially expressed during phase-specific infiltration of leukocyte subsets in human wound healing. *Am J Pathol* 153, 1849-1860 (1998)

42. A.M. Emslie-Smith, A.G. Engel: Microvascular changes in early and advanced dermatomyositis: a quantitative study. *Ann Neurol* 27, 343-356 (1990)

43. N. Goebels, D. Michaelis, H. Wekerle, R. Hohlfeld: Human myoblasts as antigen-presenting cells. *J Immunol* 149, 661-667 (1992)

44. H. Wiendl, R. Hohlfeld, B.C. Kieseier: Immunobiology of muscle: advances in understanding an immunological microenvironment. *Trends in Immunol* 26, 373-380 (2005)

45. D.S. Tews, H.H. Goebel: Expression of cell adhesion molecules in inflammatory myopathies. *J Neuroimmunol* 59, 185-194 (1995)

46. M. De Rossi, P. Bernasconi, F. Baggi, R. de Waal Malfyt, R. Mantegazza: Cytokines and chemokines are both expressed by human myoblasts: possible relevance for the immune pathogenesis of muscle inflammation. *Int Immunol* 12, 1329-1335 (2000)

47. T. Sugiura, Y. Kawaguchi, M. Harigai, K. Takagi, S. Ohta, C. Fukasawa, M. Hara, N. Kamatani: Increased CD40 expression on muscle cells of polymyositis and dermatomyositis: role of CD40-CD40 ligand interaction in IL-6, IL-8, IL-15, and monocyte chemoattractant protein-1 production. *J Immunol* 164, 6593-6600 (2000)

48. N. Signoret, A. Pelchen-Matthews, M. Mack, A.E.I. Proudfoot, M. Morsh: Endocytosis and recycling of the HIV coreceptor CCR5. *J Cell Biol* 151, 1281-1293 (2005)

49. H. Fujii, Y. Shimada, M. Hasegawa, K. Takehara, S. Sato: Serum levels of a Th1 chemoattractant IP-10 and Th2 chemoattractants, TARC and MDC, are elevated in patients with systemic sclerosis. *J Dermatol Sci* 35, 43-51 (2005)

50. L.M. Wong, S.J. Myers, C.L. Tsou, J. Gosling, H. Arai, I.F. Charo: Organization and differential expression of the human monocyte chemoattractant protein 1 receptor genes: evidence for the role of the carboxyl-terminal tail in receptor trafficking. *J Biol Chem* 272, 1038-1045 (1997)

51. M.C. Dalakas, I. Illa, J.M. Dambrosia: A controlled trial of high dose intravenous immunoglobulin infusions as treatment for dermatomyositis. *N Engl J Med* 329, 1993-2000 (1993)

52. J.L. De Bleecker, K.K. Creus, B. De Paepe: Potential therapeutic targets for idiopathic inflammatory myopathies. *Drug News Perspect* 19, 549-557 (2006)

53. J.J. Haringman, M.C. Kraan, T.J.M. Smeets, K.H. Zwinderman, P.P. Tak: Chemokine blockade and chronic inflammatory disease: proof of concept in patients with rheumatoid arthritis. *Ann Rheum Dis* 62, 715-721 (2003)

54. F. Suzuki, T. Nanki, T. Imai, H. Kikuchi, S. Hirohata S., H. Kohsaka, N. Miyasaka: Inhibition of CX3CL1 (fractalkine) improves experimental autoimmune myositis in SJL/J mice. *J Immunol* 175, 6987-6996 (2005)

55. H. Chinoy, F. Salway, N. Fertig, B.D. Tait, C.V. Oddis, W.E. Ollier, R.G. Cooper: Monocyte chemotactic protein-1 single nucleotide polymorphisms do not confer susceptibility for the development of adult onset polymyositis/dermatomyositis in UK Caucasians. *Rheumatology* 46, 604-607 (2007)

Key Words: Chemokines, Chemokine Receptors, Dermatomyositis, Polymyositis, Idiopathic Inflammatory Myopathies, Immunohistochemistry, Sporadic Inclusion Body Myositis, Review

Send correspondence to: Jan L. De Bleecker MD, PhD, Department of Neurology, Ghent University Hospital, UZ 1K12A, De Pintelaan 185, B9000 Ghent, Belgium, Tel: 3292404544, Fax : 3292404971, E-mail. jan.debleecker@ugent.be

http://www.bioscience.org/current/vol13.htm