Unexpectedly high prevalence of primary immune deficiency in fibromyalgia: serologic features and clinical correlates

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Abstract

Objective

We sought laboratory evidence of primary immune deficiency (PID), a condition known to be associated with recurrent infections and autoimmunity, in fibromyalgia (FM). We correlated laboratory findings with a clinical history of recurrent infections and reduced epidermal nerve fibre density (ENFD).

Methods

We prospectively measured serum total and subclass concentrations for IgA, IgG, IgM, IgE, and mannose-binding lectin in 72 adult FM subjects (31 "FM only;" 41 "FM+RA") and compared those results against historical controls. We also administered a novel "Lifetime History of Infections" questionnaire to all FM subjects and 40 apparently healthy, community volunteers matched for age, race, and gender. ENFD values available for 49/72 FM subjects were also correlated with immunoreactant levels.

Results

Of FM subjects, 96% (69/72) had ≥3 and 85% (61/72) had ≥4 of 9 immunoreactants below or within the lowermost quartile of historical normal values. Recurrent sinus infections occurred more often in "FM only" (p=0.06), and "FM+RA" subjects (p=0.02) than controls. "FM+RA" subjects had a significantly greater history of recurrent, severe non-sinus infections (p=0.04). The prevalence of total IgA deficiency was significantly greater in "FM only" than in "FM+RA" subjects (p=0.04). We also found a direct correlation between total IgA (p=0.02), IgA1 (p=0.005), and IgG1 (p=0.04) concentrations and ENFD in "FM only" subjects.

Conclusion

Serologic evidence of PID in FM is common and correlates with a clinical history of recurrent sinus and non-sinus infections, and reduced ENFD. This study suggests that PID may be important to diagnostic and therapeutic considerations in FM.

Key words

fibromyalgia, pain, chronic fatigue syndrome, chronic widespread pain, epidermal nerve fibre density, small fibre neuropathy, immune deficiency, primary immune deficiency, IgA, IgA subclasses, IgG, IgG subclasses, mannose-binding lectin

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Received on June 1, 2021; accepted in revised form on July 15, 2021.

Originally presented, in part, at the 2014 & 2017 Annual Scientific Meeting of the American College of Rheumatology. Arthritis Rheum 2014; 66 (10): S905, and Arthritis Rheum 2017; 69 (10): S156.

Competing interests: none declared.

Introduction

The role of primary immune deficiency (PID) in the genesis of human suffering during adulthood is much underestimated. Some aspects of this inappreciation are due to the subtlety and complexity of the immune system, while others likely reflect the better-known connection of PID with the extremes of life (1). Whatever the reasons, adult rheumatologists need to be aware of PID because of its common association with recurrent infections, autoimmunity, and neoplasia (2-5).

Herein, we present data showing that humoral immune deficiency, as evidenced by subnormal serum concentrations of certain immunoglobulin (Ig) components, a complement activating protein, mannose-binding lectin (MBL) (6), and the prevalence of a history suggestive of enhanced susceptibility to recurring infections is rather common in FM. We also discuss the implications of these findings *vis-à-vis* the pathogenesis and clinical presentation of FM.

Methods

Setting, clinical assessment, and FM subgroup assignment

Eighty consecutive subjects, between 18 and 90 years of age, referred during routine outpatient rheumatology, clinical practice, between January 2015 and May 2017, who met 1990 ACR diagnostic criteria for FM (7), were enrolled. All gave consent for the anonymous use of their health data; our facility's ethics committee (IRB) approved the study.

Subjects underwent comprehensive evaluation as described previously (8). No other degenerative or metabolic condition precluded inclusion in this study unless thought to be clinically relevant, autoimmune or chronically infectious in nature. Rheumatoid arthritis was an exception, leading instead to a separate analysis as a FM subject with concomitant RA (*i.e.* "FM+RA").

Immunoreactant and ENFD measurements

Serum levels of IgG, and IgA subclasses, total IgE, and IgM [immunoturbidimetric (IT) method], and mannose-binding lectin (MBL) [enzyme linked immunosorbent assay (ELISA)] values were determined by our reference laboratory (LabCorp Clinical Labs, San Diego, CA, USA); results were confirmed by repeat analysis 6–9 weeks later.

To define PID we used the adult lower limits of normal (LLN) and range standards suggested by Schroeder *et al.* (9). We estimated Quartile values for IgG, IgA, and IgM components by dividing their ranges into four equal parts (*i.e.* range/4 = Quartile 1-4) (10). The Ig values used to define the LLN and Q1 for IgG/A/M are listed in Figure 1. We allowed the LLN for IgE to coincide with our estimate for IgE Q1 (*i.e.* <7 IU/ml) as the concepts of LLN and Q1 for IgE are not well defined (11). MBL status was defined as: <50 ng/ml (deficient), >50 but <500 ng/ml (possibly deficient), and >500 ng/ml (normal).

Most FM subjects also underwent a 3 mm punch skin biopsy at the distal calf for determination of epidermal nerve fibre density (ENFD) and consequent small fibre neuropathy (SFN) as previously described (8). Subsequently, we correlated immunoreactant levels and ENFD.

Assessment of prior serious infections

A brief, novel, self-administered questionnaire asked each study subject to estimate the number of infections (from 0 to ≥6) sustained during their lifetimes in the following categories: sinusitis, pneumonias, urinary tract infections, pyelonephritis, recurrent skin infections, bronchitis, intestinal infections, and otitis media. To limit overestimation, we truncated the number of each at ≥6. One of the authors (XJC) reviewed this "Lifetime History of Infections" questionnaire with each subject for clarity of understanding. A diagnosis of chronic recurrent sinusitis (CRS) was accepted only if there was a history of repeated physician prescribed antibiotic therapy, ENT subspecialty confirmation, or a corroborating sinus radiographic series or CT scan.

The same questionnaire was administered to 40 apparently healthy, age (±5 years), gender and race matched community volunteers recruited from an indoor urban shopping mall approximately 5 miles from our study facility. Volunteers denied any history of rheu-

matic disease, diabetes, cancer within 5 years, or known immune deficiency. Volunteers were compensated with a $5 gift card.

Statistical analysis

Immunoreactant differences between FM groups and literature controls were calculated by Chi square; FM intergroup differences for infections were by Z Test for Two Population Proportions. Three missing immunoreactant data cells were imputed using mean value substitution (12). Correlations were made by Pearson's method. Between group comparisons for the prevalence of infections detected in FM and community controls were made using Mann Whitney U-test. Web based statistical programmes assumed a p-value ≤0.05 as significant (one tailed, unless otherwise indicated).

Results

FM subjects' demographics

Eighty FM subjects were screened; 8 were excluded (*e.g.* uncontrolled diabetes mellitus, prior cancer chemotherapy, etc.). Of 72 remaining FM subjects 31 (43%) had "FM only" and 41 (57%) had concomitant RA ("FM + RA"). The "FM only" group's mean age was 63 yrs. (range 28–72; SD ± 16); 24 (77%) were women. The "FM +RA" group's mean age was 67 yrs. (range 23–90; SD ± 13); 37 (90%) were women. Eighty-nine percent of FM subjects were Caucasian (64% European and 25% Hispanic); 11% were of other races (Asian, American Indian, African American). Twenty-two percent of "FM + RA" subjects had IgM rheumatoid factor and 25% had CCP – IgA/G. None of the 72 FM subjects were current smokers or alcoholic.

Immunoreactant data

Immunoreactant data are enumerated in Figure 1, and Tables I and II. When our FM subjects were considered as a whole, 96% (69/72) had ≥3, and 85% (61/72) had ≥4, immunoreactant levels below the LLN or within the lowermost quartile of historical normal values. IgG Subclass 3 (58.3%; 42/72) deficiency was slightly more prevalent than IgG Subclass 1 deficiency (55.6%; 40/72).

The odds ratio (OR) for a FM subject (either "FM only" or "FM + RA") having "Any Ig" deficiency (*i.e.* <LLN), compared to literature-based norms, was 1,150 (95% CI: 157 - 8399). Total IgA deficiency (*i.e.* <LLN) was significantly more prevalent in "FM Only" (41.9%) compared to "FM+RA" (19.5%) (p=0.04) (Table II). No other significant intergroup immunoreactant differences were found.

Of FM subjects who were deficient in IgG Subclass 1, 40% were also deficient in IgG Subclass 3; this association was statistically significant in the "FM + RA" subgroup (r=0.277; p=0.04). Nineteen percent of FM subjects who were deficient in IgG Subclass 2 were also deficient in IgG Subclass 4. There was a strong association between IgG Subclass 2 and Subclass 4 concentrations (values in mg/dl) in our "FM only" subjects (r=0.671; p<0.0001), and in our 72 FM subjects taken as a whole (r=0.417; p=0.0001).

Of our 71 FM subjects whose MBL values were available for analysis, 25% (18/71) had some degree of MBL deficiency (*i.e.* <500 ng/ml) (7, 18). Ten percent (7/71) had a MBL level of 50-500 ng/ml, and 15 % (11/71) had unmeasurable levels (*i.e.* <50 ng/ml) (18). There was no significant intergroup difference in the prevalence of MBL deficiency (*i.e.* <500 ng/ml) in "FM only" (8/31) compared to "FM+RA" (10/41) (p=0.89).

Forty-one percent (29/71) of our FM subjects had an elevated MBL level (>1000 ng/ml). There was no significant difference in the prevalence of elevated MBL levels (*i.e.* >2000 ng/ml) in "FM only" (13/31 elevated) compared to "FM + RA" (16/40 elevated) subgroups (p=0.44).

When we compared all FM subjects who were <60 years to those ≥60 years of age there were no significant differences in Ig or Ig subclass levels (*i.e.* we found no correlation between Ig levels and age). We also found no significant differences in Ig levels between genders for our 72 FM subjects taken as a whole (data not shown).

Sinus and other serious infections

The number of "sinus infections" recalled by our 72 FM subjects compared to normal subjects differed significantly at ≥6 total sinus infections and ≥6 "other serious infections" (*i.e.* not including sinus infections) (Fig. 3). This difference was significant for "FM + RA" *versus* apparently healthy controls' recollection of "sinus infections" (p=0.02), and "other severe infections" (p=0.003). There was also a trend towards a significant difference in the estimation of "sinus infections" in "FM only" compared to apparently healthy controls (p=0.06). Further, the number of "other severe infections" recalled by our "FM + RA" group was significantly greater than by our "FM only" group (p=0.04).

ENFD correlates

Serum levels of total IgA, IgA subclass 1, and IgG subclass 1 correlated significantly with ENFD values in our FM subjects (Table I, Fig. 2). No other immunoreactant level correlated significantly with ENFD values.

Discussion

In 1991 Riederer became the first to describe PID in an FM subject (13). He reported a 26-year-old female suffering recurrent infections "traced back to childhood," who presented with "classic symptoms of fibromyalgia." His patient had rather profound IgA deficiency (total IgA = 20 mg/dl), and IgG subclass deficiencies (total IgG = 633 mg/dl; IgG subclasses 2 and 3 <10 mg/dl; IgG subclass 4 <1 mg/dl). An array of other immunologic and inflammatory markers was unremarkable, save a "low normal" number of NK cells and a "discretely degraded" CD4/CD8 ratio of 1.02. The author concluded that the "...immunopathy in the presented [FM] case [is] not coincidental...".

More recently, Barton *et al.* (14) identified FM in 18.7% of 300 retrospectively surveyed adult patients referred because of recurrent/severe respiratory tract infections, or hypogammaglobulinaemia (mainly Ig subclass deficiency). Interestingly, the PID immunophenotype did not differ between their subjects with and without FM. Like Riederer (13), they found no discernable lymphocyte profile marker that distinguished PID

Subject	IgA1	IgA2	IgE	IgG1	IgG2	IgG3	IgG4	IgM	MBL
1	87.3	23.3	8	260	210	24	9	17	<50
2	342	67.8	3	452	177	44	4	94	2281
3	167	26.2	26	517	306	46	18	199	573
4	160	23.9	50	335	288	82	19	31	325
5	129	27.8	12	358	237	70	19	204	372
6	227	34.9	121	516	178	60	4	273	1857
7	113	14.8	7	524	173	81	13	21	2546
8	169	30.6	9	631	492	47	78	129	970
9	150	63.5	10	537	298	62	8	142	<50
10	90	37.7	58	584	111	15	9	250	363
11	61.3	34.7	37	446	334	17	24	30	<50
12	178	22.2	67	643	467	22	20	44	579
13	109	18.6	70	345	204	80	14	136	3963
14	144	83.1	90	296	408	52	32	107	1474
15	160	31.4	36	401	358	45	69	67	3202
16	184	88.6	47	393	471	55	22	42	>4000
17	147	9.7	53	254	294	17	30	15	3132
18	74.9	29.2	14	390	282	35	11	274	>4000
19	101	20	25	293	91	11	6	33	1903
20	98.5	11.8	457	598	310	75	16	212	1296
21	55.2	12	<1	503	132	50	<1	206	2703
22	153	29.6	31	552	408	66	26	80	2076
23	184	39.4	2	366	189	58	11	62	3011
24	133	27	9	528	218	21	30	79	1222
25	108	28.4	20	327	112	24	18	75	<50
26	187	52	85	452	253	45	46	107	<50
27	137	23.5	20	908	216	92	19	134	>4000
28	335	155	11	699	219	26	31	304	2513
29	201	50.8	45	483	313	23	17	145	1007
30	230	35.6	212	386	524	21	81	54	3612
31	271	49.9	101	781	404	46	43	57	1605

A. Serum Immunoreactant levels in "FM Only"

Subject	IgA1	IgA2	IgE	IgG1	IgG2	IgG3	IgG4	IgM	MBL
32*	119	6	20	545	185	24	15	61	2254
33*	121	44.7	8	544	211	13	<1	149	<50
34*	247	31.3	857	723	332	75	7	179	<50
35*			41	417	266	56	5	148	1506.4
36*	258	58.7	38	829	86	98	2	94	>4000
37*	106	48.1	7	339	402	25	17	180	<50
38*	155	39.3	19	412	351	66	33	284	1230
39*	198	25.6	143	334	394	134	21	163	683
40*	141	25.8	19	339	411	33	33	45	1091
41*	168	19.4	99	544	369	27	51	93	>4000
42*	115	8.4	<1	281	33	137	<1	42	2444
43*	321	58.8	1190	651	522	179	24	407	716
44*	176	43.4	127	365	183	31	17	139	748
45*	137	31.9	57	548	495	65	15	96	3457
46*	130	48.8	52	540	347	26	34	177	688
47*	216	73.9	27	433	421	24	32	68	>4000
48*	228	92.3	242	571	295	63	26	231	>4000
49*	191	21.4	42	397	317	37	38	72	3528
50*	146	44	31	482	722	29	6	96	2430
51*	197	24.1	4	325	254	32	6	42	>4000
52*	159	10.2	11	465	459	39	5	107	618
53*	107	31.2	79	422	359	28	54	70	3477
54*	233	70.9	14	643	317	41	58	82	1362
55*	256	33.8	103	585	376	70	12	55	1032
56*	263	56	34	528	232	83	31	126	<50
57*	161	27	32	759	244	80	44	78	179
58*	288	40.5	124	527	343	23	28	21	362
59*	81.3	13.1	6	351	123	21	16	120	1310
60*	216	34.8	31	1010	280	55	29	81	2747
61*	159	22.8	3	660	373	68	16	133	2972
62*	169	36.7	24	380	288	34	29	96	
63*	226	66.9	33	420	273	52	11	134	2812
64*	379	77.6	81	291	159	29	<1	31	1712
65*	246	32.1	1975	446	228	32	11	334	144
66*	27.4	7.4	15	336	210	32	15	43	3828
67*	150	20.1	3	380	215	20	10	79	2609
68*	139	22.8	11	390	318	21	15	134	671
69*	194	91.3	172	506	475	79	40	95	<50
70*	173	20.2	61	483	210	43	54	147	180
71*	61.4	11.7	32	500	194	66	12	115	<50
72*	103	20.1	158	326	394	27	54	122	1794

B. Serum Immunoreactant levels in "FM+RA""

Fig. 1. Immunoreactant levels in 72 FM subjects (31"FM only" [**A**] and 41 "FM+RA" [**B**]). Lower limits of normal (LLN) and ranges were those suggested for normal adults by Schroeder *et al*. (9). Ig LLN were as follows: IgG Subclass 1 (IgG1) ≤500 mg/dL, IgG2 ≤200 mg/dL, IgG3 ≤50 mg/dL, IgG4 ≤20 mg/dL, IgA1 ≤ 140 mg/dL, IgA2 ≤20 mg/dL, IgM ≤25 mg/dL (18). Ig quartile 1 (Q1) values were as follows: Q1 for IgG subclass 1 (IgG1-Q1) >500-675 mg/dL, IgG2-Q1 >200-300 mg/dL, IgG3-Q1 >50-62.5 mg/dL, IgG4-Q1 >20-40 mg/dL, IgA1-Q1 >140-210 mg/dL, IgA2-Q1 >20-27.5 mg/dL, and IgM-Q1 >25-96 mg/dL (12). Ig results are stratified into those ≤ LLN (red), those falling within Q1 (yellow), and those above Q1 (*i.e*. Q2-4) (green). LLE for IgE was <7 IU/ml. Mannose binding lectin (MBL) was defined as deficient, ≤ 500 ng/ml (red) and normal >500 ng/ml (green) (13, 22).
*denotes "FM + RA" subjects. All Ig values are mg/dL, MBL are ng/ml and IgE are IU/ml.

Table I. Immunoreactant characteristics of 72 adult FM subjects and their correlation with ENFD findings.

Meas.	IgA Total	IgA1	IgA2	IgE	IgG Total	IgG1	IgG2	IgG3	IgG4	IgM	MBL All	MBL w/o <50 >4000	ENFD
"FM Only" Group (n=31)													
Mean	203	158	39	56	1068	476	280	46	24	117	1769	1851	4.82
Std. Dev.	90	70	29	87	463	153	116	23	20	83	1350	1076	2.89
Range	67 472	55 342	9.7 155	1.0 457	456 2572	254 908	91 524	11 92	1 81	15 304	<50 >4000	325 3963	0 10.55
Median	188	150	30	36	919	452	282	46	19	94	1605	1857	4.82
ENFD Correl. n	24	19	19	19	24	19	19	19	19	19	19	15	-
ENFD Correl. r	0.49	0.58	0.18	-0.30	-0.23	0.41	0.03	-0.13	0.18	0.30	0.07	0.01	-
P (1)	**0.007**	**0.004**	0.23	0.11	0.14	**0.04**	0.45	0.30	0.23	0.10	0.39	0.48	-
P (2)	**0.01**	**0.01**	0.46	0.22	0.27	**0.08**	0.90	0.60	0.46	0.21	0.78	0.96	
"FM + RA" Group (n = 41)													
Mean	226	179	37	147	1086	488	309	52	23	121	1722	1677	5.08
Std. Dev.	92	70	22	367	449	155	128	36	17	79	1409	1124	2.86
Range	34 448	27.4 379	6 92.3	1 1975	481 2408	281 1010	33 722	13 179	1 58	21 407	<50 >4000	144 3828	0.7 10.76
Median	210	171	33	34	977	474	317	38	17	102	1434	1506	5.12
ENFD Correl. n	27	24	24	24	25	24	24	24	24	24	24	19	-
ENFD Correl. r	0.02	-0.05	0.03	0.17	0.11	0.14	0.29	-0.15	0.00	0.19	-0.12	0.11	-
P (1)	0.45	0.42	0.44	0.21	0.31	0.25	0.09	0.25	0.50	0.19	-0.12	0.11	-
P (2)	0.91	0.84	0.87	0.42	0.61	0.51	0.17	0.50	0.99	0.37	0.69	0.65	

Meas: measurement; IgA1, 2, IgG1..., etc.: IgA Subclass 1…, etc; MBL: mannose-binding lectin; MBL w/o: all MBL values excluding those <50 or >4000 ng/ml; ENFD: epidermal nerve fibre density; Std. Dev.: standard deviation; ENFD Correl. N: number of subjects' ENFD values correlated with given immunoreactant (IR) value; ENFD Correl. R: Pearson r value for IR/ENFD correlation.
P(1): *p*-value, 1 tailed; *P*(2): *p*-value, 2 tailed.

Table II. Prevalence of IR deficiency (≥2 SD below mean) in 72 adult FM subjects compared to normal population estimates†.

Immunoreactant (IR)	IR Deficiency in "FM Only" %	IR Deficiency in "FM+RA" %	IR Deficiency in All FM %	Est. Deficiency in Nml. Population %	"FM Only" *vs.* Nml. (*p*-value)	"FM+RA" *vs.* Nml. (*p*-value)
IgA (Total)	**41.9% (13/31)**	**19.5% (8/41)**	**29.2% (21/72)**	**0.17% (1/600)**	**<0.0002**	**<0.0002**
IgA1	**41.9% (13/31)**	**29.3% (12/41)**	**34.7% (25/72)**	**0.17% (1/600)**	**<0.0002**	**<0.0002**
IgA2	**16.1% (5/31)**	**17.1% (7/41)**	**16.7% (12/72)**	**0.17% (1/600)**	**<0.0002**	**<0.0002**
IgG1	**54.8% (17/31)**	**56.1% (23/41)**	**55.6% (40/72)**	**0.08% (1/1200)**	**<0.0002**	**<0.0002**
IgG2	**25.8% (8/31)**	**17.1% (7/41)**	**20.8% (15/72)**	**0.08% (1/1200)**	**<0.0002**	**<0.0002**
IgG3	**58.1% (18/31)**	**58.5% (24/41)**	**58.3% (42/72)**	**0.08% (1/1200)**	**<0.0002**	**<0.0002**
IgG4	**58.1% (18/31)**	**53.7% (22/41)**	**55.6% (40/72)**	**0.08% (1/1200)**	**<0.0002**	**<0.0002**
IgM	**9.7% (3/31)**	**2.4% (1/41)**	**5.6% (4/72)**	**0.08% (1/1200)**	**<0.0002**	**<0.0002**
MBL	**25.8% (8/31)**	**24.4% (10/41)**	**25% (18/72)**	**5 - 7%***	**<0.0002**	**<0.0002**

†Prevalence of Ig deficiency in normals from Srinivasa *et al*. (41).
*Prevalence of MBL deficiency in normals varies geographically and ethnically; listed estimate is in European Caucasians (Super *et al*. 42).
"FM Only" (n = 31); "FM+RA" (n = 41).
IR: immunoreactant; Nml.: normal; IgA1, 2, IgG, etc.: IgA Subclass 1, 2, etc.; MBL: mannose-binding lectin; Est: estimated.
The prevalence of "any deficiency" (*i.e.* ≤LLN + Q1) was: IgA Subclass 1 - 73.6% (53/72); IgA Subclass 2 - 40.3% (29/72); IgE - 11.1% (8/72); IgG Subclass 1 - 90.3% (65/72); IgG Subclass 2 - 54.2% (39/72); IgG Subclass 3 - 70.8% (51/72); IgG Subclass 4 - 84.7% (61/72); IgM - 51.4% (37/72); MBL - 25% (18/72).

subjects with FM from those without. Together, these two studies suggest that, in some adults, PID might be a necessary, though not sufficient, prerequisite to the development of FM.

Ours is the first study to prospectively quantitate humoral immunoreactant levels in consecutive, unselected adult FM subjects, and correlate these laboratory findings with clinical evidence of recurrent infections and ENFD. Surprisingly, 96% (69/72) of our subjects were either low or frankly deficient in ≥3 of 9 immunoreactants and 85% (61/72) were either low or frankly deficient in ≥4 of 9 immunoreactants (Fig. 1, Table I and II) suggesting a rather broad spectrum of humoral immune deficiencies in FM.

Identifying laboratory evidence of humoral immunodeficiency may be chal-

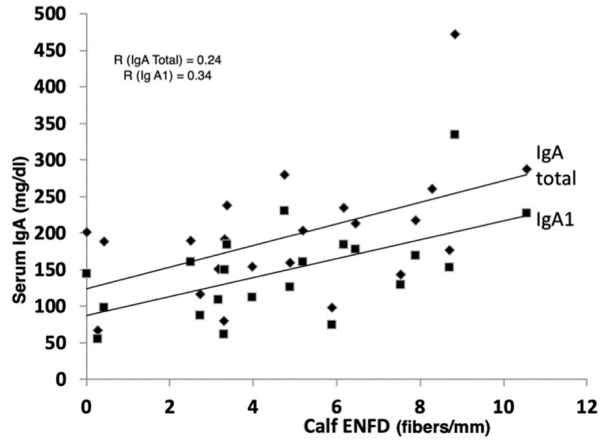

Fig. 2. Relationship between Serum IgA (Total IgA ◆ and IgA Subclass 1 ■) and Calf ENFD in "FM Only" subjects. There was a significant direct correlation of "FM Only" subjects' serum Total IgA (r=0.49, *p*=0.007) and IgA Subclass 1 (r=0.58, *p*=0.004) with ENFD. The equation of the trend line (y=mx + b, where m=slope, and b=y value when x=0) for Total IgA=14 (ENFD) + 127.5; for IgA Subclass 1=12.7 (ENFD) + 91.1. ENFD=epidermal nerve fibre density, IgA1=IgA Subclass 1.

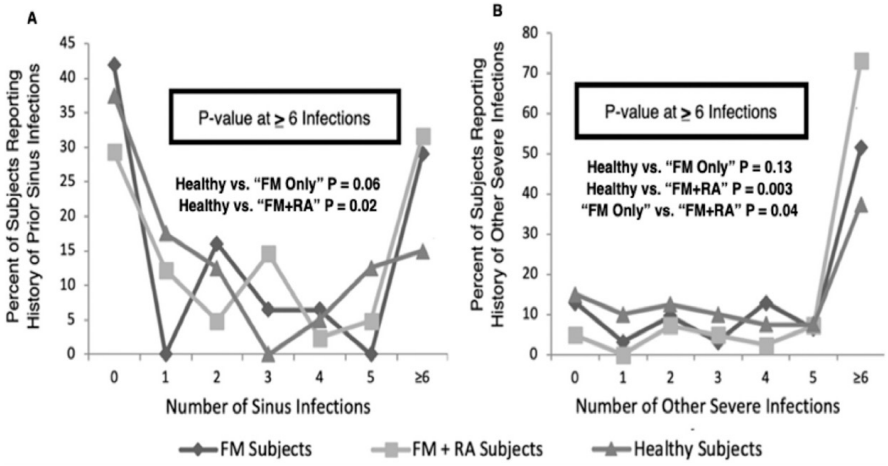

Fig. 3. Prevalence of severe infections recalled by 72 FM subjects compared to 40 healthy controls subjects. "FM only" subjects (n=31); "FM + RA" subjects (n=41).
A) depicts recall of only "sinus infections" amongst FM and control subjects.
B) depicts recall of "other severe infections" (excluding sinus infections) amongst FM and control subjects. All *p*-values are calculated at ≥6 infections reported.

lenging. due to the absence of agreed upon normal reference intervals (RI) for these immunoproteins (15). Older, "direct sampling" methods of establishing immunoreactants' RI in large cohorts of volunteers are difficult and expensive for modern laboratories or clinical investigators to reproduce de novo today.

Newer, statistically based methods of generating laboratory RI, pioneered by Hoffman (15) and others, have become popular but depend on the "fact that most of the patient specimens sent to laboratories for analysis are normal" (15). These mathematically derived RI reflect the reference laboratory's experience with "normal" immunoreactant values established using cumulative testing data generated from thousands of clinical samples. It is rare, however, for clinicians to order quantitative immunoprotein concentrations as a part of their evaluation of asymptomatic patients, rendering this RI approach vulnerable to a systematic measurement error reflecting the bimodal distribution of oft tested patient groups surveyed for these immunoreactants, *i.e*. those suspected of having unusually low or unusually high values.(16) We avoided these issues by relying on a traditional "direct observation" RI set found in a well-respected and readily available textbook of immunology (9), and investigated its clinical relevance *vis-à-vis* any history of recurring infections and ENFD in FM.

Population studies show that Ig subclass concentrations are genetically determined, generally follow a log-normal distribution, reach adult levels by mid-adolescence, and – absent a significant stressor – remain relatively fixed throughout the individual's adult life (17-21). Further, the occurrence of a particularly high or low Ig class or subclass concentration tends to co-travel with other similarly high or low immunoreactant levels (22). Several studies have also suggested that there may be minor differences in serum Ig concentrations associated with advancing age, gender, race, smoking, and alcohol use (18, 23, 24). None of these factors were important to our subjects' immunoreactant findings.

Our study populations were relatively homogenous and made up mainly of women, none of whom were smokers or alcoholic. When we compared FM subjects who were <60 years of age to those ≥60 years of age we found no correlation between Ig levels and age. We also found no significant differences in immunoreactant levels between genders for our 72 FM subjects taken as a whole, or when analysed as subgroups (data not shown). Even though some of our FM subjects might have qualified as having had common variable immune deficiency (CVID), none had the wide swings in Ig levels occasionally associated with this disorder (25).

Individuals whose Ig concentrations fall below the LLN or within Q1 are known to suffer more frequent and more severe bacterial and viral infections than those whose Ig values fall above those levels (26). For that reason, we conducted a questionnaire-based survey of our 72 FM subjects, and 40 apparently healthy community controls, using a novel "Lifetime History of Infections" questionnaire, which broadly surveyed participants' history of significant sinopulmonary, cutaneous, gastrointestinal, and urinary tract infections. We analysed our subjects' responses in two broad categories, *i.e*. sinus related, and other, serious but non-sinus related infections.

We chose to emphasise symptoms of CRS in our questionnaire because of its well-known association with PID (27). As depicted in Figure 3, we found that "FM + RA" subjects gave a history of significantly more sinus (*p*=0.04) and non-sinus infections (*p*=0.002) than

control subjects. Our "FM only" subjects claimed significantly more non-sinus infections (p=0.05) and trended toward a history of more sinus infections (p=0.06) than controls. We truncated sinus infection estimates at a maximum of ≥6 infections though many FM subjects, recalled dozens of sinus infections over their lifetime. The prevalence of ≥ 6 sinus infections recalled by controls (15%) is a figure agreeing well with U.S. national surveys for chronic rhinosinusitis, but higher than reported in Olmsted County, MN, USA. (28).

Eighteen of 72 (25%) of our FM subjects had evidence of MBL deficiency (*i.e.* MBL levels <500 ng/ml) presumably representing an admixture of subjects heterozygous (>50 but <500 ng/ml) and homozygous (<50 ng/ml) for one or more missense allele substitutions leading to MBL deficiency (6, 29). There was no significant difference in the prevalence of MBL deficiency or elevation, however, between our "FM only" and "FM + RA" groups. *Isolated* MBL deficiency is commonly thought to be clinically silent due to naturally occurring immunological redundancy, while MBL deficiency combined with other forms of immune dysfunction, such as our FM subjects' IgG subclass deficiency, is thought to be more clinically relevant (29).

We doubt that any of our subjects had idiopathic "late-onset immunoglobulin deficiency" (LOID) as described by Hermans *et al*. (30). Their subjects were, on average, younger than ours (LOID mean age 30.7 yrs. *vs*. "FM only" and "FM + RA" mean age 63 and 67 yrs., respectively). Their subjects also had a high prevalence of sinopulmonary disease, but 28% had frank bronchiectasis, and 28% had splenomegaly. Steatorrhea, cholelithiasis, and nodular lymphoid hyperplasia of the small bowel were also frequent in their LOID subjects. Furthermore, 24% of their 50 subjects developed some form of neoplasia (mainly gastric carcinoma or lymphoreticular malignancies). Some also had "arthritis," but none had FM. None of our FM subjects manifested any of these serious complications of their PID, at least within the context of our study time frame. All in all, we do not consider the clinical features of LOID as fitting our subjects' FM clinical phenotype.

The finding of PID in FM predicts other known features of immune dysfunction, such as autoimmunity and neoplastic disease (2-5). This idea might be supported in FM by recent descriptions of associated small and large fibre neuropathic injuries, with their known connections to immune dysfunction (8, 31, 32), and the greater than expected prevalence of autoimmune disorders noted in Barton *et al*. FM-PID cohort (14). A greater than expected proclivity to a variety of malignancies, including female breast, prostate, lung, and colonic cancer in FM has also been suggested (33).

Interestingly, our "FM only" subjects demonstrated a significant, positive correlation between IgA (both total IgA and IgA subclass 1) and IgG subclass 1 levels and the ENFD findings associated with a SFN (Table I, Fig. 1). It is known that the protective effects of non-complement fixing IgA, particularly IgA Subclass 1 (augmented by IgG subclass 1, 2 and 3, IgM, and IgD), are amongst the most active local agents guarding against bacterial and other microbiota related pathogens in the gastrointestinal system, particularly from the naso-oropharyngeal area to the distal ileum (34-36). It is tempting to speculate that a disruption of regional gastrointestinal homeostasis contributes to the ingress and systemic spread of noxious and antigenic substances in FM resulting in direct or indirect peripheral nervous system injury (PNS) and autoimmunity (8, 32).

It is also biologically plausible that such a peripheral SFN, principally involving nociceptive C-fibres, along with peripheral large fibre pathology (31, 32), serve as unremitting "pain drivers" in FM. These could help to explain the phenomena of spinal cord wind up and CNS sensitisation (*i.e.* "central sensitisation") so frequently postulated to be active in FM (37). Further, it would predict amelioration of the FM painful state with substances, such as IVIg, directed against small and large PNS structural and functional lesions (38). Nevertheless, in our experience, other coincident, painful nociceptive generators, both mechanical and inflammatory, need to be addressed for successful treatment of FM; thus, the importance of recognising concurrent autoimmune disorders such as the RA seen in our "FM+RA" subjects.

Our study has certain inherent limitations. We doubt, however, that significant misclassification error (*i.e.* "recall bias") for recollection of infections in our FM subjects is one of them (39). None of our FM subjects had a prior PID diagnosis, knew of our interest in PID, or had been referred for investigation of possible PID. We were also careful to shuffle our novel "Lifetime History of Infections" questionnaire in amongst routine FM intake paperwork. This brief questionnaire asked for a quantitative approximation, on a 7-point numerical scale (0 to ≥6), of the number of serious infections of 8 types (*e.g.* sinusitis, pneumonia, etc.) previously experienced. Infections recalled were truncated at ≥6 to limit overestimation, though many of our FM subjects gave a history of dozens of infections, particularly sinus infections.

Evaluation of 23-valent pneumococcal vaccination responses in our FM subjects might have better established FM immune deficiency (40). Family studies and longitudinal follow-up of the present subjects would also have been of interest. Both of these strategies, however, were beyond the scope of this study.

We have, then, herein described a substantial cohort of unselected, adult FM subjects, with and without features of RA, who had laboratory evidence of significant immunoreactant insufficiencies. In the main, these consisted of IgG subclass, IgA subclass, and MBL deficiencies. These deficiencies were associated with a significantly greater estimation of previous sinus and non-sinus infections amongst FM subjects than apparently healthy controls, suggesting that this phenomenon may be associated with clinically important translational propensities to infectious disease in FM. There was also a noteworthy correlation of IgA and IgG subclass 1 levels with ENFD. Taken as a whole, our findings suggest that a better understanding of FM may require a deeper appreciation of the immunologic milieu from which it arises.

Acknowledgments

The authors wish to acknowledge the selfless contributions of the study participants in sharing their clinical histories, and the helpful suggestions of the anonymous manuscript reviewers.

We also wish to thank Ms Stacy Carr and Angelica Parocua for their support staff professionalism.

This study is dedicated to J, M, & C.

References

1. SIMON AK, HOLLANDER GA, MCMICHAEL A: Evolution of the immune system in humans from infancy to old age. *Proc Biol Sci* 2015; 282: 20143085.
2. TODORIC K, KOONTZ JB, MATTOX D, TARRANT TK: Autoimmunity in immunodeficiency. *Curr Allergy Asthma Rep* 2013; 13: 361-70.
3. SLEASMAN JW: The association between immunodeficiency and the development of autoimmune disease. *Adv Dent Res* 1996; 10: 57-61.
4. HAAS OA: Primary immunodeficiency and cancer predisposition revisited: embedding two closely related concepts into an integrative conceptual framework. *Front Immunol* 2019; 9: 3136.
5. WEINBERG R: The Biology of Cancer. Garland Science. New York. 2006. ISBN-13:978-0815342205.
6. IP WKE, TAKAHASHI K, EZEKOWITZ, RA, STUART LM: Mannose-binding lectin and innate immunity. *Immunol Rev* 2009; 230: 9-21.
7. WOLFE F, SMYTHE HA, YUNUS MB *et al*.: The American College of Rheumatology 1990 criteria for the classification of fibromyalgia: report of the multicenter criteria committee. *Arthritis Rheum* 1990; 33: 160-72.
8. CARO XJ, WINTER EF: Evidence of abnormal epidermal nerve fiber density in fibromyalgia: clinical and immunologic implications. *Arthritis Rheumatol* 2014; 66: 1945-54.
9. SCHROEDER HW JR, WALD D, GREENSPAN NS: Immunoglobulin Structure and Function. *In*: PAUL WE (Ed.): Fundamental Immunology, 7th Ed, New York, Lippincott 2013: 123-49.
10. YULE GU, KENDALL MG: An Introduction to the Theory of Statistics. 14th Ed. Charles Griffin & Co. London. 1958; 140-2.
11. KLINK M, CLINE MG, HALONEN M, BURROWS B: Problems in defining normal limits for serum IgE. *J Allergy Cllin Immunol* 1990; 85: 440-4.
12. KARAHALIOS A, BAGLIETTO L, CARLIN JB, ENGLISH DR, SIMPSON JA: A review of the reporting and handling of missing data in cohort studies with repeated assessment of exposure measures. *BMC Med Res Methodol* 2012; 12: 96-106.
13. RIEDERER J: Fibromyalgiesyndrom mit IgA- und IgG-Subklassenmangel [Fibromyalgia syndrome with IgA and IgG subclass deficiency]. *Med Klin (*Munich*)* 1991; 86: 547-9.
14. BARTON JC, BERTOLI LF, BARTON JC, ACTON RT: Fibromyalgia in 300 adult index patients with primary immunodeficiency. *Clin Exp Rheumatol* 2017; 35 (Suppl. 105): 68-73.
15. HOFFMANN RG: Statistics in the practice of medicine. *JAMA* 1963; 185: 864-73.
16. ZHANG Y, MA W, WANG G, LV Y, PENG Y, PENG X: Limitations of the Hoffmann method for establishing reference intervals using clinical laboratory data. *Clin Biochem* 2019; 63: 79-84.
17. JOLLIFF CR, COST KM, STIVRINS PC *et al*.: Reference intervals for serum IgG, IgA, IgM, C3, and C4 as determined by rate nephelometry. *Clin Chem* 1982; 28: 126-8.
18. CASSIDY JT, NORDBY GL, DODGE HJ: Biologic variation of human serum immunoglobulin concentrations: sex-age specific effects. *J Chron Dis* 1974; 27: 507-16.
19. BÁTORY G, JANCSÓ A, PUSKÁS É, RÉDEI A, LENGYEL E: Antibody and immunoglobulin levels in aged humans. *Arch Gerontol Geriatr* 1984; 3: 175-88.
20. SHAKIB F, STANWORTH DR: Human IgG subclasses in health and disease. (A review). Part I. *La Ricerca Clin Lab* 1980; 10: 463-79.
21. SHAKIB F, STANWORTH DR: Human IgG subclasses in health and disease. (A review). Part II. *Ric Clin Lab* 1980; 10 :561-80.
22. CASSIDY JT, NORDBY GL: Human serum immunoglobulin concentrations: prevalence of immunoglobulin deficiencies. *J Allergy Clin Immunol* 1975; 55: 35-48.
23. RITCHIE RF, PALOMAKI GE, NEVEUX LM, NAVOLOTSKAIA O: Reference distributions for immunoglobulins A, G, and M: A comparison of a large cohort to the world's literature. *J Clin Lab Anal* 1998; 12: 371-7.
24. GONZALEZ-QUINTELA A, ALENDE R, GUDE F *et al*.: Serum levels of immunoglobulins (IgG, IgA, IgM) in a general adult population and their relationship with alcohol consumption, smoking and common metabolic abnormalities. *Clin Exp Immunol* 2008; 151: 42-50.
25. SELIGMANN M, AUCOUTURIER P, DANON F, PREUD'HOMME JL: Changes in serum immunoglobulin patterns in adults with common variable immunodeficiency. *Clin Exp Immunol* 1991; 84: 23-7.
26. WALSH EE, FALSEY AR: Humoral and mucosal immunity in protection from natural respiratory syncytial virus infection in adults. *J Infect Dis* 2004; 190: 373-8.
27. MCGEADY SJ, INCAUDO GA: Immunodeficiency and Sinus disease. *In*: CHANG CC, INCAUDO GA, GERSHWIN (Eds.) Diseases of the Sinuses. 2nd Ed. Springer, New York, 223-46.
28. SHASHY RG, MOORE EJ, WEAVER A: Prevalence of the chronic sinusitis diagnosis in Olmsted County, Minnesota. *Arch Otolaryngol Head Neck Surg* 2004; 130: 320-3.
29. TURNER MW: The role of mannose-binding lectin in health and disease. *Mol Immunol* 2003; 40: 423-9.
30. HERMANS PE, DIAZ-BUXO JA, STOBO JD: Idiopathic late-onset immunoglobulin deficiency. Clinical observations in 50 patients. *Am J Med* 1976; 61: 221-37.
31. CARO XJ, WINTER EF: The role and importance of small fiber neuropathy in fibromyalgia pain. *Curr Pain Headache Rep* 2015; 19: 55-62.
32. CARO XJ, GALBRAITH RG, WINTER EF: Evidence of peripheral large nerve involvement in fibromyalgia: a retrospective review of EMG and nerve conduction findings in 55 FM subjects. *Eur J Rheumatol* 2018; 5: 104-10.
33. MCBETH J, SILMAN AJ, MACFARLANE GJ: Association of widespread body pain with an increased risk of cancer and reduced cancer survival: a prospective, population-based study. *Arthritis Rheum* 2003; 48: 1686-92.
34. CHEN K, MAGRI G, GRASSET EK, CERUTTI A: Rethinking mucosal antibody responses: IgM, IgG and IgD join IgA. *Nat Rev Immunol* 2020; 20: 427-41.
35. BRANDTZAEG P, BAEKKEVOLD ES, FARSTAD IN *et al*.: Regional specialization in the mucosal immune system: what happens in the microcompartments? *Immunol Today* 1999; 20: 141-51.
36. JOHANSEN F-E, BRAATHEN R, MANTHE E *et al*.: Regulation of the mucosal IgA system. *In*: KAETZEL CS (Ed.): Mucosal Immune Defense: Immunoglobulin A. Springer, New York, 2007, 111-43.
37. HARTE SE, HARRIS RE, CLAUW DJ: The neurobiology of central sensitization. *J Appl Behav Res* 2018; 23: e12137.
38. CARO XJ, WINTER EF, DUMAS AJ: A subset of fibromyalgia patients have findings suggestive of chronic inflammatory demyelinating polyneuropathy and appear to respond to IVIg. *Rheumatology* (Oxford) 2008; 47: 208-11.
39. COUGHLIN SS: Recall bias in epidemiologic studies. *J Clin Epidemiol* 1990; 1: 87-91.
40. ORANGE JS, BARLOW M, STIEHM ER *et al*.: Use and interpretation of diagnostic vaccination in primary immunodeficiency: A working group report of the Basic and Clinical Immunology Interest Section of the American Academy of Allergy, Asthma & Immunology. *J Allergy Clin Immunol* 2012; 130 (3 Suppl.): S1-24.
41. SRINIVASA BT, ALIZADEHFAR R, DESROSIERS M, SHUSTER J, PAI NP, TSOUKAS CM: Adult primary immune deficiency: what are we missing? *Am J Med* 2012; 125: 779-86.
42. SUPER M, GILLIES SD, FOLEY S, SASTRY K, SCHWEINLE JE, SILVERMAN VJ, EZEKOWITZ RA: Distinct and overlapping functions of allelic forms of human mannose binding protein. *Nat Genet* 1992; 2: 50-5.