Review
Immune mechanisms in the pathogenesis of idiopathic inflammatory myopathies

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Abstract

Idiopathic inflammatory myopathies (IIMs), comprising polymyositis, dermatomyositis, and inclusion-body myositis, are characterized by inflammatory cell infiltrates in skeletal muscle tissue, muscle weakness, and muscle fatigue. The cellular infiltrates often consist of T lymphocytes and macrophages but also, in some cases, B lymphocytes. Emerging data have led to improved phenotypic characterization of the inflammatory cells, including their effector molecules, in skeletal muscle, peripheral blood, and other organs that are frequently involved, such as skin and lungs. In this review we summarize the latest findings concerning the role of T lymphocytes, B lymphocytes, dendritic cells, and other antigen-presenting cells in the pathophysiology of IIMs.

Introduction

Idiopathic inflammatory myopathies (IIMs) are characterized by mononuclear inflammatory cell infiltrates in skeletal muscle tissue, by muscle weakness, and by muscle fatigue. They are often subclassified into three major groups on the basis of clinical and histopathological differences: polymyositis, dermatomyositis, and inclusion-body myositis. The cellular infiltrates in muscle tissue are mainly composed of T lymphocytes and macrophages but also, in some cases, B lymphocytes. This observation, together with frequently detected autoantibodies particularly in polymyositis and dermatomyositis, suggests that the inflammatory myopathies are immune-mediated; they are believed to be triggered by environmental factors in genetically susceptible individuals. The varying clinical features and the different predominating histopathological features such as localization and phenotypes of inflammatory infiltrates, or rimmed vacuoles as seen in inclusion-body myositis, suggest that there are different pathophysiological mechanisms leading to myositis. Despite these differences the inflammatory molecules produced in muscle tissue are highly similar in chronic inflammatory myopathies, suggesting that some molecular pathways are shared between the subsets of inflammatory myopathies.

In the inflammatory myopathies there are also signs of microvascular involvement. The involvement of microvessels was first reported in patients with dermatomyositis as capillary loss and recognized by the presence of the membrane attack complex (MAC) [1,2]. Later, activated capillaries with increased expression of adhesion molecules (intercellular cell-adhesion molecule-1 and/or vascular cell-adhesion molecule-1) and IL-1α were also seen in patients without skin rash, in polymyositis and inclusion-body myositis. Damage or activation of blood vessels could indicate that the microvessels are targets of the immune reaction in some subsets of patients with IIM.

It has long been recognized that the inflammatory cell infiltrates and muscle fiber damage are patchy and are sometimes not detected in muscle biopsies. This is a clinical problem in the diagnostic procedure. Moreover, the lack of correlation between the degree of inflammatory infiltrates and muscle weakness has led to a search for mechanisms other than immune-mediated muscle fiber damage that could cause muscle weakness. One such non-immune mechanism, endoplasmic reticulum stress, has been proposed, on the basis of observations both from human studies and from an animal model for myositis, the major histocompatibility complex (MHC) class I transgene [3]. These non-immune mechanisms have been addressed in a recent review paper [4].

New data are constantly emerging, leading to improved characterization of the phenotypes of the inflammatory cells and their effector molecules that are expressed in IIMs, not

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APC = antigen-presenting cell; DC = dendritic cell; ICOS = inducible co-stimulator; ICOS-L = ICOS ligand; IFN = interferon; IIM = idiopathic inflammatory myopathy; IL = interleukin; ILD = interstitial lung disease; MAC = membrane attack complex; MHC = major histocompatibility complex.

only in the major target organ, the skeletal muscle, but also in peripheral blood and in other organs that are frequently involved, such as skin and lungs. This increasing knowledge has a great potential to improve our understanding of the role of these inflammatory cells in disease mechanisms in IIMs. In this review we summarize the latest findings concerning the role of T lymphocytes, B lymphocytes, dendritic cells, and other antigen-presenting cells (APCs) in the pathophysiology of IIMs.

T lymphocytes
T lymphocyte function
T lymphocytes recognize antigens on APCs through the T-cell antigen receptor in a MHC-restricted fashion. Peptides from intracellular pathogens proliferating in the cytoplasm are carried to the cell surface by MHC class I molecules and presented to cytotoxic (CD8+) T lymphocytes, which once fully activated have the capacity to lyse infected target cells. In contrast, peptide antigens from pathogens in intracellular vesicles, and those derived from ingested extracellular bacteria and toxins, are carried to the cell surface by MHC class II molecules and presented to CD4+ T helper cells. These can then differentiate into effector cells, such as T_H1, T_H2, and T_H17 cells [5]. Pathogens that accumulate inside macrophages and dendritic cells (DCs) tend to stimulate the differentiation of T_H1 cells and the production of IgG antibodies by B lymphocytes. Conversely, extracellular antigens tend to stimulate the production of T_H2, which can subsequently stimulate the production of IgA and IgE. T_H17 is a recently described effector T lineage that has been suggested to contribute to chronic inflammatory settings. CD8+ T lymphocytes do not have as distinct sublineages and are cytotoxic cells working in a perforin/granzyme-dependent manner; interestingly, CD4+ lymphocytes can sometimes also display cytotoxic effector functions.
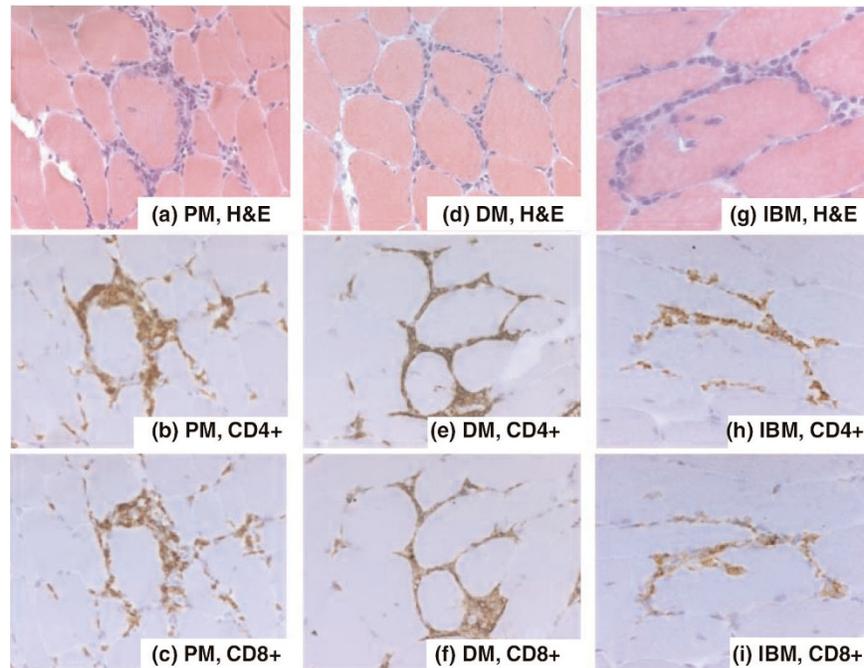
T lymphocytes in idiopathic inflammatory myopathies
Although prominent T lymphocyte infiltrates are not always found in muscle biopsies, two types of cellular infiltrate have been recognized in IIMs, one being endomysial inflammatory infiltrates consisting mainly of CD8+ T lymphocytes and macrophages invading non-necrotic muscle fibers expressing MHC class I antigens [6-8]. These are typically, but not exclusively, found in inclusion-body myositis and polymyositis. The other type of mononuclear cell infiltration is perivascular/perimysial and has become a characteristic of dermatomyositis; it consists predominantly of CD4+ T lymphocytes, occasionally together with B lymphocytes and macrophages [6,9]. The deposition of complement components is also mainly localized to the perivascular regions of muscular or cutaneous lesions. However, the 'classical' T lymphocyte picture in IIM is, as the authors say, much more complex and an oversimplification of reality [6,9]. Independent of T lymphocyte localization, their presence suggests an involvement of the adaptive immune system in these disorders (Figures 1 and 2).

Cytotoxic CD8+ T lymphocytes can release three different cytotoxic proteins: perforin, granzyme, and granulysin. It is known from earlier studies that in polymyositis and inclusion-body myositis, CD8+ T lymphocytes and macrophages surrounding the non-necrotic muscle fibers expressing MHC class I antigen do express perforin. Perforin may cause a leak in the sarcolemmal surface through which granzymes could invade the sarcoplasm to initiate muscle fiber necrosis [10-12]. Recently, granulysin has also been demonstrated in both polymyositis and inclusion-body myositis [13]. The presence of granulysin-expressing CD8+ T lymphocytes tended to correlate with steroid resistance in polymyositis [13]. Interestingly, perforin/granzyme-expressing CD4+ T lympho-cytes have also been demonstrated [10,14]. A schematic summary of the potential role of different immune cells in the context of chronic muscle inflammation is presented in Figure 3.

The T lymphocyte repertoire in blood seems to differ between polymyositis and dermatomyositis [15]. In peripheral blood of active dermatomyositis, a decreased percentage of CD3+ and CD8+ T lymphocytes and decreased IFN-γ expression by CD4+ and CD8+ T lymphocytes but an increase in B lympho-cytes and IL-4-producing CD4+ T lymphocyte frequencies were found. These features were not seen in the inactive form of the disease. In patients with polymyositis with relapsing disease, markedly perturbed T cell repertoires were seen. The expanded T lymphocytes clonally displayed a memory phenotype, expressed intracellular perforin, and responded to stimulation by IL-2, which indicates that they have the potential for reactivation under appropriate conditions [16]. This suggests that a continuous autoantigen-driven process might be prominent in this disease and that in patients with polymyositis a relapse would more probably be associated with the reactivation of clones, which are present at disease onset, rather than with the emergence of new ones. The concept that clonally expanded muscle-infiltrating CD8+ T lymphocytes could recirculate into the blood is true not only for patients with polymyositis but also for those with inclusion-body myositis [17]. Another possibility for the recirculation of T lymphocytes seen in polymyositis and inclusion-body myositis might be that the same antigen, for example a virus, could independently induce the same expansion in the blood and in the muscle tissue if the antigen is present at both sites.

In patients with IIMs, T lymphocytes are found not only in inflammatory muscle tissues or blood but also in lungs in patients with interstitial lung disease (ILD), which is a frequent manifestation in patients with polymyositis and dermatomyositis; this was found in up to 60 to 70% of such patients when sensitive techniques such as high-resolution computed tomography and pulmonary function tests were employed [18]. ILD seems to be less common in patients with inclusion-body myositis, although no reports are available in which newly diagnosed patients have been investigated for
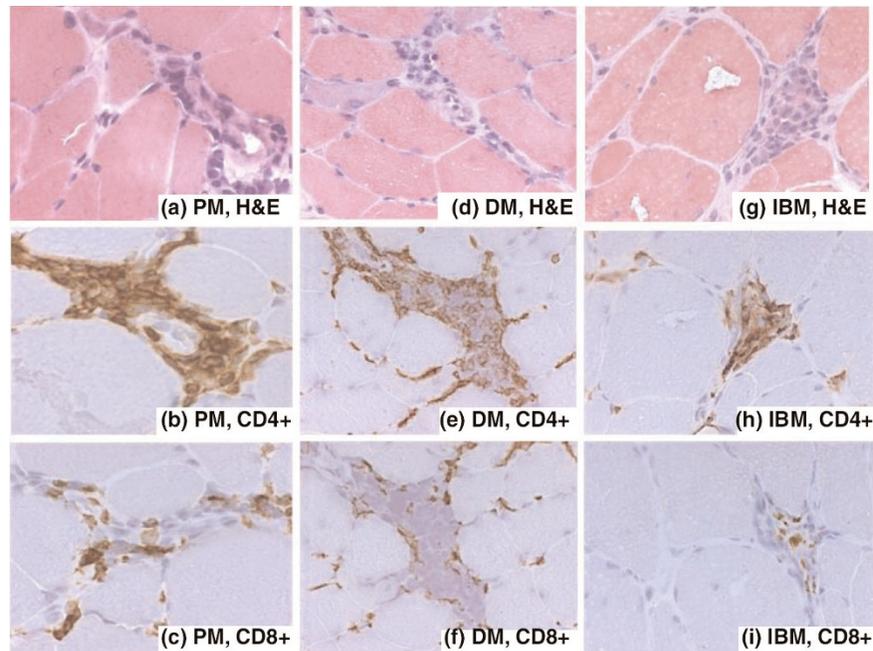
Figure 1

Endomysial localization of CD4$^+$ and CD8$^+$ T lymphocytes. Immunohistochemical staining of samples from patients with polymyositis (PM) **(a–c)**, dermatomyositis (DM) **(d–f)**, and inclusion-body myositis (IBM) **(g–i)**. **(a)** Hematoxylin and eosin (H&E) staining to localize inflammatory cell infiltrates in a patient with polymyositis. **(b)** CD4$^+$ T lymphocytes stained with a monoclonal SK3 mouse IgG$_1$ antibody (Becton Dickinson, San Jose, CA, USA) in the same area as in (a) but further down in the biopsy. **(c)** CD8$^+$ T lymphocytes stained with a monoclonal SK1 mouse IgG$_1$ antibody (Becton Dickinson) in a consecutive section to that in (b). **(d)** H&E staining to localize inflammatory cell infiltrates in a patient with dermatomyositis. **(e)** CD4$^+$ T lymphocytes stained with a monoclonal SK3 mouse IgG$_1$ antibody in the same area as in (d) but further down in the biopsy. **(f)** CD8$^+$ T lymphocytes stained with a monoclonal SK1 mouse IgG$_1$ antibody in a consecutive section to that in (e). **(g)** H&E staining to localize inflammatory cell infiltrates in a patient with inclusion-body myositis. **(h)** CD4$^+$ T lymphocytes stained with a monoclonal SK3 mouse IgG$_1$ antibody in the same area as in (g) but further down in the biopsy. **(i)** CD8$^+$ T lymphocytes stained with a monoclonal SK1 mouse IgG$_1$ antibody in a consecutive section to that in (h). Original magnifications: ×250 (a–f) and ×312.5 (g–i).

lung involvement with these techniques. In the lungs CD8$^+$ T lymphocytes were distributed both in and around lymphoid follicles and in the walls of normal-appearing alveoli, whereas CD4$^+$ T lymphocytes and B lymphocytes were seen in association with lymphoid follicles. CD4$^+$ T lymphocytes have also been demonstrated in reconstructed thick alveolar walls [19]. The CD4$^+$/CD8$^+$ ratio in bronchoalveolar lavage fluid was low [20,21]; most of the CD8$^+$ T lymphocytes were of HLA-DR$^+$ [21] and CD25$^+$ [22] types, suggesting that they were activated. In a small study [23], a low CD4$^+$/CD8$^+$ ratio was seen in bronchoalveolar lavage fluids in all patients with radiographic signs of ILD early in the disease course of patients with polymyositis or dermatomyositis. The function of these T lymphocytes is not known. It can only be speculated that they might interact with antigens (from viruses or other sources) expressed in various pulmonary cells (Figure 3). It is interesting in this context that there is a reported tissue variation of histidyl-tRNA synthetases with a higher expression in normal lungs than in other organs, and that patients with autoantibodies against histidyl-tRNA synthetase – anti-Jo-1 antibodies – have ILD in close to 100% of cases.

Furthermore, a restricted accumulation of T lymphocytes expressing selected T-cell antigen receptor V-gene segments was recorded in skeletal muscle and lung but not in peripheral blood, which suggests a common target antigen in these organs [23]. Moreover, a role for histidyl-tRNA synthetase in the disease mechanism is supported by the observation that this antigen can serve as a chemokine for DCs and T lymphocytes when cleaved by certain proteases [24].

Even though the histopathological picture in polymyositis and dermatomyositis is often different, a similar clonal expansion of T lymphocytes is seen in bronchoalveolar lavage fluid [25]. In addition, T lymphocytes have been extracted from muscle tissue of these patients. The established T cell lines showed a variable proportion of CD4$^+$ and CD8$^+$ T lymphocytes, which did not correlate with diagnosis [26]. Examples of CD4$^+$ and CD8$^+$ T lymphocyte localization in polymyositis, dermato-myositis, and inclusion-body myositis are shown in Figures 1 and 2. As demonstrated here, a similar pattern with a mixture of cell populations and both endomysial and perimysial localization may be found despite the diagnosis of myositis.
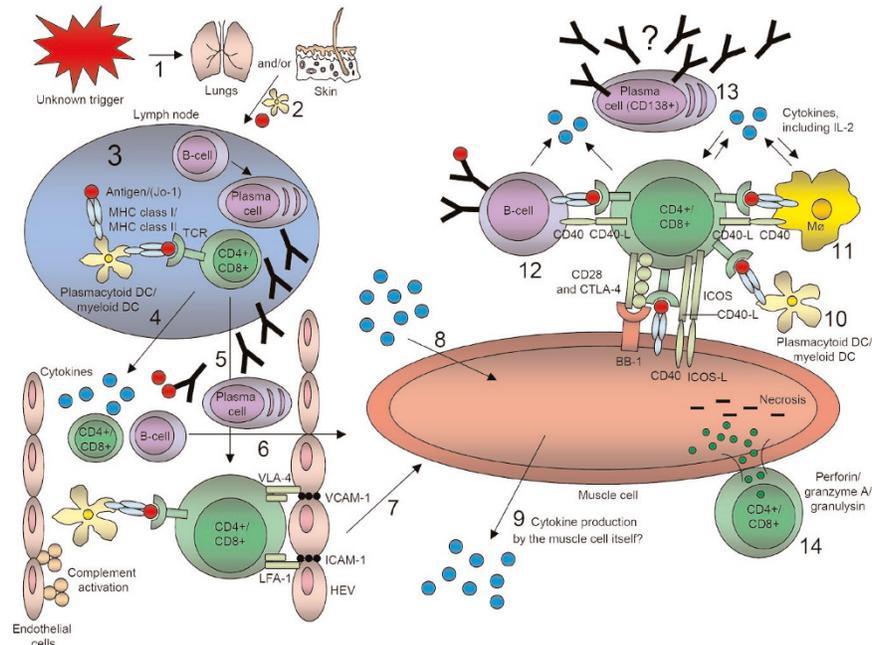
Figure 2

Perivascular localization of CD4+ and CD8+ T lymphocytes. Immunohistochemical staining of samples from patients with polymyositis (PM) **(a–c)**, dermatomyositis (DM) **(d–f)**, and inclusion-body myositis (IBM) **(g–i)**. **(a)** Hematoxylin and eosin (H&E) staining to localize inflammatory cell infiltrates in a patient with polymyositis. **(b)** CD4+ T lymphocytes stained with a monoclonal SK3 mouse IgG_1 antibody in the same area as in (a) but further down in the biopsy. **(c)** CD8+ T lymphocytes stained with a monoclonal SK1 mouse IgG_1 antibody in a consecutive section to that in (b). **(d)** H&E staining to localize inflammatory cell infiltrates in a patient with dermatomyositis. **(e)** CD4+ T lymphocytes stained with a monoclonal SK3 mouse IgG_1 antibody in the same area as in (d) but further down in the biopsy. **(f)** CD8+ T lymphocytes stained with a monoclonal SK1 mouse IgG_1 antibody in a consecutive section to that in (e). **(g)** H&E staining to localize inflammatory cell infiltrates in a patient with inclusion-body myositis. **(h)** CD4+ T lymphocytes stained with a monoclonal SK3 mouse IgG_1 antibody in the same area as in (g) but further down in the biopsy. **(i)** CD8+ T lymphocytes stained with a monoclonal SK1 mouse IgG_1 antibody in a consecutive section to that in (h). Original magnifications: ×312.5 (a–c, g–i) and ×250 (d–f).

When evaluating the presence of CD8+ T lymphocytes in biopsies it is important to consider which reagents have been used. Although the CD8 molecule is the classic lineage marker for cytotoxic T lymphocytes, this is only true if the CD8 molecule consists of an α and a β subunit (CD8$\alpha\beta$) and not if it is a CD8$\alpha\alpha$ homodimer. The homodimer can sometimes be found on activated CD4+ T lymphocytes [27], especially in the gastrointestinal tract. Thus if the reagent used to detect CD8+ T lymphocytes is an antibody against the α subunit it could detect both classical CD8+ T lymphocytes but also activated CD4+ T lymphocytes. This information was not always available when immunophenotyping of lymphocytes in muscle tissue was presented. However, if the analysis includes the detection of a cytotoxic agent this caveat is partly avoided. A summary of selected T lymphocyte studies in IIMs is presented in Table 1.

A role of T lymphocytes in polymyositis and dermatomyositis is further supported by the clinical improvement after treatment with immunosuppressive drugs that are known to affect T lymphocytes. In contrast, patients with inclusion-body myositis rarely display improved muscle function after treatment with immunosuppressive drugs, which is why the role of T lymphocytes in disease mechanisms is more questionable in this form of myositis. Therapeutic agents that do not solely target T lymphocytes, but in all mentioned examples affect T lymphocyte populations, have been shown to be effective in polymyositis and dermatomyositis; these are methotrexate, cyclosporin A, tacrolimus, and anti-thymocyte globulin. Methotrexate is one of the most commonly used second-line immunosuppressive drugs given to patients with IIM. It is known to be well tolerated and effective in polymyositis and dermatomyositis, although no placebo-controlled trials have yet been performed [28]. There are also case series showing beneficial effects of tacrolimus and anti-thymocyte globulin [29,30]. In addition, topical cutaneous tacrolimus therapy has also effectively been applied to skin lesions in patients with dermatomyositis [31]. Although the clinical improvement with these drugs could indicate that T lymphocytes have a role in polymyositis and dermatomyositis, there are no data available to show that these therapies have effects on inflammatory cell infiltrates or molecular expression in muscle tissue that correlate with the clinical effects, which would strengthen such a hypothesis.

Figure 3

Hypothetical involvement of T lymphocytes, B lymphocytes, and dendritic cells (DCs) in idiopathic inflammatory myopathies. (1) An unknown trigger (for example viral infection or ultraviolet radiation) in the respiratory tract or through the skin leads to the cleavage of histidyl-tRNA synthetase by granzyme B through antiviral CD8+ T lymphocytes in the lungs. (2) Immature DCs carry receptors on its surface that recognize common features of many pathogens. When a DC takes up a pathogen in infected tissue it becomes activated and migrates to the lymph node. (3) Upon activation, the DC matures into a highly effective antigen-presenting cell (APC) and undergoes changes that enable it to activate pathogen-specific lymphocytes in the lymph node. T lymphocytes become activated and B lymphocytes, with active help from CD4+ T lymphocytes, proliferate and differentiate into plasma cells. (4) Activated DCs, T lymphocytes, and B lymphocytes could release cytokines into the bloodstream. (5) The activated T lymphocyte, on which the DC-MHC–antigen complex is bound, itself binds to specialized endothelial cells called high endothelial venules (HEV). For this purpose it uses the VLA-4 (very late activation antigen-4) and LFA-1 (lymphocyte function associated antigen-1) molecules on its surface to interact with adhesion molecules (vascular cell-adhesion molecule-1 (VCAM-1) and intercellular cell-adhesion molecule-1 (ICAM-1)) on HEVs, where they can penetrate into peripheral lymphoid tissues. (6,7) Naïve T lymphocytes and B lymphocytes that have not yet encountered their specific antigen circulate continuously from the blood into the peripheral lymphoid tissues. (8,9) Various cytokines from the bloodstream or produced locally could affect the muscle tissue or cell in many different ways. However, it is not clear whether the muscle cell itself could produce and release cytokines. (10-12) DCs, macrophages (Mϕ), and B lymphocytes can interact with T lymphocytes in various ways. T lymphocytes could possibly also bind to muscle cells through inducible co-stimulators (ICOS), CD40 ligand (CD40-L), CD28, and CTLA-4 (CD152) on T lymphocytes to ICOS ligand (ICOS-L), CD40, and BB-1 antigen on the muscle cell. In that fashion, the muscle cell would function as an APC. (13) Plasma cells (CD138+) can be found in the muscle tissue of certain subgroups of patients with idiopathic inflammatory myopathy, but whether these cells could produce autoantibodies locally is not yet known. (14) T lymphocytes have been shown to bind in close contact with muscle cells and to release perforin, granzyme A, and granulysin, which may cause necrosis of muscle tissue or cells.

B lymphocytes

B lymphocyte function

B lymphocytes have a major role in the immunological pathogenesis of autoimmune diseases. Not only can their differentiated progeny, plasma cells [32,33], synthesize and secret large quantities of immunoglobulins, they could also regulate other cell types, secrete cytokines, and present antigens. B lymphocyte activation requires both binding of an antigen by surface immunoglobulin – the B-cell receptor – and an interaction with antigen-specific T lymphocytes (CD4+). CD4+ T lymphocytes then can induce vigorous B lymphocyte proliferation and direct the differentiation of the clonally expanded progeny of naïve B lymphocytes into either plasma cells or memory B lymphocytes. Both cytokines released by CD4+ T lymphocytes and somatic hypermutation of V-region genes can influence antibody isotype switching or the antigen-binding properties of the antibody, respectively, resulting in the production of antibodies of various isotypes that can be distributed to various body compartments.

B lymphocytes in idiopathic inflammatory myopathies

In 1984 and 1990, Arahata and Engel showed that B lymphocytes were more frequent in perivascular sites than in endomysial sites [6,9]. Furthermore, B lymphocytes were more common in muscle tissue from patients with dermatomyositis than from those with polymyositis or inclusion-body myositis. This observation was further supported by another study in which perivascular B lymphocytes were only found in patients

Table 1

Selected articles on immunohistochemical localization of inflammatory cells in idiopathic inflammatory myopathies

Reference	Polymyositis	Dermatomyositis	Inclusion-body myositis
T lymphocytes			
[9]	53 patients. T lymphocytes were least abundant at perivascular and most abundant at endomysial sites. The CD4/CD8 ratio was highest at perivascular sites and lowest at endomysial sites. The proportion of T lymphocytes estimated to be Ia+, or activated, was nearly twice as high at endomysial sites as at perivascular sites	31 patients. The proportion of T lymphocytes was lowest at perivascular sites and highest at endomysial sites. The CD4/CD8 ratio was highest at perivascular sites and lowest at endomysial sites. Many inflammatory cells display the Ia marker, but only one-sixth of perivascular lymphocytes and one-fifth of endomysial T lymphocytes was Ia+, or activated	48 patients. T lymphocytes were least abundant at perivascular sites and most abundant at endomysial sites. CD4/CD8 ratio was highest at perivascular sites and lowest at endomysial sites. The proportion of T lymphocytes estimated to be Ia+, or activated, was nearly twice as high at endomysial sites as at perivascular sites
[8]	7 patients. Endomysial infiltrates of T lymphocytes were predominant. Partly invaded non-necrotic cells were also seen; some of these fibers also expressed MHC class I	21 patients. Severe muscle fiber necrosis, predominant perivascular T lymphocyte infiltrates, fibrosis and perifascicular atrophy. MHC class I were localized to perifascicular fibers	–
[11]	5 patients. Perforin was co-localized with granzyme A and occasionally invaded non-necrotic muscle fibers. The percentage of perforin+ cells among the endomysial CD8+ cell population was 9.9%	5 patients. There were very few perforin+ and granzyme A-positive cells in DM	2 patients. Perforin was co-localized with granzyme A and occasionally invaded non-necrotic muscle fibers. The percentage of perforin+ cells among the endomysial CD8+ cell population was 12.5%
[10]	5 patients. Co-localization of perforin with CD8, CD4, CD3, and CD2 was analyzed. The general distribution of inflammatory cells was endomysial. The overall CD4/CD8 ratio was 1.1 and the total number of CD8+ inflammatory cells in 9 random microscopic fields was 236. More than 90% of perforin+ cells were CD2+CD3+, and perforin was expressed both in non-invasive interstitial T lymphocytes and in autoinvasive CD8+ T lymphocytes. Of all perforin+ cells, 60% were CD8+ and 40% CD4+. Conversely, 75% of the CD8+ cells and 50% of the CD4+ cells were perforin+. 43% of the CD8+ T lymphocytes that contacted a muscle fiber expressed perforin vectorially toward the target muscle fiber	4 patients. Co-localization of perforin with CD8, CD4, CD3, and CD2 was analyzed. The general distribution of inflammatory cells was perivascular and perimysial. The overall CD4/CD8 ratio was 1.2 and the total number of CD8+ inflammatory cells in 9 random microscopic fields was 167. More than 90% of perforin+ cells were CD2+CD3+, and 50% of all perforin+ cells were CD4+ and 50% were CD8+. Conversely, 80% of the CD4+ cells and 90% of the CD8+ cells were perforin+. Perforin was distributed randomly in the cytoplasm of the inflammatory T lymphocytes	–
[13]	17 patients. The numbers of CD4, CD8, and CD56 cells per 1,000 muscle fibers at endomysial sites were 275 ± 140, 355 ± 150, and 25 ± 13 (means ± SD) in all cases. Granulysin was expressed in the cytoplasm of infiltrating cells, mostly CD4+ and CD8+ cells, only a few CD56+ cells at a higher level in endomysial sites than in perivascular sites. Notably, steroid-resistant patients with PM had a higher frequency of double-positive CD8 and granulysin at endomysial sites than steroid-resistant patients with IBM	–	7 patients. The numbers of CD4, CD8, and CD56 cells per 1,000 muscle fibers at endomysial sites were 250 ± 120, 330 ± 200, and 31 ± 16 (means ± SD) in all cases. Granulysin was expressed in the cytoplasm of infiltrating cells, mostly CD4 and CD8+ cells, only a few CD56+ cells at a higher level at endomysial sites than at perivascular sites
[61]	2 patients. CD8+ T lymphocytes were found to invade muscle fibers co-expressing ICOS-L and MHC class I. The endomysial ICOS-positive CD8+ and CD4+ T lymphocytes were seen at a similar frequency and ratio to those in patients with IBM	2 patients. Most ICOS and ICOS-L positive cells were localized to the perimysial regions and connective tissue. ICOS-expressing CD4+ and CD8+ T lymphocytes were infrequent and present only in the perimysium and around blood vessels, but not among autoinvasive T lymphocytes	20 patients. CD8+ T lymphocytes were found to invade muscle fibers co-expressing ICOS-L and MHC class I co-expressing muscle fibers. ICOS-L expression was found on the surface of almost all fibers, including healthy myofibers away from inflammation. 1/20 or 2/20 autoinvasive CD8+ T lymphocytes were double-positive for ICOS and CD8. A similar percentage of CD4+ T lymphocytes, partly found in close contact with CD8+ T lymphocytes, also showed ICOS double immunoreactivity

Table 1 (continued)

Reference	Polymyositis	Dermatomyositis	Inclusion-body myositis
B lymphocytes			
[9]	53 patients. B lymphocytes were most abundant at perivascular sites and least abundant at endomysial sites; however, only few B lymphocytes were expressed in comparison with patients with dermatomyositis	31 patients. B lymphocytes were most abundant at perivascular sites and least abundant at endomysial sites	48 patients. B lymphocytes were most abundant at perivascular sites and least abundant at endomysial sites; however, only few B lymphocytes were expressed compared with dermatomyositis
Polymyositis, dermato-myositis, and inclusion-body myositis, [34]; dermato-myositis, [49]	3 patients. Occasional scattered B lymphocytes (CD19 and CD20) with a mean density of 2.8 cells/mm² were seen. Plasma cells (CD138) showed a mean density of 11.0 cells/mm². CD138+ cells were located predominantly in the endomysium. There were 3.9-fold more plasma cells than B lymphocytes	No data were given for 5 patients with dermatomyositis who were investigated for B lymphocytes (CD19 and CD20) and plasma cells (CD138). However, a comparison was done with another study in which it was shown that IBM muscle tissue had 0.84-fold B lymphocytes and 4.3-fold plasma cells	16 patients. Occasional scattered B lymphocytes (CD19 and CD20) with a mean density of 4.2 cells/mm² were seen. Plasma cells (CD138) showed a mean density of 17.2 cells/mm². CD138+ cells were located predominantly in the endomysium. There were 4.1-fold more plasma cells than B lymphocytes
Dendritic cells			
Polymyositis and inclusion-body myositis, [48]; dermato-myositis, [49]	10 patients. Collections of myeloid DCs (BDCA-1+) were present in 90%, widely distributed across the section with additional high-density accumulations. High-density accumulations were typically endomysial and either surrounded myofibers and sometimes invaded apparently non-necrotic myofibers. They were also expressed in dense collections of cells (always some CD3+ cells in these dense collections) between myofibers. Hardly any plasmacytoid DCs (BDCA-2+) were seen	14 patients. Plasmacytoid DCs (BDCA-2+) were seen in 10/14 patients and were mainly localized to endomysial and previavascular locations	20 patients with IBM were investigated. Collections of myeloid DCs (BDCA-1+) were present in 17/20 patients widely distributed across the section with additional high-density accumulations. These high-density accumulations were typically endomysial and either surrounded myofibers and sometimes invaded apparently non-necrotic myofibers. They could also be expressed in dense collections of cells (always some CD3+ cells in these dense collections) between myofibers. Hardly any plasmacytoid DCs (BDCA-2+) were seen
Dermato-myositis, [49]; inclusion-body myositis, [48,49]	–	14 patients. MxA was expressed in perifascicular areas. Eight patients showed MxA expression by myofibers in a preferentially perifascicular distribution in both atrophic and normal-sized fibers but also in capillaries and other blood vessels in 13/14 patients. In regions of perifascicular atrophy all myofibers were positive	20 patients. A quantitative study has been done between patients with IBM and DM who had not received immunosuppressive treatment. The numbers of myeloid and plasmacytoid DCs were similar in perivascular and perimysial areas in IBM. However, the endomysial locations of myeloid DC numbers were a mean of 3-fold higher than those of plasmacytoid DCs. In contrast, in DM there were 16-fold and 3-fold more plasmacytoid DCs than myeloid DCs in endomysial and perivascular sites, respectively
[47]	6 patients. Immature DCs (CD1a) were mainly found in lymphocytic infiltrates in all patients. Mature DCs (DC-LAMP/CD83) were localized to perivascular infiltrates surrounding muscle fibers	6 patients. Immature DCs (CD1a) were mainly found in lymphocytic infiltrates in all patients. Mature DCs (DC-LAMP/CD83) were localized to perivascular infiltrates surrounding muscle fibers	–

The references were selected according to immunohistochemical data of localization of T lymphocytes, B lymphocytes, and dendritic cells (DCs) in skeletal muscle tissue of patients with polymyositis (PM), dermatomyositis (DM), and inclusion-body myositis (IBM). MHC, major histocompatibility complex; ICOS, inducible co-stimulator; ICOS-L, ICOS ligand.

with dermatomyositis but not in patients with polymyositis or inclusion-body myositis [8]. In contrast, a recent study by Greenberg and colleagues [34] demonstrated the presence of differentiated B lymphocytes in the form of CD138+ plasma cells predominantly in the endomysium of muscle tissue of patients with polymyositis and inclusion-body myositis (no patients with dermatomyositis were included in this study). A local antigen-driven response has been shown to be present in inflammatory myopathies. Clonal expansion, class-switched significant somatic mutation, and variation of local B lymphocyte and plasma cell maturation could occur within the skeletal muscle [35]. These characteristics are hallmarks of an antigen-driven response, which would mean that T lymphocytes are not the only cell that could drive an intramuscular antigen-specific autoimmunity in inflammatory myopathies.

In peripheral blood, activated B lymphocytes (RP105-negative B cells) were distinctly increased in patients with dermatomyositis in comparison with those with polymyositis [36]. In another study, peripheral blood mononuclear cells from patients with dermatomyositis, but not from those with with polymyositis, produced significant amounts of immunoglobulins *in vitro* [37].

Thus, earlier studies based on cellular expression in muscle tissue and, to some extent, the investigations of peripheral blood suggest that B lymphocytes are important in dermatomyositis but may have a less important role in polymyositis. However, more recent data using staining markers to detect plasma cells indicated a role of B lymphocytes in polymyositis and inclusion-body myositis as well [34]. Autoantibodies are present in 60 to 70% of patients with polymyositis or dermatomyositis, supporting a role for B lymphocytes in these disease entities, but less often in patients with inclusion-body myositis. Some autoantibodies found in patients with myositis, such as anti-Ro/SSA, anti-La/SSB, anti-Scl-70, and anti-U1 ribonucleo-protein, are also found in other autoimmune diseases, whereas others, especially anti-synthetase antibodies (for example anti-histidyl-tRNA synthetase or anti-Jo-1), anti-Mi2, and anti-signal-recognition particle are more specific for myositis. The myositis-specific autoantibodies are often associated with distinct clinical manifestations, such as the anti-synthetase syndrome characterized by myositis, ILD, arthritis, Raynaud's phenomenon, and skin changes called 'mechanic's hands'. The most frequent myositis-specific autoantibody is anti-Jo-1, which is more common in patients with polymyositis but may also be present in patients with dermatomyositis [38,39]. The newly discovered autoantibody anti-p155 seems to be associated more often with dermatomyositis and para-neoplastic dermatomyositis, and its frequency is similarly high in children (29%) and adults (21%) (with para-neoplasy the frequency is 75%) [40].

There are also reports on autoantibodies in patients with inclusion-body myositis. In one of these, an increased frequency of serum monoclonal antibodies reactive to a muscle constituent was demonstrated [41].

The functional role of plasma cells in muscle in polymyositis and inclusion-body myositis is not yet fully elucidated. B lymphocytes and plasma cells could, beside antibody secretion or their role as APCs, function as stimulatory cells for other immune cells. In dermatomyositis, CD4+ T lymphocytes could release IL-17 and IFN-γ after both polyclonal and oligoclonal activation to acquire a plasma cell-like morphology that is associated with a high secretory activity, similar to that of plasma cells secreting immunoglobulins [42]. The different role of immune cells in the context of muscle inflammation is presented in Figure 3.

More recent support for a role of B lymphocytes is the good clinical effect seen with B cell depletion. This was first reported in a pilot study that included patients with corticosteroid-refractory dermatomyositis who were treated with rituximab (Rituxan®), an anti-CD20 monoclonal antibody that is approved for treatment of some rheumatic diseases [43]. Later short-term beneficial effects with rituximab were also demonstrated in a case report of two patients with refractory polymyositis and one with dermatomyositis [44].

So far, only few studies have investigated and further addressed the roles of B lymphocytes and plasma cells in myositis. It is, however, certain that further research focusing on the functional role of B lymphocytes and specific autoantibodies in IIMs is warranted; this could provide pivotal insights in the disease mechanisms for a possible future specific targeted treatment strategy. A comparison of expression in B lymphocytes in IIMs is presented in Table 1.

Dendritic cells and other antigen-presenting cells
Dendritic cell function
Tissue DCs that have internalized particulate and soluble antigens at the site of inflammation are induced to mature, and an innate immune response is triggered. These cells are activated through receptors that signal the presence of pathogen components or cytokines. DCs are also responsible for T lymphocyte activation by migrating to the lymph node and providing both antigen presentation and co-stimulation. After maturation, DCs lose their ability to capture new antigens and thus there is a continuous flow of DCs from tissue to draining secondary lymphoid organs after challenge with antigen. In their mature activated form, DCs are the most potent APCs for naïve T lymphocytes. Both macrophages and B lymphocytes also have the capacity to function as APCs, but the ability of DCs to take up, process, and present a variety of pathogens and antigens makes them the most important activators of naïve T lymphocytes.

Dendritic cells and other antigen-presenting cells in idiopathic inflammatory myopathies
The presence of T lymphocytes in all subsets of IIMs indicates a permanent immune response that requires the

presence of APCs. DCs are central to the development of innate and adaptive immune responses. Two main classes of DC have been classified, myeloid and plasmacytoid DCs. Myeloid DCs are potent APCs and have a function in the adaptive immune system. They are capable of capturing, processing, and presenting antigens and thereby stimulating lymphocytes to a specific immune response. In contrast, plasmacytoid DCs are important in the innate immune system and can produce large amounts of IFN-α and IFN-β, both of which have several functions including the stimulation of cells to produce specialized protein as a defense against pathogens. IFN-α can transform healthy monocytes into cells with properties of DCs; this was shown in sera from patients with active systemic lupus erythematosus [45]. IFN-α can also contribute to plasma cell differentiation and could therefore be important in the generation and sustenance of antibody responses [46].

Until recently, only few data were available on DCs in IIMs, but the results of some very recent studies have partly revealed important insights on this issue. Physiologically, DCs do not appear in normal muscle tissue, whereas the use of new markers for immature and mature DCs (CD1a and DC-LAMP/CD83, respectively) enabled the immature DCs to be detected in lymphocytic infiltrates in both polymyositis and dermatomyositis muscle tissue samples [47]. Local DCs have recently been demonstrated in all subsets of IIMs [48]: in muscle specimens from patients with inclusion-body myositis and patients with polymyositis, myeloid DCs were present in substantial numbers, frequently surrounding and sometimes invading intact myofibers. They were part of a dense collection of cells that also included T lymphocytes. In dermatomyositis muscles, an increased number of plasmacytoid DCs was found in comparison with the amount of myeloid DCs [48]. The importance of the plasmacytoid DCs in activating the type I interferon system in dermatomyositis was suggested by its association with the transcriptional response of the IFN-α and IFN-β inducible genes and by the fact that the interferon-induced MxA protein was expressed in muscle tissue of these patients [49]. MxA expression was demonstrated in capillaries and in perifascicular myofibers, which are characteristic sites of dermatomyositis pathology [49].

We have also found MxA expression in muscle tissue in dermatomyositis, but also in polymyositis and inclusion-body myositis, supporting a role of the type 1 interferon in all subsets of myositis. We therefore could not confirm a specific MxA staining pattern able to be used as a diagnostic tool that then could distinguish dermatomyositis from the other subsets of myositides, as suggested by Greenberg and colleagues [34]; this therefore needs to be pursued further. A comparison between expression patterns of DCs in IIMs is presented in Table 1.

However, the detailed function of both myeloid and plasmacytoid DCs in IIMs has still not been fully clarified.

Greenberg and colleagues [34] proposed two hypotheses: first, the presence of myeloid DCs invading the non-necrotic-seeming myofiber regions suggests the occurrence of active phagocytosis, endocytosis, pinocytosis, or receptor-mediated uptake of antigen, activities for which these cells are specialized; and second, that some of these cells are actively presenting antigen and activating T lymphocytes locally within muscle tissue rather than in the lymph node [48].

In addition, there is some evidence that chemokine receptors on DCs can promote autoimmune reactions. This is supported by the observation mentioned above that some autoantigens, such as aminoacyl tRNA synthetases, may exhibit chemotactic properties for activated monocytes, T lymphocytes, and immature DCs (not mature DCs). They could therefore have a capacity to attract inflammatory cells, including immature DCs, to infiltrate affected muscle cells. Taken together, these results suggest that antigens delivered to receptors on immature DCs are potent immunogens capable of breaking self-tolerance and able to induce autoimmune diseases [24,50].

As mentioned above, macrophages and B lymphocytes can also function as APCs. In addition to those, other cell types can acquire this function, although usually without reaching the same efficacy as a 'professional' APC [51]. Emerging evidence from experiments *in vitro* and *in vivo* suggests that muscle fibers could function as antigen-specific cells [52-54]. A schematic summary of the different roles of immune cells in the context of muscle inflammation is presented in Figure 3.

Whether muscle cells actually do function as APCs still remains uncertain. Muscle fibers are incapable of expressing the 'classical' co-stimulatory molecules B7-1 and B7-2, but some studies indicate that skeletal muscle cells and human myoblasts still have the possibility of acting as APCs with a cell-to-cell contact between BB-1 antigen on the one hand, and CD28 and CTLA-4 on CD4+ or CD8+ T lymphocytes on the other [53-55]. However, whether muscle cells that express BB-1 simultaneously could express MHC class I and/or class II antigens is still not clarified. Furthermore, cDNA for the BB-1 antigen has not been isolated. BB-1 is selectively induced by treatment of myoblasts with pro-inflammatory cytokines such as IFN-γ or tumor necrosis factor [55]. As both these cytokines have been detected in muscle tissue from patients with IIM [56-58], the muscle environment in the inflamed muscle could possibly provide the necessary signals on muscle fibers for an antigen presentation to T lymphocytes.

Once a naïve T lymphocyte has been activated, it expresses several proteins to sustain or modify the co-stimulatory signal for the clonal expansion and differentiation of T lymphocytes. Several co-stimulatory signal systems have been identified in muscle tissue from patients with IIM, such as CD40–CD40 ligand (CD40-L), inducible co-stimulator (ICOS)–ICOS ligand (ICOS-L), B7RP-1, B7h, and B7-H2. When a co-stimulatory

receptor is bound by its ligand, an activating signal is transmitted to the T lymphocyte. This also activates the APC to express B7 molecules, which further stimulate T lymphocyte proliferation. Interestingly, muscle cells and myofibers have been shown to express some of these co-stimulatory signals, namely CD40-L and ICOS-L (ICOS-L both expressed and functional [59]) during pathological conditions [59,60]. Schmidt and colleagues [61] demonstrated that muscle fibers expressing MHC class I, taken from patients with inclusion-body myositis, caused an upregulation of ICOS–ICOS-L molecules in association with enhanced perforin expression by autoinvasive CD8$^+$ T lymphocytes, which could exert a cytotoxic effect. This further supports the hypothesis that muscle fibers could work as APCs.

Conclusion

The molecular basis of IIMs in humans, as in many other autoimmune rheumatic diseases, is heterogeneous, involving several complex cellular components that probably contribute to differences in disease susceptibility, clinical and histopathological phenotype, and severity. Although this heterogeneity makes the study of the pathogenesis of IIMs extraordinarily complex, it might also provide distinct avenues for novel therapeutic interventions. Controlling the immune response is as complex as its launching. An essential feature of physiological immune response is its self-limitation, by which it is attenuated by several mechanisms. We have only just started to understand the orchestrated life of T lymphocytes, B lymphocytes, and DCs in IIMs, but there are still many unanswered questions about how this usually effective system can go awry and result in false immune-mediated reactions.

On the basis of detailed immunohistochemical studies on muscle biopsies, two major types of inflammatory infiltrate were observed: endomysial and perivascular/perimysial. In endomysial infiltrates there was a striking dominance of CD8$^+$ T lymphocytes, which could even be the predominating infiltrating cell type, followed by macrophages and CD4$^+$ T lymphocytes. These infiltrates often surrounded non-necrotic fibers and sometimes seemed to invade the fibers (Figure 1). This observation suggests an immune reaction that targets muscle fibers. The perivascular infiltrates, in contrast, were dominated by CD4$^+$ T lymphocytes and macrophages, and sometimes the presence of B lymphocytes suggested an immune reaction that targets microvessels (Figure 2). A role for B lymphocytes as well as one for CD4$^+$ T lymphocytes in the pathogenesis of IIMs is supported by frequently detected autoantibodies in polymyositis and dermatomyositis but less often in inclusion-body myositis. These autoantibodies are both non-specific (frequently also being found in other autoimmune disease) and myositis-specific [38,39]. A role for CD4$^+$ T lymphocytes in the disease mechanism is further supported by the genetic association with HLA-DRB1*0301, DQA1*0501, and DQB1*0201, which was particularly seen for subgroups of patients with autoantibodies.

The endomysial infiltrates were reported to be characteristic features of polymyositis and inclusion-body myositis, whereas the perivascular infiltrates were associated with patients with dermatomyositis. However, there are cases with a less distinct localization of infiltrates or with combined endomysial and perivascular cellular infiltrates [3,6]. Moreover, in some cases the inflammatory cell infiltrates are diffusely spread in the tissue, whereas in other cases the infiltrates are very small or are not found at all. In addition, the perivascular changes may be seen in patients without a skin rash, whereas endomysial infiltrates are occasionally seen in cases with a skin rash.

Taken together, these results indicate that there may be two major pathways: one leading to cellular infiltrates with predominating endomysial localization, and another with a predominantly perivascular localization often with microvessel involvement and capillary loss. The latter is more often seen in patients with a skin rash and dermatomyositis, but there seems to be an overlap between clinical phenotypes, histopathology, and immunotypes. These observations suggest that there might be more than just one factor that determines the histopathological and clinical phenotypes, for example genes and environment.

During the past few years, the results of several advanced histopathological, molecular, functional, and medical studies have provided new data that could be of importance in understanding the immune mechanisms in IIMs. Taken together, they demonstrate the complexity of involvement of the immune system in these diseases and suggest that both the innate and adaptive immune systems are involved in dermatomyositis, polymyositis, and inclusion-body myositis. This complexity of T lymphocyte populations in muscle tissue in clinical subsets of myositis was demonstrated in the original observations of different cellular subsets in muscle tissue by Arahata and Engel [6,7,9] and is exemplified in Figures 1 and 2. These observations make it necessary to revise the 'old historical' hypothesis on the pathogenesis of IIMs. Recently a dispute over the most appropriate and accurate diagnostic criteria has erupted, including the importance of the histopathological picture and the localization of immune cells [62]. Other phenotypes such as autoantibody may also be important for the classification of disease subsets that will help to enhance our understanding of disease mechanisms and thereby improve the treatment and prognosis for these patients. A revision of classification criteria has recently been started in an international interdisciplinary collaboration that we believe will facilitate these efforts.

Competing interests

The authors declare that they have no competing interests.

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