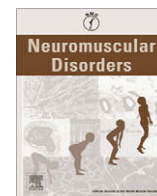




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Workshop report

International Workshop on Inclusion Body Myositis held at the Institute of Myology, Paris, on 29 May 2009

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1. Introduction

This 1-day workshop was organized by *Olivier Benveniste* and *David Hilton-Jones* and assembled 20 clinicians, pathologists and researchers (see the list of participants) from four countries (UK, USA, Austria, France). This workshop followed one held in London 1 year earlier with some common participants [1] in order to reinforce a collaborative interface between several international groups actively investigating the clinical features, pathogenesis and treatment of sporadic inclusion body myositis (sIBM). To date, all drugs tested in randomized clinical trials (RCT) appear to be comparable to placebo [2–9]. Nevertheless, new molecules are emerging [10,11]. The assessment of potential new treatments is hampered by difficulties in the design of trials, relating in part to the slowly progressive nature of the disorder and uncertainties about the natural history, and the low incidence and prevalence of the disease. Evaluation of required sample size for future sIBM trials has been calculated from the results of two studies using two doses (30 and 60 µg/weekly) of βIFN (without any difference in strength between the 57 placebo or IFN treated patients) [2,3]. For a two-arm, 6-month therapeutic intervention, aiming to show arrest of disease progression, it would require 208 subjects per group [2,3]. The enrolment of such a number of patients would be achievable only in a collaborative international multicentre study. Treatments that slow, rather than arrest, progression would need larger numbers, and also longer studies with associated increased costs. The first part of the meeting reviewed the currently widely used diagnostic criteria proposed by RC Griggs et al. [12]. The need for consensus with respect to pathological assessment of muscle biopsy specimens, particularly immunohistochemistry, was agreed. The second part of the meeting was devoted to defining the outcome measures for such trials, and finally we explored the possibilities for international RCTs.

2. Diagnostic criteria of sIBM

Olivier Benveniste presented the preliminary results of a study called “Natural history and history under treatment of sIBM: the Pitié-Salpêtrière/Oxford study”. Patients were included on the basis of typical clinical features at presentation and on long-term review, with pathological features not inconsistent with the diagnosis of sIBM (in other words, the type of patient seen in everyday practice). One hundred and thirty-six patients (57% males) were included. First symptoms started at 61 [55–69] years and were limb muscle weakness in 87%, swallowing difficulties in 4% or both in 12%. A delay between symptom onset and diagnosis of 52 [28–99] months was observed. Considering entry criteria for future sIBM trials, this study noted that 30% of the patients had had an initial incorrect diagnosis (PM in 17%, muscular dystrophy in 3%, atrophic lateral sclerosis in 2%). Griggs et al. [12] defined *definite IBM* as patients exhibiting inflammatory myopathy characterized by mononuclear cell invasion of non-necrotic muscle fibers, vacuolated muscle fibers and amyloids deposits within muscle fibers (by fluorescent method of identification) or 15–18 nm tubulofilaments by electron microscopy. *Possible IBM* was considered when the muscle biopsy shows inflammation but not the additional pathological features, and patients have characteristic clinical features. Dalakas and Needham [13,14] introduced in their revised IBM diagnostic criteria the following: (1) amyloid-like filaments can immunoreact with various amyloid-protein-related antibodies (Abs) [13], (2) definite IBM is confirmed by the biopsy showing all the features described by Griggs with in addition also COX-negative fibers and upregulation of MHC-I expression, (3) a third category of possible IBM was defined for those with an atypical pattern of weakness and incomplete biopsy criteria [14]. Seventy percent of the patients in the Paris/Oxford study had a diagnosis of *probable* sIBM according to the criteria of Dalakas or Needham [13,14] (clinical phenotype of sIBM and muscle biopsy showing inflammation (or major histocompatibility complex (MHC) class I antigen upregulation) and rimmed vacuoles but without evidence of amyloid deposits), 24% with *possible* sIBM (clinical phenotype of sIBM, inflammation without vacuoles or vice versa), and only 6% with *definite* sIBM (inflammation, rimmed vacuoles and amyloid deposits) – but the main reason was that routine assessment of the latter (electron microscopy, Congo-red-positive or crystal violet amyloid

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deposits, or immunohistochemistry with various amyloid-protein-related Abs) had not been performed routinely.

David Hilton-Jones presented a review of the clinical features and diagnostic criteria of sIBM [12,13,15–18]. sIBM was initially delineated as a specific disorder on the basis of the identification of certain pathological changes, including rimmed vacuoles, in patients with treatment-resistant polymyositis (PM). It was later realised: (a) that these pathological changes were not specific and (b) that patients with sIBM had a strikingly selective pattern of muscle involvement, that differed from PM, with early weakness of distal muscles (especially the finger flexors), and greater weakness of quadriceps than iliopsoas [12]. Later, accumulation of amyloid and other proteins was recognised as being a common finding. The still widely used Griggs criteria defined *Definite sIBM* at a pathological level, requiring the demonstration of amyloid deposits or tubulofilaments as well as the presence of rimmed vacuoles and partial invasion [12]. *Possible sIBM* was defined on the basis of characteristic clinical features and muscle showing partial invasion but not rimmed vacuoles, amyloid deposition or tubulofilaments. In routine clinical practice many patients fall into the possible sIBM category (although extensive testing for amyloid is often not performed). This may reflect sampling error, or it may be that the currently recognised canonical biopsy features (rimmed vacuoles, amyloid, filaments) are late features of the disease in which case excluding patients lacking these features from drug trials may bias against patients more likely to respond to treatment. That clinical features alone may define the diagnosis is supported by recent observations by Chahin and Engel [19].

David Hilton-Jones therefore proposed revised diagnostic criteria (Table 1) in which clinical features alone (with pathological features that are supportive and not inconsistent with the diagnosis) allow a category of “Clinically defined sIBM”, whereas those with the canonical biopsy features, with appropriate clinical features, represent “Pathologically defined sIBM”. Both groups of patients should be eligible for clinical trials with separate analysis of response to treatment. Those with “Possible sIBM” should be excluded from trials, but in practice most will probably, on long-term follow up, prove to indeed have sIBM.

Janice Holton presented a review of pathological features and diagnostic criteria of sIBM. The Griggs pathological criteria for the diagnosis of definite and possible sIBM were reviewed [12]. More recent observation has stressed that an important pathological finding in sIBM is the increased expression of MHC-Class I on muscle fibres, with infiltration of such fibres by CD8-positive T lymphocytes [20]. Recently proposed diagnostic criteria [14] were considered in which a diagnosis of definite sIBM requires charac-

teristic clinical features, invasion of non-necrotic muscle fibres by CD8-positive T lymphocytes, upregulation of sarcolemmal MHC-Class I expression, the presence of rimmed vacuoles with intracellular amyloid deposition or ultrastructural confirmation of tubule-filamentous material. Cytochrome oxidase deficient fibres may be an additional pathological feature. Needham has suggested that, probable sIBM may be diagnosed when there are typical clinical features but the only supportive pathological feature is infiltration of non-necrotic muscle fibres by CD8-positive T lymphocytes and possible sIBM is considered when the clinical syndrome is atypical and the biopsy features are incomplete [14]. The typical light-microscopy and ultrastructural features of sIBM were demonstrated. Accumulation in muscle fibres of a number of proteins typically associated with neurodegenerative diseases has been demonstrated using immunohistochemistry in recent years [21]. However, clear recommendations for a diagnostic strategy incorporating such immunohistochemical data have not been established.

Caroline Sewry discussed the difficulties of the pathological differential diagnosis when the typical features of sIBM are not clearly apparent. The pathological differentiation of a myofibrillar myopathy from sIBM, in particular, can often be difficult. The importance of always relating the pathology to the clinical features was emphasised, and inclusion and exclusion criteria discussed by others. Inflammatory infiltrates and invasion of otherwise healthy looking fibres by T cells are considered the hallmark of PM and sIBM, but absence of inflammation in a single sample does not exclude the diagnosis of sIBM. Similarly, the distribution of T cells, and proportion of T cell subtypes does not always give a clear indication of sIBM. In addition, inflammation is not specific for inflammatory myopathies and can occur to varying degrees in several neuromuscular disorders, including dysferlinopathies, fascio-scapulothoracic dystrophy, other muscular dystrophies, and occasionally in myofibrillar myopathies. Sarcolemmal and internal immunolabelling of MHC-class I antigen is recognised as a useful indicator of inflammatory disease [22], and can be seen in the absence of inflammatory cells. This, however, is not specific for inflammatory disorders and can occur in Xp21 muscular dystrophies, dysferlinopathies, and to a variable degree on some fibres in myofibrillar myopathies, and other myopathic conditions. Regeneration also has to be considered as high levels of MHC-I expression is a feature of regenerating fibres whatever the underlying disorder. The significance of low to moderate levels of MHC-I is not clear, nor the effect of drug therapy on MHC-I expression, including corticosteroids and statins. Vacuoles are also not specific for sIBM and can occur in a wide variety of disorders, including

Table 1
Revised diagnostic criteria for sIBM.

| Clinical and laboratory features Consistent with IBM | Classification Pathologically defined IBM | Pathological features Endomysial exudate Partial invasion Vacuoles Congo-red or crystal violet or hyperphosphorylated tau (SMI-31) p62/SQSTM1 or TDP43 or 15–18 nm filaments |
|---|--|--|
| Duration >6 months Age >30 years EMG consistent Quads weakness > hip flex and FF weakness > should abd | Clinically defined IBM | Endomysial exudate or ↑ MHC1 no Congo-red or SMI-31 or 15–18 nm filaments |
| Duration >6 months Age >30 years EMG consistent Quads weakness > hip flex or FF weakness > should abd | Possible IBM | Endomysial exudate or ↑ MHC1 no Congo-red or SMI-31 or 15–18 nm filaments |

myofibrillar myopathies. The number of vacuoles that are considered significant in sIBM is not known, and it is not clear whether all vacuoles in sIBM are the rimmed type or if some are unrimmed as in myofibrillar myopathies. Similar accumulation of proteins, and their detection with various techniques, occurs in both sIBM and myofibrillar myopathies, and the application of the most suitable techniques was discussed. There remains considerable uncertainty with respect to specificity and sensitivity of the identification of these aggregations of proteins. It was concluded that there is a need to assess and identify the most useful tools, in particular the application of Congo-red vs crystal violet and the application of a variety of antibodies. The need to consider methods and the source of antibodies, was emphasised. Studies are in progress to select the most useful methods for differential diagnosis, and to provide updated guidelines for the assessment of muscle biopsies.

Odile Dubourg presented data from a pilot study on the immunohistochemical pattern of different markers of degenerative changes in sIBM: SMI31 (SMI-31R, Covance, dilution 1/1000), SMI-310 (SMI-310R, Covance, dilution 1/1000), Ubiquitin (Z0458, Dako, dilution 1/1000), APP (MAB 348, Millipore, dilution 1/1000) and Beta A4 (Clone 6F/3D, Dako, dilution 1/50). Immunohistochemistry was performed on frozen sections of 22 definite sIBM, 12 possible sIBM, 15 pathological controls (7 PM, 3 dysferlinopathies, 2 calpainopathies, 2 unspecified LGMD, one Bethlem myopathy) and 6 normal controls. Age at biopsy was similar between definite (64.8 ± 11.3 , 52–89 year) and possible sIBM (67.7 ± 12.2 , 55–88 year). She reported muscle fibers with SMI31-immunoreactivity (IR) in 100% of the definite sIBM cases but in none of the possible sIBM cases. SMI31 showed non-specific IR in PM and dystrophic muscles. Ubiquitin deposits were present in 77% of the definite sIBM cases, 16% of the possible sIBM cases but also in some cases with PM/DM and dystrophies, showing that this marker is less specific than SMI31. It was negative in normal control muscles. Beta A4, APP and SMI310 IR were observed in a smaller proportion of the definite sIBM cases (13.6%, 12.5% and 18.7%, respectively). All these markers were negative in diseased and normal control muscles. By calculating the percentage of IR fibers in the definite sIBM cases, we observed that SMI31 only was positive in more than 1% of the fibers (3.9 ± 3.2 , 0.5–12.5%) indicating that it was the more sensitive marker among those tested. We concluded that SMI31 is the more sensitive marker for the diagnosis of definite sIBM among the markers tested. Finally, we showed some examples of immunohistochemistry with TDP43 (TAR DNA-binding protein 43, 10782–2-AP, ProteinTech Group, dilution 1/100) on frozen muscle sections, which seems to be an interesting marker in sIBM and plan to test it in the whole series.

Julia Wanschitz presented a pilot study on markers of muscle regeneration in sIBM. Using immunohistochemical and fluorescence techniques she analysed the expression profile of myogenic regulatory factors Pax7, MyoD and Myogenin of myogenic progenitor cells in muscle biopsies from 32 patients with idiopathic inflammatory myopathies (IIM) including histologically confirmed sIBM ($n = 12$, mean age 67.2 years), patients with clinical features of sIBM and signs of inflammation on muscle biopsies but lacking vacuoles or inclusions (classified as possible sIBM, $n = 10$, mean age 64.3 years) and patients with treatment-responsive myositis ($n = 10$, mean age 50.7 years) compared with five patients with limb-girdle muscular dystrophy (LGMD, mean age 40.2 years) and five individuals without myopathy as negative controls (mean age 66.2 years). The process of muscle regeneration, visualized by the percentage of neonatal myosin-, vimentin-, and CD56-expressing myofibers was studied in relation to the extent of inflammation, deposition of age-related proteins and the duration of the disease.

In a preliminary analysis she found significantly increased numbers of Pax7 and myogenin positive nuclei in typical sIBM and treatment-responsive myositis compared to LGMD and negative controls, whereas possible sIBM did not differ from LGMD and controls. In contrast, expression of MyoD was significantly increased in myositis, but not in sIBM (with or without typical muscle biopsy findings). The percentages of neonatal myosin-, vimentin-, and CD56-expressing myofibers were higher in typical sIBM and myositis than in non-typical sIBM, LGMD and controls, but biopsies from treatment-responsive myositis had significantly higher numbers of neonatal myosin- and vimentin-expressing regenerating muscle fibers than all other groups. In addition, expression of myogenic regulatory factors and the frequency of regenerating fibers appeared to correlate with the intensity of inflammation, but decreased with the age of patients and the duration of the disease. These results demonstrate a substantial increase of myogenic progenitors in typical sIBM and myositis. However, increased expression of MyoD and higher percentages regenerating muscle fibers in myositis may reflect differences in the dynamic process of myoregeneration, which might be more efficient in treatment-responsive myositis than in sIBM, but these staining methods do not allow distinction between definite and possible sIBM.

Olivier Boyer presented preliminary results of his recent prospective, pilot myoARRAY study (PI: Isabelle Marie and Olivier Boyer). He reminded us that several large-scale muscle transcriptome analyses have suggested that gene arrays may be of help for the diagnosis of myositis but the diagnostic value of this approach has not been assessed in a prospective study. Forty-nine muscle samples from patients with an indication of muscle biopsy for suspicion of myositis were assayed for gene expression of a series of more than 700 non-redundant genes previously described to be differentially expressed in different forms of muscle diseases including myositis. Preliminary results indicate that a set of genes (notably HLA-class I and related genes) can be identified that distinguishes patients with myositis from patients with other forms of muscle disease or controls. Nevertheless, among the group of patients with myositis, sIBM could not be identified by a distinct pattern of gene expression, consistent with the original study of Greenberg et al. [23]. It was then discussed that the presence of inflammatory infiltrates and upregulation of HLA are features not only of sIBM but also of other forms of myositis such as PM and dermatomyositis (DM). Thus, it is understandable that the gene expression pattern of these different types of myositis may be similar. Further work will validate the results using quantitative RT-PCR, compute a synthetic index of the data and evaluate its specificity and sensitivity for the diagnosis of myositis, and compare blood and muscle transcriptomes.

Valerie Askanas presented observations from her group at the University of Southern California (USC) Neuromuscular Center considering the important pathologic diagnostic criteria for sIBM:

1. Light-microscopic histochemistry and immunocytochemistry

Characteristic light-microscopic pathologic features of sIBM evident with the Engel-Gomori trichrome stain [24] include: (a) muscle fibers with one or several, irregular and various-sized, vacuoles on a given 10- μ m thick fresh-frozen cross-section and (b) various degrees of lymphocytic inflammation, with some macrophages. In contrast to what is commonly stated, only some of the vacuoles appear rimmed by a trichrome-reddish material (reviewed by Askanas and Engel [25–27]), while many of the vacuoles do not have a conspicuous reddish rim and appear “empty” (these must be distinguished from freeze-artefacts). In a given section, 60–80% of the sIBM vacuolated muscle fibers, mainly in their non-vacuolated cytoplasm, contain foci positive with Congo-red, thioflavin-s and crystal violet identifying amyloid in β -pleated

sheet conformation (reviewed in [25–27]). Because amyloid deposits can be very small and sometimes present in only a few muscle fibers, they can often be missed if examined only by Congo-red visualized through polarizing filters, thioflavin-s, or crystal violet. Analyzing Congo-red staining by a non-experienced eye through polarizing filters is rather difficult, and may account for some erroneous negative results recently reported in the literature. Her fluorescence-enhanced Congo-red technique greatly facilitates identification of amyloid deposits [28].

Currently she recommends the following morphologic criteria to diagnose sIBM:

- (1) Engel-Gomori trichrome staining to visualize vacuolated muscle fibers, mononuclear-cell-inflammation, and the occasional ragged-red fibers.
- (2) The Askanas Fluorescence-enhanced Congo-red staining to visualize intra-muscle fiber congophilia (but crystal violet staining is important to differentiate sIBM from myofibrillar myopathies, see below).
- (3) Immunocytochemical non-fluorescence (streptavidin HRP) staining for the presence of paired-helical filaments (PHFs), which utilizes antibody recognizing p62 protein. P62 is a shuttle protein carrying ubiquitinated proteins for the degradation by both the 26S proteasome and autophagosomal-lysosomal systems [21,29].

In sIBM muscle fibers, by light-microscopy p62 is present in the form of various-sized clearly demarcated inclusions, often in a linear or squiggly pattern, which closely co-localize with phosphorylated tau [29]. By immuno-electron-microscopy p62 immunodecorates clusters of paired-helical filaments (PHFs) [29]. Phosphorylated-tau containing PHFs are very prominent in sIBM muscle fibers, and are prominently present in both vacuolated and non-vacuolated muscle fibers [30,31].

By the nature of its very intense staining at the edges of the PHFs clusters [29], p62 immunoreactivity in sIBM muscle fibers is very strong, and it is now her preferred immunostaining to pathologically diagnose sIBM. This staining replaced SMI31-immunoreactivity (previously recommended by her [32], because, recently, since the Sternberger company (Sternberger Monoclonals, Inc., Baltimore, MD), has been acquired by Covance (Covance, Denver, PA), the SMI31 antibody has been very weak requiring much stronger concentration and a longer incubation time, both of which result in some non-specific staining, including a non-specific staining of the nuclei. However, correctly performed p62 reaction does not recognize nuclei by light-microscopy.

- (4) Ubiquitin immunoreactivity within muscle fibers can be also be used [33].

Ubiquitin-positive inclusions can be identified in both fresh-frozen and formalin-fixed paraffin-embedded muscle biopsies of sIBM patients, but not in any other inflammatory myopathies [33,34].

There are other light-microscopy aspects of sIBM muscle biopsies that are characteristic and important, but not diagnostic for sIBM. For example: (a) mitochondrial abnormalities, which include: (a) ragged-red fibers [35] and (b) cytochrome-c-oxidase (COX) negative muscle fibers are more common in sIBM than expected for the patient's age. (b) Small angular muscle fibers, which are histochemically dark with the pan-esterase and NADH-tetrazolium-reductase reactions, are always present and are indistinguishable from those seen in denervation. They are generally considered indicative of "recent-denervation", and may contribute to the clinical weakness (reviewed by Askanas and Engel [25–27]).

2. Ultrastructural abnormalities of sIBM vacuolated, and some non-vacuolated, muscle fibers

Characteristic are PHFs, often in clusters, strikingly resembling PHFs of AD brain, being 15–21 nm diameter and containing phosphorylated tau (reviewed in detail by Askanas and Engel [25]). PHFs are present in both vacuolated and non-vacuolated muscle fibers. In 2–6% of sIBM muscle nuclei there are clusters (inclusions) of 15–21 nm "tubulofilaments", which in favorable sections are seen to be paired-helical filaments like those in the cytoplasm.

The sIBM vacuolated muscle fiber cytoplasm, and often cytoplasm of the non-vacuolated muscle fibers, also contain amyloid- β -positive: (a) collections of 6–10 nm filaments; (b) fine flocculomembranous material; and (c) amorphous material [36]. Those preferentially contain amyloid- β 42 [37]. Myelin-like whorls and other lysosomal debris are present in the vacuolated fibers. Ultrastructurally abnormal mitochondria containing paracrystalline inclusions are occasionally present.

3. General Comments, including differentiation of sIBM from PM and myofibrillar myopathies

- (1) An often-asked question relates to how many Congo-red-positive, and/or specifically-immuno-stained fibers in a given cross-section of a muscle biopsy are needed to make a definite diagnosis of sIBM. V. Askanas considers sIBM diagnosis to be very probable, if, in the milieu of inflammation and of 1–2-vacuolated muscle fibers, there are two fluorescence-enhanced Congo-red-positive muscle fibers, and two p62-immunopositive muscle fibers; and definite, if, there are three or more fluorescence-enhanced Congo-red-positive muscle fibers, and 3 or more p62-immunopositive muscle fibers. She does not recommend TDP43 as diagnostic criterion of sIBM, since in her experience it is much less sensitive and less abundant than p62 immunoreactivity (D'Agostino et al., 2009, to be published).
- (2) Regarding pathologic differentiation between sIBM and PM, in addition to the above, which, in her hands never occur in PM, she also recommends alkaline phosphatase (AP) staining of perimysial connective tissue. In sIBM, perimysial connective tissue, even in regions of active disease, lacks the typically-strong AP-positivity seen in similarly-active regions of PM and DM, which is attributable to active fibroblasts (reviewed in [38]).
- (3) The most characteristic feature of myofibrillar myopathies on Engel trichrome staining are masses of a dense filamentous green material, which do not occur in sIBM. Regarding other pathologic differences between sIBM and myofibrillar myopathies, the latter do not exhibit: (a) inflammation; (b) typical crystal violet or thioflavin S positivity; and by electronmicroscopy, clusters of paired-helical filaments or tubulofilamentous inclusions.

2.1. Concluding remarks concerning sIBM diagnosis criteria

In routine clinical practice, in about 1/3 of clinically suspected sIBM cases, some key pathological features (as defined above and in Table 1) currently considered essential for a diagnosis of definite sIBM are missing. Most frequently, these missing features are vacuoles and/or evidence of misfold proteins accumulation, the latter because many routine laboratories do not undertake appropriate studies. In this situation, the pathological features do not permit distinction between sIBM and PM, a condition known to respond to conventional immunosuppressive drugs. In many cases, the clinical presentation may nevertheless help to distinguish between these two disorders (notably by the asymmetry, presence of early distal muscle weakness, and the

selective involvement of quadriceps (Table 1) in sIBM), but these features can be absent, notably in the early stages of the disease. In order to confirm the diagnosis of sIBM, the group considers that: (1) electronic microscopy is no longer needed, even as entry criteria for a future clinical trial in sIBM, because other equally sensitive and specific techniques, more easily applied in routine laboratories are available; (2) immunostaining with myogenic regulatory factors or transcriptome approaches (see above) are still not useful for diagnosis purpose; (3) the detection of amyloid deposits (Congo-red or crystal violet) and/or of other sIBM characteristic accumulated proteins by immunohistochemistry (SMI31, TDP43 and/or p62) is required and should be added to the routine set of analysis, and must be incorporated into the entry criteria for any future sIBM trials. These immunostaining techniques can detect misfold proteins accumulation in the absence of any vacuoles. However, their precise specificity (noting positivity in other vacuolated myopathies) and sensitivity (e.g. p62 or TDP43 labeling in the early phase of the disease and/or in absence of vacuoles) remain to be determined and for this reason a multicentre study to clarify these issues is in progress at the London MRC Centre for Neuromuscular Disease, John Radcliffe Hospital (Oxford), UCL Institute of Neurology (USA) and the Institut de Myologie, Hôpital Pitié-Salpêtrière (France).

In the proposed group of “Clinically defined sIBM” patients (Table 1), the group recommends considering repeating the biopsy, preferentially in an affected muscle as determined by clinical assessment and possibly aided by muscle imaging or EMG. Nevertheless, since the canonical biopsy features (rimmed vacuoles, amyloid, filaments) may be late features of the disease, excluding patients lacking these features from drug trials may bias against patients more likely to respond to treatment, the group recommends that both “Clinically defined sIBM” and “Pathologically defined sIBM” (Table 1) patients should be eligible for clinical trials with separate analysis of response to treatment. Those with “Possible sIBM” (Table 1) should be excluded from trials.

3. Measuring outcomes for RCT

The second part of the meeting concerned the clinical and immunological monitoring of sIBM patients for future trials.

Jean-Yves Hogrel presented a pilot study on a subgroup of 22 untreated patients (mean age 70) from the “Oxford/Pitié-Salpêtrière project” evaluating their strength and mobility by different myometric methods (dynamometric measurements for handgrip, wrist, elbow, ankle and knee flexion and extension) and functional scales (6 min walk test with accelerometer, Walton, Karnofsky, Rivermead Mobility Index). He showed that muscle strength was significantly lower for the patients compared to their age and sex matched healthy controls. The weakest functions for the upper limb were handgrip, wrist flexion and elbow flexion (<40% of normal). For the lower limb, ankle flexion, knee extension and flexion are the weakest (<40% of normal). Interestingly, for the upper limb, the non-dominant side was significantly weaker than the dominant one, suggesting that muscle activity may delay the disease progression, as also noted by Needham and Mastaglia [14]. The 6 min walk test was significantly reduced in the ambulatory patients ($n = 19$) compared to controls. This finding correlated with the strength of ankle extension, knee flexion and knee extension. A significant correlation was observed between strength and duration of the disease for knee extension. As a global picture, flexors were more affected than extensors; and the non-dominant side was more affected than the dominant side for the upper limb. The 6 min walk test is a good functional test for assessing global impairment in ambulatory patients. The same population will be assessed 9 months later to evaluate the progression of the disease.

Yves Allenbach presented a pilot study on the same 22 patients and controls looking for different immunological markers relevant for the immunomonitoring of sIBM patients during RCT (regulatory T cells (Treg), TH1/TH2/TH17 lineage). Peripheral blood mononuclear cells were analyzed using flow cytometry. Muscle biopsies of 10 sIBM patients were tested for the presence of Treg cells using semi quantitative analysis. No significant difference was observed between sIBM patients and controls concerning the number of lymphocytes, T cells and the T cells repartition (CD4+ and CD8+) in blood cells. The mean percentage of CD3+CD4+IFN γ + among CD3+CD4+ T cells in IBM and control groups was respectively 14.15 ± 1.960 (SEM) and 14.34 ± 1.420 $p = 0.93$, whereas the mean percentage of CD3+CD8+IFN γ + among CD3+CD8+ was higher in IBM patients (61.38 ± 3.701) compared to controls (47.00 ± 5.3) $p = 0.029$. The mean percentage of CD3+CD4+IL-17+ among CD3+CD4+T cells in IBM patients (0.28 ± 0.04) compare those obtained in the control group (0.40 ± 0.07) was not statically different $p = 0.19$. The percentage of Treg (CD3+CD4+CD25+CD127-FOX3+) among CD4+ T cells was lower in the IBM group (5.4 ± 0.2) compare to controls (6.4 ± 0.3) $p = 0.033$. Treg cells (FOXP3+) were present in all muscle infiltrates of IBM patients. Together these results show that the profile of the immune systemic response in IBM patients is engaged in a TH1 lineage and not in TH17. Treg cells are decreased in the systemic compartment whereas they are present in the muscle of IBM patients suggesting that they are unable to control the immune response in the muscle. Strategies that can increase their number or function may be new therapeutic approaches...

Mike Hanna reviewed the clinical trials already performed in sIBM and considered possible therapeutic targets and the need for the sIBM community to reach consensus regarding the approach and design of future trials. Although RCTs' should be the gold standard there are relatively few that have been adequately performed in sIBM. Treatments that have been tried fall into three main categories: (1) drug treatments, (2) non-drug treatments (exercise, adaptations, psychosocial interventions) and (3) symptom specific (e.g. dysphagia). Focussing on drug treatments, one problem is that given that the precise aetiology sIBM is not known it is problematic to identify the correct therapeutic target. This aetiological uncertainty has meant that several targets have been considered and include: (1) inflammatory/immunological, (2) mitochondrial, (3) oxidative stress, (4) muscle wasting and (5) protein aggregation. A wide range of agents have been tried in a variety of clinical trials, many of which are non-RCTs, and results are difficult to interpret. Immunological agents tried include; steroids, azathioprine, cyclophosphamide, chlorambucil, methotrexate (MTX), cyclosporine, mycophenolate, total body irradiation, plasma exchange, intravenous immunoglobulins (IVIg), anti T lymphocyte Ig, alemtuzumab and etanercept [14]. Other agents have targeted oxidative stress (anti-oxidants vitamin E), mitochondria (carnitine, con-enzyme Q10), and muscle wasting (oxandrolone). More recently protein aggregation has been targeted (rapamycin, arimocloamol) [39,40]. Most of these studies are non-RCTs. There have only been nine RCTs to date- three with IVIg, one with MTX, one with oxandrolone, one with MTX and anti T lymphocyte immunoglobulin and one with MTX and azathioprine [2–9]. Corticosteroids have never been trialled in an RCT although here is a general view based on clinical experience that steroids are not effective in IBM. The data from IVIg in the RCTs that have been undertaken are slightly conflicting. Dalakas [5] observed function improvement in 28% over 3 months, Walter [6] observed stabilisation in 90% over 3 months. But there is little evidence of substantial or long-term benefit and whether such treatment is justified has been questioned [41]. This review of the somewhat confused literature of treatment trials in sIBM with very few RCTs indicates that the sIBM clinical research community needs to reevaluate the

position and ideally make recommendations regarding future trials. Points to consider include: (1) should we retest any of the “old” agents for which evidence is inconclusive (e.g. IVIg) or should we only test new agents and if so which new agents? (2) The diagnostic entry criteria into trials have varied and consensus upon which criteria should be used is required. The possibility of including clinically defined sIBM needs to be considered if this indeed represents an earlier stage of the disease which may potentially be more amenable to treatment (see earlier). The need to exclude hereditary IBM requires further evaluation as the frequency of hIBM gene mutations in the sporadic IBM population is not determined. (4) Which outcome measures and end points to use, and over what period of time, is not clearly established and has varied in different trials making direct comparisons between them problematic. A clearly validated set of outcome measures for sIBM, to be used in future trials is required. This should include patient reported outcome measures (PROM) as has recently been strongly advised in all clinical trials by the FDA. Any PROM used in a clinical trial must satisfy minimum performance criteria and clinically meaningful content must be established through qualitative evaluation. Currently there is no established PROM for sIBM. The choice of the outcome measure should also take into consideration the primary purpose of the trial e.g. proof-of-principle vs efficacy trial. The IBM functional rating scale (FRS), adapted from the motor neurone disease FRS, has recently been proposed as a validated outcome measure in IBM patients that correlated well with multiple other end point parameters [42]. The duration of the trial is very important considering sIBM is a slowly progressive disease which may stabilize for short periods of time [43].

Overall there may important challenges in order for future clinical trials in sIBM to generate positive or negative results that can be relied upon. Ideally the sIBM community should work together to devise a “tool kit” of minimum criteria relating to the design and conduct of clinical trials in sIBM that take into account the above considerations. The wide-spread adoption of such a “tool kit” for sIBM trials will mean that patients with sIBM are less likely to enter poorly designed trials not powered to address the intended question. Furthermore, it is suggested that this sIBM community considers making recommendations regarding the prioritisation of which agents should be trialled.

4. Therapeutic approaches and future trials

Before the final discussion, *Marinos Dalakas* reviewed current understanding of the immunopathology of the disease and how this might aid the development of novel approaches to treatment [11].

sIBM is clearly a very complex disorder. In addition to T-cell-mediated cytotoxicity identical to that observed in PM, the disease has degenerative features consisting of vacuoles and accumulation of stressor and amyloid-related proteins in muscle fibers not invaded by T cells. This observation suggests that in IBM two processes act independently or in concert with each other: a primary immune process and a degenerative one. The scenario that the disease begins with the accumulation of misfolded proteins, which then act as neoantigens to trigger a cytotoxic lymphocytic attack on muscle fiber, remains untested. Although the factors triggering immune and protein dysregulation or vacuolar formation remain unknown, a primary inflammatory process that leads to degeneration is favored by Dalakas [13,44] because of the following:

- (a) T cell invasion of non-necrotic fibers is found early and in higher frequency than the Congo-red-positive fibers [45],

- (b) uniform expression of class I MHC products at the surface of most muscle fibres is characteristic of PM and IBM whereas in DM this phenomenon may be evident only in the perifascicular or other random regions [22,46]. Ubiquitous expression of MHC-class I does not occur in limb-girdle dystrophy, denervating diseases or metabolic myopathies (except in regenerating fibres or in fiber invaded by macrophages and lymphoid cells), which makes MHC immunostaining a very helpful diagnostic tool,
- (c) the cytokine-induced upregulation of MHC-I occurs early and is capable of triggering cell stress and degeneration [13]. Most importantly, endomysial inflammation alone can cause muscle destruction and clinical weakness, as seen in PM; whether the tiny β -amyloid deposits are sufficient alone to trigger muscle degeneration in humans remains unclear. Further, these accumulations are not unique to sIBM, because they are also observed to a similar extent in other vacuolar myopathies such as myofibrillar myopathies and genetic disorders. What appears unique to sIBM however, compared to other chronic vacuolar myopathies, is the strong presence of primary inflammation and autoimmune markers mentioned above,
- (d) frequent association with other autoimmune diseases, auto-antibodies, monoclonal proteins and immunodeficiency [13],
- (e) immunogenetic association with DR β *0301, DQ β 1*0201 alleles and the B8-DR3-Dr52-DQ2 haplotype [47]; the latter associated with earlier disease onset, and
- (f) the occurrence with HIV- and HTLV-I infection. It is now becoming clear that the association of retroviruses with sIBM is more than a coincidence because more than 30 cases of HIV/HTLV-1 positive patients with sIBM have been reported or are known to us [48–52]. In these seropositive patients, the disease appears before the age of 50 but several years after the first manifestations of the retroviral infection, suggesting that in HIV-positive patients who live longer and harbor the virus for several years the disease is more frequently recognized. The clinical phenotype and muscle histology in HIV-IBM patients is identical to the retroviral-negative sIBM. The predominant endomysial cells are CD8+ cytotoxic T cells which, along with macrophages, invade or surround MHC-1-antigen –expressing non-necrotic muscle fibers [53,54]. Using in situ hybridization, PCR, immunocytochemistry and electronmicroscopy, viral antigens could not be detected within the muscle fibers but only in occasional endomysial macrophages [48,50,53,54]. Molecular immunological studies using tetramers have shown that retrovirus-specific cytotoxic T cells, whose T cell receptor contains amino acid residues for specific HLA/viral peptides are recruited within the clonally expanded T cells and invade muscle fibers [51,55,56]. We have interpreted these observations to suggest that in HIV-1 and HTLV-1 IBM there is no evidence of persistent infection of the muscle fibers with the virus or viral replication within the muscle, but, rather that the chronic retroviral infection, in genetically susceptible individuals, triggers a persistent inflammatory process that leads to sIBM [51,56].

4.1. Reconciling the roles of inflammation and degeneration

Regardless of whether the primary event is a dysimmune or protein dysregulation process, there is now strong evidence that in sIBM pro-inflammatory cytokines not only correlate with the intramuscular accumulation of amyloid, phosphorylated tau, ubiquitin and α B-crystallin but also induce tau phosphorylation, amyloid aggregates and stressor proteins such as α B crystallin [57]. Cytokines also stimulate myofibers to produce inflammatory

mediators in an autoamplificatory mechanism, enhancing further the chronicity of inflammation, amyloid formation and cell stress.

4.2. Experience with conventional immunotherapies

Although a number of patients with IBM might show a transient response to corticosteroid therapy, the majority do not. Methotrexate, ciclosporin, azathioprine or mycophenolate mofetil are largely ineffective (although some patients might initially respond to some degree). IVIg can provide some benefit to a small proportion of patients for a period of time, especially for those with dysphagia but its overall effect is disappointing.

The resistance of sIBM to these therapies has been used to argue that the degenerative process, rather than the inflammatory one, might be more relevant. A lack of agents that target degeneration, however, has prompted the suggestion that strategies that aggressively suppress inflammation by targeting T cells and molecules implicated in MHC-activation complex could still have a role in suppressing muscle degeneration, through their effects on cell stress and protein misfolding. Based on the interaction of inflammation and degeneration noted above, the concept of 'neuroinflammation', using sIBM as a model, was introduced. Accordingly, the use of potent anti-inflammatory agents as a means of suppressing the accumulation of potentially toxic or stressor molecules within the muscle fibers warrants investigation.

4.3. Experience with alemtuzumab

With this aim in mind, a proof-of-principle trial with alemtuzumab, a humanized monoclonal antibody that causes long-lasting depletion of peripheral blood lymphocytes, was performed to examine if the drug would reduce endomysial T cells and favourably alter the natural course of sIBM [11]. In the study, 13 patients with established sporadic sIBM received 0.3 mg/kg per day alemtuzumab for 4 days. Six months after therapy, the overall decline in patients' muscle strength was only 1.9%, compared with the 14.9% decline seen during the 12-months that preceded the start of therapy. Apart from this short-term stabilization of the disease, no substantial improvement was seen in strength, as only four patients reported improvements of 4–19%. Subjectively, however, 6 of the 13 patients reported improved performance of daily activities. Repeated muscle biopsies showed a mean reduction in lymphocytes of 50% ($P < 0.008$), a finding most prominent in those patients with improved strength, and reduced messenger RNA expression of stressor molecules including Fas, Mip-1a and α B-crystallin; by contrast, messenger RNA levels of desmin, a regeneration-associated molecule, were increased. These observations can be considered either discouraging or encouraging. The discouraging news is that, in spite of an observed reduction in T cells and in some stressor molecules in the muscle, the patients' strength did not significantly improve, which suggests that the disease is more complex than thought and some might argue against immune factors being of major importance in the disease process. Conversely, the more encouraging observation is that the reduction of these molecules was associated with short-term disease stability, which suggests that long-term therapy with potent anti-T-cell agents, if they are proved safe for long-term use, might produce clinically meaningful changes. Ideally, agents that affect both degeneration and inflammation might be better suited to the treatment of sIBM than agents that target either alone.

5. Concluding remarks

Some phase II trials with the use of innovative therapies have been accomplished [10,11], or are planned, not only in the field

of immunosuppressive drugs (campath, anti-CD3, rapamycin), but also with a β_2 -agonist with anabolic properties (formoterol), with myostatin blocking-antibodies/peptides, and with anti-protein aggregation drugs (bapineuzumab, rember). By their concept, these trials need the inclusion of less than 30 patients so can be undertaken by collaboration between one or two regional centres. Depending on the specific aims and expectations of each study, assessments might include immunological issues both in the blood and the muscle, myometric measures (including simple, cheap reproducible measures such as grip strength), MRI parameters and functional scales (PROM, 6 min. walk test). When one of these drugs successfully completes a phase II study (i.e. no serious side effects and promising efficacy), it will move to a phase III RCT (presumably against placebo). The calculated size of such a trial will be around 200 patients (as noted in the Introduction), necessitating then a multicentre study for the recruitment and evaluation of the patients. Because the availability of outcome measurements may vary from a centre to one other, robust, simple, reproducible and repeatable outcomes must be chosen. Functional scales, PROM, and simple clinical tests realisable during a clinic which can be combined in a composite index are the most promising. On the basis of collaborative discussions between the different investigators of a future multicentre trial, these primary and secondary outcomes will be defined after consensus.

The workshop ended on these general considerations. Future workshops will be needed prior to a multicentre RCT.

6. List of participants

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