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1 Short communication

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3 **IgG1 Subclass Restriction and Biochemical Peculiarities of Monoclonal**
4 **Immunoglobulins in Scleromyxedema**

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43

44 **Declaration of Interest:** none

45

46 **Running Title:** Monoclonal IgG1 in scleromyxedema

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SUMMARY

49 **Background:** Scleromyxedema (SME) is a rare mucinosis associated with monoclonal
50 gammopathy. Several biochemical peculiarities of monoclonal immunoglobulins (Ig) in SME
51 patients were reported in case reports or short series, such as IgG λ over-representation,
52 cationic migration, and partial deletion.

53 **Methods:** Monoclonal immunoglobulins (Ig) from the serum of 12 consecutive patients
54 diagnosed with scleromyxedema (SME) were analyzed using electrophoretic and
55 immunoblotting techniques.

56 **Results:** All monoclonal Ig from 12 SME were of IgG1 subclass, with an overrepresentation
57 of the lambda-type light chain and a cationic mobility on standard zone electrophoresis, as
58 compared with 21 cases of monoclonal gammopathy of undetermined significance (MGUS)
59 of IgG1 subclass. Reactivity with specific monoclonal antibodies demonstrated no evident
60 deletion of the heavy chain constant domains, which was also confirmed by analysis of Ig
61 heavy chain molecular weight on a purified monoclonal component from one case.

62 **Conclusions:** Significant isotype restriction of both heavy and light chains, and peculiar
63 biochemical properties suggest that monoclonal Ig might be involved in pathophysiological
64 events of SME.

65

66 **Keywords:** scleromyxedema, monoclonal gammopathy, immunoglobulin, IgG subclass

67

68 **Abbreviations:** MGUS: monoclonal gammopathy of undetermined significance, SDS-PAGE:
69 sodium dodecyl sulfate-polyacrylamide gel electrophoresis, SME: scleromyxedema

70

71

INTRODUCTION

72 Scleromyxedema (SME) is a rare systemic mucinosis associated with a monoclonal
73 gammopathy (MG) that belongs to the recently described group of Monoclonal Gammopathy
74 of Cutaneous Significance (MGCS) [1]. It is characterized by a histological triad including
75 intense dermal mucin deposition, increased fibroblast proliferation, and fibrosis, and is
76 responsible for a scleroderma-like syndrome associated with generalized papular eruption [2].
77 Various systemic manifestations are common in SME, including cutaneous, neurological, and
78 articular involvements. Dermato-neuro-syndrome (DNS), an acute encephalopathy leading to
79 seizure, coma, and death, is the most severe complication of the disease. Despite the rare
80 association of SME with hematologic malignancy (10 - 15%) [3], mortality remains high
81 among reported SME cases [2,3].

82 The almost constant occurrence of MG in SME has made it a diagnostic criterion along with
83 cutaneous eruption, the histological triad on skin biopsy, and the absence of a thyroid
84 dysfunction [2,4]. However, a direct or indirect role of the monoclonal immunoglobulin (Ig)
85 in SME pathogenesis remains uncertain. Treatments aimed at eradicating a monoclonal
86 plasma cell proliferation proved efficient in certain cases [3,5,6].

87 Intrinsic biochemical peculiarities of monoclonal Ig in SME patients were reported. A
88 cationic migration and IgG λ restriction was found in 5 cases [7]. In 1978, Kitamura et al. [8]
89 found a low molecular weight (110 kDa) monoclonal IgG, suggesting deletion of a part of the
90 Fd fragment, in a single case of SME. Subsequent studies showed that the monoclonal
91 component is always of the IgG class with an over-representation of the lambda-type light
92 chain [2,3].

93 Here, we identified the IgG subclass and light chain type of the monoclonal component in 12
94 SME cases, and assessed their biochemical peculiarities, including mobility on zone
95 electrophoresis and presence of constant domains of heavy-chains.

96

97

MATERIALS AND METHODS

Patients

99 Serum samples were collected from 12 consecutive patients diagnosed with SME, recruited
100 from 9 French centers between June 2017 and November 2018 by MINHEMON French
101 network (dysimmune disorders associated to hemopathies and cancers). Samples were kept at

102 -20°C until analyses. As a control group for electrophoretic migration, 39 sera from
103 consecutive patients with MGUS were collected at the Saint Antoine hospital (Paris). All
104 analyses, including electrophoresis and immunochemical studies, were part of the routine
105 biological follow-up of patients.

106 **Characterization of isotypes and electrophoretic mobility of serum monoclonal Ig**

107 Serum monoclonal Ig were identified by standard immunofixation (Sebia, France).
108 Electrophoretic mobilities of monoclonal IgG were determined by measuring their retention
109 factor (Rf) relative to albumin. Then, relative mobilities of monoclonal IgG were normalized
110 against the Rf of a single serum IgG (SME patient #7) serving as an internal standard.

111 **Characterization of IgG subclasses and presence of heavy chain constant domains**

112 Monoclonal IgG subclass typing and reactivities for heavy chain constant domains CH1,
113 CH2, and CH3 were performed using a home-made immunoblotting method [9]: appropriate
114 dilutions of serum samples were submitted to thin-layer agarose gel zone electrophoresis and
115 transferred by single pressure (15 g/cm² for 10 minutes) onto a 0.2 µm nitrocellulose
116 membrane. The membrane was then blocked for 30 minutes in 5% skimmed milk in
117 phosphate buffered saline pH 7.4 (PBS) and cut into strips that were incubated for 60 minutes
118 at room temperature with appropriate primary mouse monoclonal antibodies diluted in 0.05%
119 Tween-20, 0.02% bovine serum albumin PBS. The monoclonal antibodies were specific for
120 kappa and lambda light chains, and IgG1, IgG2, IgG3, and IgG4 heavy chain isotypes (clones
121 HP6053, HP6054, NL16, GOM2, ZG4, and RJ4, respectively) and for IgG constant domains
122 (anti-CH1: TM15, ZB8, HP6044; anti-CH2: G7C, HP6018; anti-CH3: X3A8, HP6017). After
123 washing with 0.05% tween-PBS at 4°C, strips of nitrocellulose membrane were incubated
124 with alkaline phosphatase-conjugated rabbit polyclonal anti-mouse IgG diluted in 0.05%
125 tween-20, 0.02% BSA/PBS. Strips were washed as before, and revealed by chromogenic
126 reaction with tetrazolium nitroblue / bromo-chloro-indolyl phosphate in 0.1 M NaCl 0.1 M
127 MgCl₂ 0.1 M TRIS/HCl buffer pH 9.5.

128 **Purification of monoclonal Ig and heavy and light chain molecular weight determination**

129 The monoclonal IgG could be purified from the serum of one SME case and from a control
130 IgG1 myeloma patient, using ion exchange chromatography. Serum samples were
131 fractionated on a diethylaminoethyl (DEAE)-Trisacryl column in 10 mM TRIS/HCl buffer pH
132 7.8 with a 0 to 0.1 M NaCl linear gradient. Protein fractions were concentrated by
133 ultrafiltration and purity was assessed by standard thin layer agarose zone electrophoresis at
134 pH 8.6. Purified (> 95%) fractions were reduced with 10 mM dithiothreitol in 1% sodium

135 dodecyl sulfate (SDS) loading buffer at 95°C for 3 minutes, and 1 µg protein of each was
136 fractionated by 12% SDS polyacrylamide gel electrophoresis (SDS-PAGE).

137 **Statistical methods**

138 Frequencies of monoclonal IgG1 and λ-type IgG were compared to those in a published series
139 of MGUS using Fisher's exact test. Electrophoretic mobilities were compared using Mann-
140 Whitney-Wilcoxon's test. Differences with a p value < 0.05 were considered significant.
141 Computations were performed using R software.

142

143

RESULTS

144 Patients were aged 29 - 75 years at the time of the study (mean: 61.2 ± 16.8 years) with a sex
145 ratio of 1 (Table 1). Three patients presented with DNS and the remaining 9 had non-severe
146 forms of SME.

147 All 12 monoclonal Ig from the SME patients were of IgG1 subclass (Table 1 and Figure 1B),
148 including 7 with a lambda and 5 with a kappa light chain. The IgG1 restriction was significant
149 as compared with the occurrence of IgG1 in the largest published series of MGUS (155/241
150 (63%); p = 0.009) [10], while the predominance of lambda light chain isotype in SME (7/12,
151 58%) was not statistically significant as compared with the same series of MGUS (89/241,
152 37%) (p = 0.22).

153 In SME patients, the electrophoretic migration of the monoclonal component was
154 significantly more cationic (median [IQR]: 1.00 [0.96 – 1.03]) than the 21 MGUS that
155 displayed a monoclonal IgG1 (median [IQR]: 0.95 [0.85 – 0.98]; p = 0.03; Figure 1A).

156 Analyses of constant domain reactivity with a panel of CH1-, CH2- and CH3-specific
157 monoclonal antibodies by immunoblotting led to conclude on the absence of any deletion of
158 the heavy chain constant region in all 12 studied SME-associated monoclonal IgG1 (Figure
159 1B). This was confirmed by the SDS-PAGE analysis of one SME-associated monoclonal
160 IgG1 kappa that we could purify, as it revealed normal sizes of both heavy and light chains
161 (Figure 2).

162

163

DISCUSSION

164 This study demonstrates a strict IgG1 restriction of SME-associated monoclonal
165 immunoglobulins, and it confirms that most of them have a high isoelectric point as suggested
166 by their cationic migration on agarose zone electrophoresis.

167 The bias toward IgG class and lambda type light-chain in SME MG was already noted by
168 James et al. [7] but subclasses of IgG have not been analyzed, as far as we know. The actual

169 importance of the IgG1 restriction in SME should be confirmed in a larger series, but it
170 already appears quite significant in the present study.

171 The cationic electrophoretic migration of SME-associated monoclonal immunoglobulins has
172 been evoked in small series or in a single case [7,8]. To our knowledge, this is the first
173 statistical demonstration of cationic electrophoretic migration of SME monoclonal Ig as
174 compared with classical MGUS. We found that this electrophoretic property is independent of
175 the IgG1 restriction of SME-associated monoclonal component.

176 On the other hand, we did not confirm the results from Kitamura et al. [8] suggesting a
177 deletion of the Fd portion (most likely the CH1 domain) of a monoclonal IgG from one SME
178 case. Indeed, all constant domains of the heavy chains were immunoreactive in our
179 immunoblotting studies, while 1 to 3 distinct antibodies were tested for each domain. This
180 was confirmed in one case whose monoclonal component could be purified and analyzed by
181 SDS-PAGE, revealing normal sized heavy and light chains.

182 The almost constant presence of a MG in SME, isotype restriction and basic nature of the
183 monoclonal component pinpoint the potential role of a monoclonal plasma-cell proliferation
184 in the pathophysiology of SME. However, a direct pathogenic role of such hematologic event
185 and the bone marrow microenvironment remains unknown. The efficacy in severe cases of
186 new treatments targeting putative plasma cell clones with concomitant hematological and
187 clinical response supports the hypothesis of a direct or indirect role of these cells in the SME
188 pathogenesis [3,5,6]. An old study has shown that serum factor(s), but not the monoclonal
189 immunoglobulin alone, could stimulate *in vitro* fibroblast proliferation [11,12]. A hypothesis
190 that has been suggested to explain this phenomenon could be the presence of an unknown
191 circulating cofactor that could stimulate proliferation in association with the monoclonal
192 immunoglobulin. Biochemical changes such as glycosylation and/or modifications of the
193 amino acid sequence of the immunoglobulin could affect its electric charge and explain the
194 strong association between cationic migration and SME. The great efficacy of high dose
195 intravenous immunoglobulins in SME [2,3,13] could be explained by their
196 immunomodulatory functions, or by increased clearance of the monoclonal Ig or its complex
197 with a co-factor. Further studies, based for example on mass spectrometry analyses of the
198 serum secretome, as well as functional experiments using purified monoclonal Ig with and
199 without other serum fractions on human *in vitro* fibroblast cultures, are required for better
200 understanding the implication of these peculiar monoclonal Ig in the pathophysiology of
201 SME.

202

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244

245 **Table 1.** Demographic, clinical, and biological characteristics of 12 patients with
 246 scleromyxedema

Patient	Gender/Age	Severity	Monoclonal Ig isotype	Electrophoretic mobility (normalized value)
1	H/60	Non severe	IgG1 λ	Medium/cathodic (0.98)
2	F/29	Severe, DNS, cardiomyopathy	IgG1 λ	Cathodic (1.00)
3	H/75	Non severe, cutis laxa-like eruption, MBL	IgG1 κ	Cathodic (1.02)
4	H/73	Non severe	IgG1 λ	Cathodic (1.01)
5	F/71	Non severe	IgG1 λ	Cathodic (1.02)
6	F/29	Severe, DNS	IgG1 κ	Medium (0.92)
7	F/75	Non severe	IgG1 λ	Cathodic (1.00)
8	F/69	Non severe	IgG1 κ	Cathodic (1.10)
9	M/51	Non severe	IgG1 λ	Medium (0.89)
10	M/53	Non severe	IgG1 κ	Anodic (0.86)
11	M/55	Severe, recurrent DNS	IgG1 κ	Cathodic (1.11)
12	F/75	Non severe	IgG1 λ	Cathodic (1.05)

247

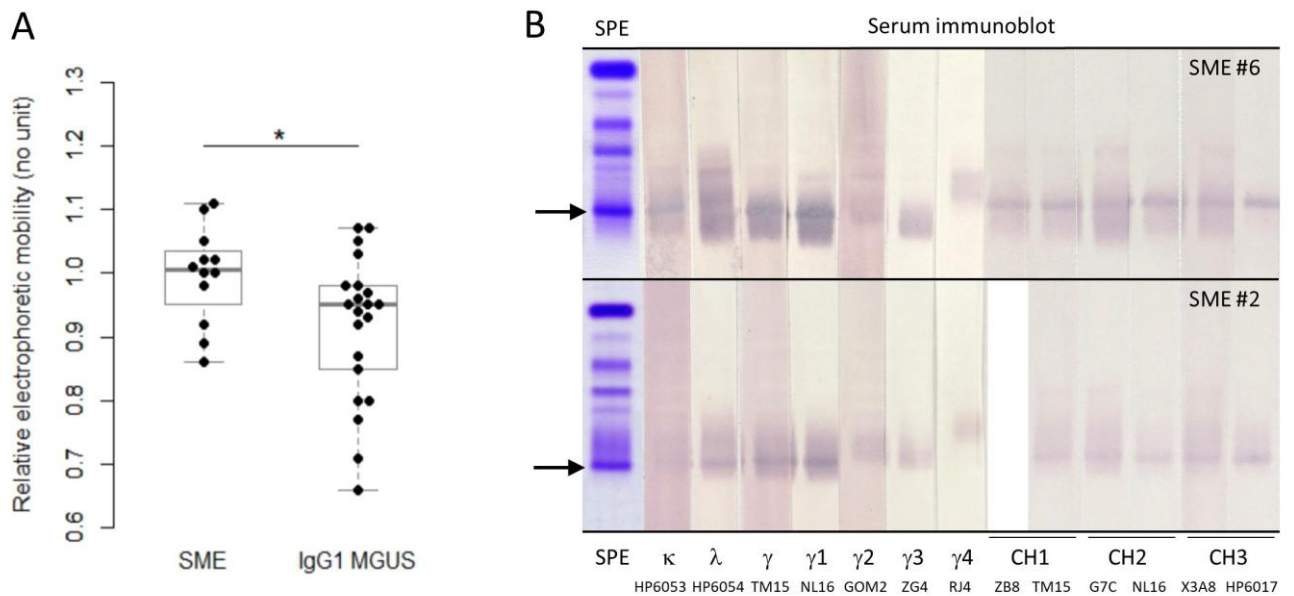
248 DNS: dermato-neuro syndrome; Non severe disease: absence of DNS or mucinous
 249 cardiomyopathy; MBL: monoclonal B lymphocytosis; Severe disease: presence of DNS or
 250 mucinous cardiomyopathy

251

252 **Figure legends**

253

254 **Figure 1.** (A) Comparison of electrophoretic mobilities of serum monoclonal IgG from 12
 255 patients with scleromyxedema (SME) and 21 subjects with IgG1 MGUS (*: $p < 0.05$). (B)
 256 Immunoblotting analysis of IgG subclasses, light chain type and antigenic reactivity of CH1,
 257 CH2 and CH3 domains of serum samples from 2 patients with SME (used antibody clones are
 258 mentioned below corresponding strips; SPE: serum protein electrophoresis).



259

260 **Figure 2.** Apparent molecular weight of purified monoclonal IgG1 kappa from patient #6, as
 261 compared with a purified myeloma IgG1 kappa. (A) Agarose zone electrophoresis showing
 262 serum and DEAE-purified fraction from patient #6. (B) 12% SDS-PAGE under reducing
 263 conditions of purified monoclonal IgG1 kappa from a scleromyxedema patient (SME #6) and
 264 a multiple myeloma IgG1 kappa (MM control), showing normal sized heavy chain (black
 265 arrow) and light chain (white arrow).

