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Inflammatory and autoimmune manifestations in X-linked carriers of chronic granulomatous disease in the United Kingdom

To the Editor:

X-linked chronic granulomatous disease (XL-CGD) due to a defect in the nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase complex subunit gp91^{phox} encoded by *CYBB* impairs neutrophil respiratory oxidative burst (NROB). In the United Kingdom, the incidence is approximately 1 in 125,000 of which 70% are X-linked.[1] Patients experience recurrent, severe bacterial and fungal infection[2] as well as inflammatory and autoimmune complications,[3] which cause significant morbidity and mortality.

XL-CGD female carriers have a dual phagocyte population due to lyonization, with 20% to 80% functioning phagocytes.[4]

Reports of XL-CGD female carriers with discoid lupus erythematosus, photosensitivity,[4] gastrointestinal symptoms, chorioretinitis, and autoimmune phenomena are published.[4] We performed a detailed health survey of XL-CGD carriers identified from families known to the UK CGD registry[1] and via the CGD Society. Carriers were confirmed by a dual phagocytic cell population measured by the nitroblue tetrazolium test or dihydrorhodomine flow cytometric assay. The presence of gastrointestinal symptoms and symptoms related to systemic lupus erythematosus (SLE) defined by American Rheumatology Association (ARA) diagnostic criteria[5] were specifically sought. An estimate of neutrophil function was measured at the recruiting center using dihydrorhodomine, at enrollment. Available previous results were recorded. Antinuclear, antigastric parietal cell, antimitochondrial, and anti–smooth muscle autoantibodies were measured by standard routine methodologies.

Of 94 XL-CGD carrier families identified from the UK registry, 81 XL-CGD carriers, from 62 families, were recruited; complete data were available for 61 (see Table E1 in this article's Online Repository at www.jacionline.org).

Median NROB at enrollment was 47% (range, 7% to 94%), compared with a historical median of 52%, performed at any time before study commencement. Most subjects had an NROB of 20% to 60%: there was no significant correlation with carrier age.

Skin symptoms were reported by 63 (79%) subjects, most frequently photosensitivity. Other symptoms included a malar, lupus-like rash, and eczema (see Table E2 in this article's Online Repository at www.jacionline.org). Adult acne was present in 8 (10%), reminiscent of CGD folliculitis. There was no significant difference in median NROB in affected versus nonaffected subjects. Skin abscesses were reported in 14 (17%), in whom the NROB was significantly lower than in nonaffected carriers ($P = .0088$).

Gastrointestinal symptoms were reported by 40 carriers, most frequently abdominal pain and diarrhea. Most did not have a defined diagnosis: 3 had inflammatory bowel disease. Fifteen were investigated for symptoms (see Table E3 in this article's Online Repository at www.jacionline.org). Four had undergone appendicectomy, but histologic results were not available. Thirteen were investigated; histologic features of Crohn disease were reported in 2 and colitis was reported in 1—similar to those reported in CGD colitis. The NROB was lower in symptomatic carriers (abdominal pain, diarrhea, rectal bleeding), but did not reach statistical significance ($P = .08$). The NROB was significantly lower in subjects affected by diarrhea ($P = .009$) (Table I). The presence of colitis in an index case correlated with symptoms in the related carriers ($P = .03$).

Features of SLE (see Table E4 in this article's Online Repository at www.jacionline.org) were compared with a cohort of European patients with SLE.[6] Photosensitivity, oral ulcers, Raynaud phenomenon, and arthritis occurred more frequently in carriers than in the reference cohort. There was no significant difference in the ratio of normal neutrophil function in symptomatic compared with nonsymptomatic subjects (see Table E5 in this article's Online Repository at www.jacionline.org).

Fifty-seven percent of carriers (n = 45) met 3 or more ARA SLE criteria, and more than 25% met 4 or more, consistent with SLE (Table E5), but only 18% had been diagnosed with a lupus-like disorder, most of whom were not labeled as having SLE. There was no significant difference in NROB when considering the number of ARA criteria met ($P = .6$ by 1-way ANOVA). A positive antinuclear antibody was infrequently encountered as one of the ARA SLE criterion. Clinical manifestations of SLE that were unusual were nephropathy, serositis, and neurological involvement.

Those in whom joint symptoms were elicited reported episodic inflammation, pain, and redness, associated with extreme fatigue, suggesting an inflammatory rather than degenerative pattern. More carriers who reported joint symptoms were autoantibody positive when tested (19% vs 14%).

Autoantibody results were available for 51 participants: 42 had a negative panel, 12 had 1, and 2 had 2 or more positive autoantibodies. The antinuclear antibody (ANA) was positive (>1:40) in 8 carriers, with 4 demonstrating positive antigastric parietal cell antibody and 1 each demonstrating antimitochondrial and anti–smooth muscle autoantibodies, respectively. There was no clear correlation with symptoms (Tables I and E5).

TABLE I. Percentage of normal neutrophil function and autoantibody positivity in XL-CGD carriers with and without symptoms

Symptom	No. affected (%)	Mean NROB in the affected group	Mean NROB in the unaffected group	Paired t test P value	Autoantibody positivity in the affected group (%)	Autoantibody positivity in the unaffected group (% positive)
GI symptoms						
Any GI symptom	40 (50)	44	52	.08	16	
Abdominal pain	25 (31)	40	52	.05	10	
Diarrhea	24 (30)	37	54	.009	10	
Rectal bleeding	17 (21)	44	50	.16	4	
Gastrointestinal diagnoses						
Inflammatory bowel disease/CGD colitis	3 (4)	16	NA		0	
Irritable bowel syndrome	8 (9.8)	44			40	
Other	2 (2.5)	32			0	
No formal GI diagnosis	56 (69)	48			10	
Photosensitivity	57 (74)	46	49	.3	25	5
Oral ulcers	57 (74)	46	54	.13	17	24
Raynaud phenomenon	27 (35)	49	46	.3	13	14
Joint symptoms	48 (62)	47	47	.46	19	14
Fatigue	37 (51)	43	51	.08	23	30

GI, Gastrointestinal; *NA*, not applicable/available.

The NROB was higher (62%) in carriers meeting none of the ARA criteria for SLE compared with 43% in those with 4 or more (Table E5). Thirteen received hydroxychloroquine treatment.

We report wide-ranging inflammatory and autoimmune symptoms in XL-CGD carriers, many of which overlap with clinical presentations of patients with CGD. The most striking finding was that 26% of carriers met 4 or more of the ARA SLE diagnostic criteria and only 18% had been diagnosed with a lupus-like disorder. This has not been previously described as such a common problem in this population. Anecdotally, carriers with SLE-like symptoms were told that they did not have SLE because autoantibodies were negative. The phenotype is more extensive than previously highlighted features of discoid lupus, and the pattern is different to the reference Euro-lupus cohort, who had more organ involvement. Previous reports have referred to a "lupus-like" disease in XL-CGD carriers due to the lack of positive "lupus autoantibodies."[4,7] However, symptom pattern may suggest a different process, forming part of the spectrum of disease of SLE, rather than "classical" SLE.[8] Failure to associate symptoms with CGD, or with an SLE variant, has led to inadequate treatment and ongoing symptoms.

Reports of XL-CGD carriers with extreme X-inactivation suffering from inflammatory bowel disease have been published.[9] We demonstrate that bowel symptoms are more prevalent than previously recognized although few were investigated. There was a significant correlation between carriers and index case experiencing gastrointestinal symptoms, suggesting an association of symptoms with a shared environment.

Our study suggests that symptomatic XL-CGD carriers warrant further investigation and treatment. All known UK families were approached for this study, thus limiting bias toward only symptomatic carriers. Identifying carriers most at risk of developing symptoms was not possible, because there was no clear correlation with degree of neutrophil function or with detection of autoantibodies by immunofluorescence, although the association of bowel symptoms within families is intriguing.

We recommend that symptomatic XL-CGD carriers be managed proactively, and reviewed by a suitably qualified specialist physician.

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Mast cell–derived prostaglandin D$_2$ attenuates anaphylactic reactions in mice

To the Editor:

Anaphylaxis is a severe and life-threatening allergic reaction. As of the last decade, 0.05% to 2% of the general population worldwide experienced an anaphylactic reaction during their lifetime, and approximately 2% of these subjects died as a result of the reaction.[1]

Clinical and experimental studies have shown that vascular hyperpermeability is a hallmark of anaphylaxis.[2,3] Activated mast cells release vasoactive mediators, such as histamine and platelet-activating factor, which induce the initial anaphylactic response of vascular hyperpermeability by causing vasodilation, endothelial barrier disruption, or both. Systemic vascular hyperpermeability and plasma leakage into local tissues lead to systemic reactions, including increased hematocrit levels and hypotension.

Prostaglandin D$_2$ (PGD$_2$) is produced by the activity of COX and hematopoietic PGD synthase (H-PGDS). Because a previous study demonstrated that mast cells strongly express H-PGDS and produce PGD$_2$ on a large scale,[4] we hypothesized that mast cell–derived PGD$_2$ makes some pathophysiologic contribution to anaphylaxis, although the exact nature of this contribution is unknown.

We first examined anaphylactic vascular hyperpermeability under conditions in which PGD$_2$ was present or absent. Intravenous injection of the antigen dinitrophenyl (DNP) into the DNP-IgE–sensitized mouse ear induced the extravasation of intravenously injected dye in wild-type (WT) mice (Fig 1, A and B). Intradermal injection of compound 48/80 (C48/80) also induced extravasation in WT mice (Fig 1, C and D). A higher degree of dye extravasation was observed in H-PGDS–deficient (Hpgds$^{-/-}$) mice than in WT mice (Fig 1, A and B). Hpgds$^{-/-}$ mice also exhibited a stronger increase in hematocrit levels, more severe hypotension, and deeper and longer hypothermia compared with WT mice in response to systemic mast cell–activating stimulation (Fig 1, E and F, and see Fig E1 in this article's Online Repository at www.jacionline.org). In contrast, overexpression of H-PGDS (HTG) attenuated the C48/80-induced hypothermia (Fig 1, G). These results suggest that H-PGDS–derived PGD$_2$ inhibits vascular hyperpermeability and systemic responses in patients with anaphylaxis.

Mast cell–induced anaphylactic responses are largely attributable to histamine release and subsequent nitric oxide (NO) production from endothelial cells.[5,6] C48/80 stimulation produced comparable increases in plasma levels of histamine in the 2 lines of mice (see Fig E2, A, in this article's Online Repository at www.jacionline.org), and pretreatment with the histamine H$_1$

receptor blocker diphenhydramine abolished the C48/80-induced hyperpermeability and hypothermia (see Fig E2, B-D). H-PGDS deficiency did not influence the sensitivities to histamine (see Fig E2, E-G). Lack of the endothelial NO synthase gene also abolished the C48/80-induced anaphylactic responses (see Fig E3, A and B, in this article's Online Repository at www.jacionline.org). Moreover, pretreatment with the NO synthase inhibitor N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME) led to a nonsignificant inhibition of dye extravasation and hypothermia in WT mice, whereas it significantly inhibited the responses in Hpgds$^{-/-}$ mice (see Fig E3, C-E), suggesting increased NO production in the PGD$_2$-absent condition.

Immunostaining results indicated that mast cells localized with α-smooth muscle actin (α-SMA)–positive and platelet endothelial cell adhesion molecule (PECAM) 1–positive vessels and that their numbers were comparable between WT and Hpgds$^{-/-}$ mice (see Fig E4, A and B, in this article's Online Repository at www. jacionline.org). The perivascular mast cells strongly expressed H-PGDS in the ears of WT but not Hpgds$^{-/-}$ mice (Fig 1, H). We then generated mast cell–specific H-PGDS–deficient mice (Mcpt5^{Cre}Hpgds$^{fl/fl}$) by crossing mast cell protease 5 (MCPT5)-Cre transgenic mice and H-PGDS floxed mice (see Fig E4, C-E and Fig E7 in this article's Online Repository at www.jacionline. org). Compared with the control mice (Hpgds$^{fl/fl}$), Mcpt5^{Cre} Hpgds$^{fl/fl}$ mice exhibited enhanced vascular hyperpermeability without PGD$_2$ production in response to C48/80 (Fig 1, I-K). On the other hand, Mcpt5^{Cre}Hpgds$^{fl/fl}$ mice exhibited a slight but nonsignificant increase in C48/80-induced hypotension and hypothermia compared with the Hpgds$^{fl/fl}$ mice (Fig 1, L and M).

There are 2 major types of mast cells: connective tissue–type mast cells (CTMCs) and mucosal-type mast cells (MMCs). CTMCs typically reside in the skin and the peritoneal cavity, whereas MMCs are predominant in the mucosal layer of the lung and intestine. Because the protease MCPT5 is mainly expressed in CTMCs but is rarely expressed in MMCs, Mcpt5^{Cre}Hpgds$^{fl/fl}$ mice lack H-PGDS in CTMCs. MCPT5^{Cre}iDTR$^+$ mice, in which CTMCs are selectively depleted by diphtheria toxin, exhibit a strong reduction of anaphylactic hypothermia while still exhibiting a small response because of residual MMCs.[7] MMC-derived PGD$_2$ can also be involved in anaphylaxis suppression.[8]

We next examined the contribution of 2 distinct PGD$_2$ receptors to the attenuation of anaphylaxis. Systemic DP deficiency (Dp$^{-/-}$, see Fig E7) but not CRTH2 receptor deficiency (Crth2$^{-/-}$) significantly increased dye extravasation compared with that seen in WT mice (Fig 2, A and B). Administration of PGD$_2$ or a DP agonist inhibited the C48/80-induced vascular hyperpermeability (Fig 2, C and D, and see Fig E5 in this article's Online Repository at www.jacionline.org). There is another endogenous DP ligand, PGD$_1$, which has much lower affinity than PGD$_2$. However, the high dose of PGD$_1$ (1 μg) did not inhibit anaphylactic hyperpermeability (see Fig E5). The DP agonist also attenuated hypothermia in both mouse lines (Fig 2, E). Although it is reported that DP activation induces vasodilation in isolated vessels of the human lung,[9] it did not influence the blood pressure of mice in this regimen (see Fig E6 in this article's Online Repository at www.jacionline.org). Anaphylactic vascular hyperpermeability depends on endothelial G$_q$/G$_{11}$/intracellular calcium ([Ca^{2+}]$_i$)–induced NO production.[3,6] Activation of the Gs-coupled DP receptor increases intracellular cyclic AMP, which activates protein kinase A and decreases [Ca^{2+}]$_i$ levels. Results

TABLE E1. Baseline demographic characteristics of XL-CGD carriers

No. of carriers	81
% Alive	97.5
Median age (y) (range)	42.5 (3-77)
Ethnicity (% white British)	96
Median age of index case (y) (range)	12 (1-43)
Relationship to index case (% mothers)	69
Exclusions	
Deceased index case	4
Non-UK resident	11
New mutation in index case	5

TABLE E2. Skin and infective manifestations in XL-CGD carriers

Manifestation	No. of carriers (%)
Photosensitivity	56 (74)
DLE/malar rash	30 (40)
Eczema	11 (14)
Psoriasis	3 (4)
Adult acne	8 (10)
Erythema multiforme	2 (3)
Dermatitis	5 (7)
Allergic/hives	5 (7)
Rosacea	5 (7)
Malignancy	1 (1)
Any significant infection	19 (23.5)
Fungal infection	1 (1.2)
Pneumonia	4 (4.9)
Meningitis	2 (2.5)
Recurrent skin abscesses	14 (17)
Sinus infection	2 (3)
Lymphadenitis	4 (5)
Alopecia	6 (8)

DLE, Discoid lupus erythematosus.

TABLE E3. Gastrointestinal investigations in XL-CGD carriers

Carrier	Investigation	Investigation finding
3	Colonoscopy	Colitis consistent with CGD colitis
	Endoscopy	Hiatus hernia
4	Colonoscopy	Minor, nonspecific inflammation (reported as not significant)
11	Labeled white cell	Normal
	Small-bowel follow-through endoscopy	Normal
	USS	Hiatus hernia and gastritis
		Normal
13	Gastroscopy	Normal duodenal biopsy
		Telangiectasia
		Normal GI tract
20	USS	Normal
21	Colonoscopy	Crohn disease
34	Body scan	Diverticular disease
36	Colonoscopy	No significant abnormality
39	Colonoscopy	Crohn disease
		Patchy inflammatory abscesses with cryptitis and crypt abscesses. No convincing granulomas. Consistent with mild/moderate chronic inflammation
	Sigmoidoscopy	Mild nonspecific inflammation
		Indeterminate colitis
	Colonoscopy and endoscopy	Patchy inflammatory changes. Acute inflammation with cryptitis and crypt abscesses. Mild chronic inflammation in left colon. No convincing granulomas.
		The appearances are of patchy mild and moderate active chronic inflammation, in keeping with clinical history of Crohn disease
42	Sigmoidoscopy	Normal
53	Endoscopy	Esophagitis (early Barrets)

GI, Gastrointestinal; *USS*, ultrasound scan.

TABLE E4. SLE symptoms in XL-CGD carrier cohort compared with published data of European SLE cohort*

Problem	Euro-Lupus cohort, n (%)	CGD carrier cohort, n (%)	P value
Malar rash	311 (31.1)	30 (39)	.07
Photosensitivity	229 (22.9)	57 (74)	**<.01**
Oral ulcers	125 (12.5)	57 (76)	**<.01**
Raynaud phenomenon	163 (16.3)	27 (35)	**<.01**
Arthritis	481 (48.1)	48 (62)	**.01**
Serositis	160 (16.0)	3 (3.8)	*.003*
Nephropathy	279 (27.9)	3 (3.8)	*<.001*
Neurological involvement	194 (19.4)	3 (3.8)	*<.005*
Death	68 (6.8)	2 (3)	*<.0001*

Boldface type signifies more significant in the CGD carrier group, italic type signifies more significant in the Euro SLE group.

*O'Neill S, Cervera R. Systemic lupus erythematosus. Best Pract Res Clin Rheumatol 2010;24:841-55.

TABLE E5. The number of ARA criteria for the diagnosis of SLE met, percentage with positive autoantibodies positivity, and mean percentage of normal neutrophil function in XL-CGD carriers

No. of ARA criteria met	No. of XL-CGD carriers	Autoantibody positivity (%) (% carriers ANA positive)	NROB (mean)
0	10	0 (0)	62
1	9	11 (0)	54
2	17	12 (12)	43
3	24	17 (4)	47
4+	21	33 (24)	43

TABLE E6. Autoimmune features in XL-CGD carriers with NROB in affected and unaffected groups and autoantibody positivity

Symptom	No. affected (%)	Mean NROB in the affected group	Mean NROB in the unaffected group	Paired *t* test *P* value	Autoantibody positivity (% positive in affected)	Autoantibody positivity in the unaffected group (% positive)
Photosensitivity	57 (74)	46	49	.3	25	5
Oral ulcers	57 (74)	46	54	.13	17	24
Raynaud phenomenon	27 (35)	49	46	.3	13	14
Joint symptoms	48 (62)	47	47	.46	19	14
Fatigue	37 (51)	43	51	.08	23	30