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RESEARCH ARTICLE

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Unveiling the value of C-reactive protein as a severity biomarker and the IL4/IL13 pathway as a therapeutic target in recessive dystrophic epidermolysis bullosa: A multiparametric cross-sectional study

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Abstract

Patients with recessive dystrophic epidermolysis bullosa (RDEB) experience numerous complications, which are exacerbated by inflammatory dysregulation and infection. Understanding the immunological mechanisms is crucial for selecting medications that balance inflammation control and immunocompetence. In this cross-sectional study, aiming to identify potential immunotherapeutic targets and inflammatory biomarkers, we delved into the interrelationship between clinical severity and systemic inflammatory parameters in a representative RDEB cohort. Encompassing 84 patients aged 1–67 and spanning all three Epidermolysis Bullosa Disease Activity and Scarring

María José Escámez and Rosa Sacedón contributed equally to this study.

Index (EBDASI) severity categories, we analysed the interrelationship of infection history, standard inflammatory markers, systemic cytokines and Ig levels to elucidate their roles in RDEB pathophysiology. Our findings identify C-reactive protein as an excellent biomarker for disease severity in RDEB. A type 2 inflammatory profile prevails among moderate and severe RDEB patients, correlating with dysregulated circulating IgA and IgG. These results underscore the IL4/IL13 pathways as potential evidence-based therapeutic targets. Moreover, the complete inflammatory scenario aligns with *Staphylococcus aureus* virulence mechanisms. Concurrently, abnormalities in IgG, IgE and IgM levels suggest an immunodeficiency state in a substantial number of the cohort's patients. Our results provide new insights into the interplay of infection and immunological factors in the pathogenesis of RDEB.

KEYWORDS
cytokines, immunodeficiency, immunoglobulins, inflammation, recessive dystrophic epidermolysis bullosa

1 | INTRODUCTION

Recessive dystrophic epidermolysis bullosa (RDEB) is a genodermatosis caused by loss-of-function pathogenic variants in *COL7A1* that disrupt the anchorage of epithelia to dermis or *lamina propria*, which affects skin and mucosae. Due to the extreme fragility of these tissues, patients with RDEB are subject to recurrent blistering, the burden of tissue regeneration, inflammation and risk of infection from birth.[1] Phenotypical manifestations of RDEB point to a pleiotropic impact of the loss of COLVII function and the heterogeneity of disease expression, even in patients with the same pathogenic variants, suggest the existence of modifier factors, whether genetic or environmental.[2,3]

Anaemia,[4,5] pruritus,[6] pain,[7] malnutrition[8–10] and fibrosis[11] are chronic complications in RDEB exacerbated by inflammation. Conventional treatments often prove to be inefficient.[12] The inflammatory status in RDEB [13–19] and autoimmunity against skin antigens[15,20–23] have been investigated. However, their specific roles in the progression of the disease remain unclear. Infection and sepsis are among the primary causes of hospitalization and death in RDEB patients,[24–26] highlighting the critical importance of maintaining immunocompetence as part of patient management.

Current clinical practice provides a battery of anti-inflammatory drugs that could mitigate the negative consequences of abnormal inflammatory responses in RDEB. However, due to the delicacy of the balance between inflammatory mediators and, considering the underlying risks,[27] a thorough understanding of the immunologic mechanisms involved is required for selecting the most appropriate drug.

This is the first correlational multiparametric analysis between inflammatory mediators and markers of progression performed in a representative severity-stratified cohort of 84 patients with RDEB, in which C-reactive protein (CRP) is identified as the best biomarker of severity and type 2 inflammatory response as a potential therapeutic target. We also propose *Staphylococcus aureus* infection as a possible trigger for immune system distortion and disease progression. Data also support the coexistence of a humoral immunodeficiency as an aggravating mechanism worth exploring further in RDEB.

2 | MATERIALS AND METHODS

This study was approved by the Ethics Committee at Hospital Universitario La Paz (HULP, Code: PI-4690). Written informed consent was obtained from all subjects and/or legal guardians.

2.1 | Study design, patients and data collection

Observational, non-interventional, cross-sectional study conducted in the RDEB cohort visited at HULP, a Spanish EB Reference Unit. EB diagnosis followed international criteria.[28] Recruitment took place May–December 2021. All registered RDEB patients ($n=93$) were invited. Clinical data and samples were collected at their regularly scheduled follow-up visit.

A single dermatologist scored disease severity by the 'Epidermolysis Bullosa Disease Activity and Scarring Index' (EBDASI) and categorized following stablished cut-off.[29] Demographic data, percentage of body surface area affected (BSA), presence of active cutaneous infection (ACI), history of cutaneous infection microbiologically confirmed (HCIC) and hospitalization because of infection (HHI) were also recorded.

2.2 | Systemic parameter quantification

Blood samples were only obtained when the patient's general health status was compatible. Laboratory parameters CRP, procalcitonin (PCT), platelet and white blood cell (WBC) absolute count were analysed at Hospital la Paz facilities. IgA, IgG, IgM and IgE levels were

measured at the Immunology Lab of the San Carlos Specific following standardized protocols validated for diagnosis. Commercial kits (Merck, Madrid, Spain) were used for determining the levels of IFNγ, IL1β, IL4, IL6, IL10, IL13, IL17A, IL31, IL33, vascular endothelial growth factor A (VEGFA), tumour necrosis factor (TNF) (MILIPLEX MAP, Human TH17 Magnetic Bead Panel AG-14 K-10), nerve growth factor (NGF) (Human Adipokine Magnetic Bead Panel 2) and IL-37/IL-1F7 (Magnetic Bead Panel IV). Although the levels of analytical parameters were classified according to standard clinical criteria (Table S1), those of cytokines were analysed using as reference an age- and sex-matched cohort of healthy controls (HC) (apparently healthy, without declared disease, $n=71$) provided by the Community Blood and Tissue Center of Asturias and the Biobank for Biomedical and Public Health Research of the Valencian Community (IBSP-CV Biobank). To avoid bias, sex- and age-matched HC and patient cytokine quantification were performed in the same experimental setting cytokine levels above 95th percentile of HC (95th PCTL) were defined as increased unless this value was far above the maximum range found in patients with mild disease. In these cases, we used the 90th PCTL of HC as cut-off point.

Blood samples to complete the analytical study were obtained from 82 of the 84 patients (77 in the case of cytokines) due to difficult venous access and/or recommended extraction volume limits for sick children.[30] Cytokine levels detected in one patient were extremely high, with the highest being up to 100 times the maximum found in the entire cohort. The disproportionately high levels observed did not correspond to any identifiable clinical cause, suggesting they were likely due to an analytical error and were therefore excluded from the statistical analysis.

2.3 | Statistical analysis

Data analysis and visualization were performed with Microsoft Excel 365, GraphPad Prism 8.0.2 and SPSS 25.0. Normal distribution was assessed by Kolmogorov–Smirnov–Lilliefors ($n>30$) or Shapiro–Wilk tests ($n<30$). The association between numerical data was evaluated by the Spearman's rank correlation coefficient (ρ). Quantitative variables were compared by the Mann–Whitney U test (two categories) or the Kruskal–Wallis (>2 categories) tests followed by the Dunn's test. For qualitative variables, Pearson's chi-Squared test (or Fisher's exact test) was applied. The mean, standard deviation (SD) and range were tabulated. Individual data, median and interquartile range (IQR) were plotted. p-values (p) <0.05 were considered statistically significant.

3 | RESULTS

3.1 | Patient stratification

All 84 recruited subjects (40 females) aged 1–67 years completed this study (Figure S1, Table S2). Pathogenic variants, RDEB clinical subtype and complications displayed by patients in this cohort will be reported elsewhere (Quintana-Castanedo et al, manuscript in preparation). According to EBDASI, 15% ($n=13$), 27% ($n=22$) and 58% ($n=49$) of the patients exhibited mild, moderate and severe scores, respectively (Table S2). Mild EBDASI was preferentially observed in the youngest children. A weak association between age and BSA was detected ($\rho=0.26$, $p=0.033$). No sex differences were found in severity scores.

3.2 | C-reactive protein as a biomarker of severity in RDEB

Serum CRP and IL6 levels and their correlation, were assessed as non-specific inflammatory markers (Figure 1A–C). Elevated CRP was found in 73% of RDEB patients with a significant association with EBDASI categories, mainly in severe (98%, 48/49) and moderate (55%, 12/22) but rarely in mild patients (Figure 1A). A statistically significant and strong semilogarithmic association was found for CRP with BSA and EBDASI scores (and sub-scores) (Figure 1D). Therefore, in this cohort, a significantly higher risk of systemic inflammation (CRP >5 mg/L) could be predicted ($p<0.0001$) in patients with an EBDASI over 95 points (activity>12, damage>70) or a BSA > 20%. Likewise, significantly high IL6 concentrations were found in 68% of patients (53/78), and were clearly more prevalent in moderate (70%, 14/20) and severe (83%, 38/46) than in mild patients (1/12) (Figure 1B). Also, IL6 significantly correlated with all severity scores analysed (Figure 1E). Although IL6 is considered the main inducer of hepatic CRP synthesis, their correlation was weak and a non-negligible number of patients had elevated CRP and IL6 within control range (13/78) or vice versa (9/78) (Figure 1C).

3.3 | Type 2 cytokines predominate among moderate/severe RDEB patients

To characterize inflammatory response and its relationship with disease severity, the serum levels of 13 cytokines (including IL6) were quantified. Levels of all cytokines except IL37 were significantly increased in RDEB (Figure 2A). All of them but TNF, IL37, vascular VEGFA and NGF were influenced by severity categories. In mild patients, only TNF was increased. All cytokines, except IL37 and VEGFA, were elevated in moderate/severe cases; VEGFA only rose in severe cases. Cytokine levels showed no significant differences between moderate and severe categories. IL4, IL13 and IFNγ were the only cytokines that showed statistically significant differences when comparing mild and moderate patients. Remarkably, the correlation between type 2 cytokine levels, such as IL13 and IL4, with BSA, EBDASI score (and sub-scores) and CRP was weak/moderate but significant (Figure 2B). IL10 showed a weak correlation with severity markers and none with CRP. No differences in cytokine level distribution were found related to sex or age.

Over 60% of patients displayed high levels of IL6, IL13 and IL4 and approximately 50% had elevated IL1β, IL10 or IL31. IFNγ or IL33 were high in 40% of the cohort (Table S3). Only 30% had elevated IL17A, and four patients had markedly high levels of IL17A (700–2360 pg/mL, Figure 2A). During the study, one of them suffered a severe scalp pyodermitis caused by *S. aureus* infection, which resolved after standard antibiotic treatment. However, the other three patients showed no signs of cutaneous infection either at the time of the study or in the following months.

3.4 | IL6, IL4 and IL13 are the most relevant interactive cytokines in RDEB

We explored cytokine network in RDEB. In Table 1, Spearman's test correlation coefficients (ρ) between cytokine levels are depicted by a system of graded colours. The most relevant interactions occurred among the cytokines IL6, IL13, and IL4, which exhibited strong and significant correlations with each other. Additionally, these cytokines showed notable correlations with other type 2 cytokines,

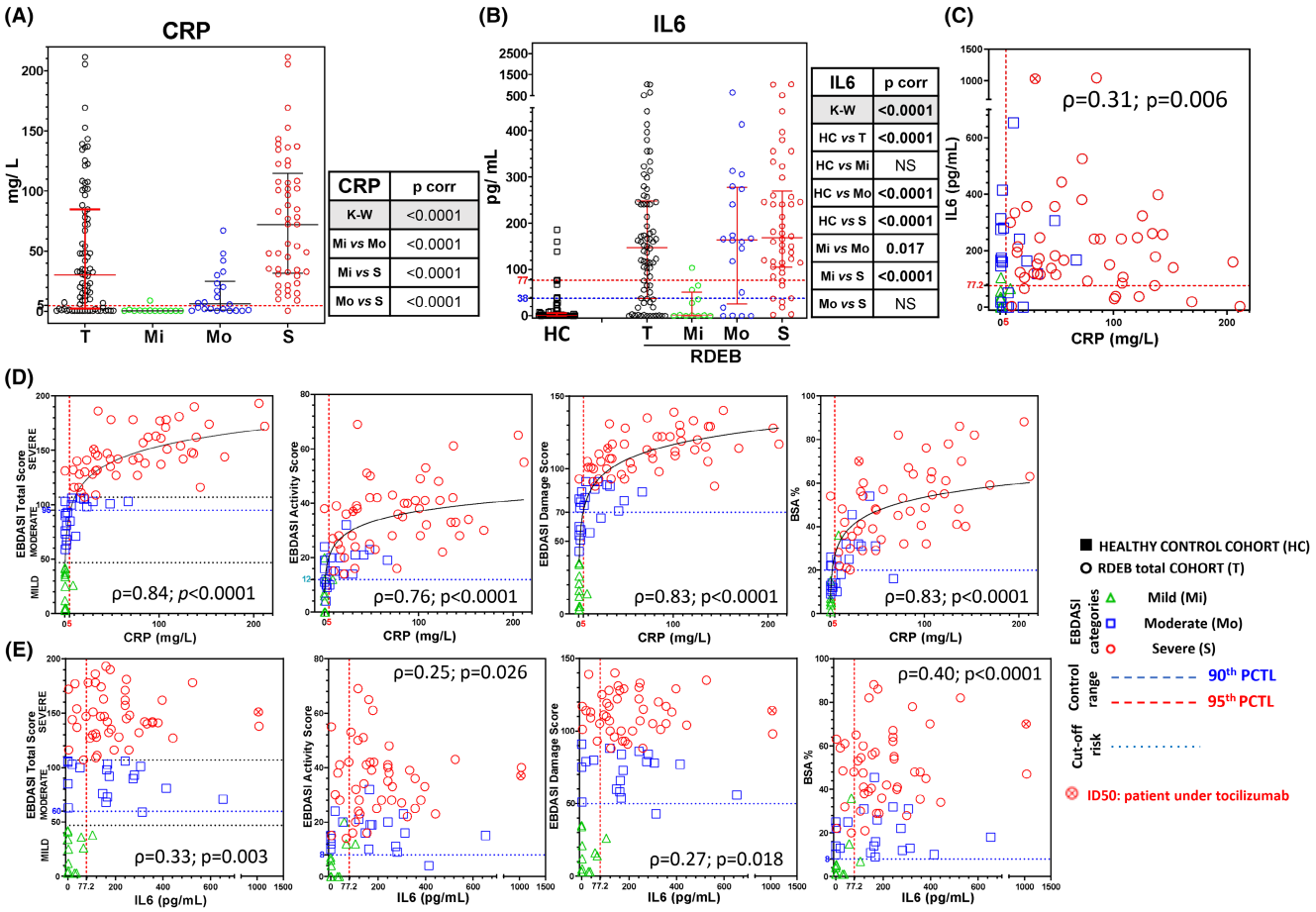
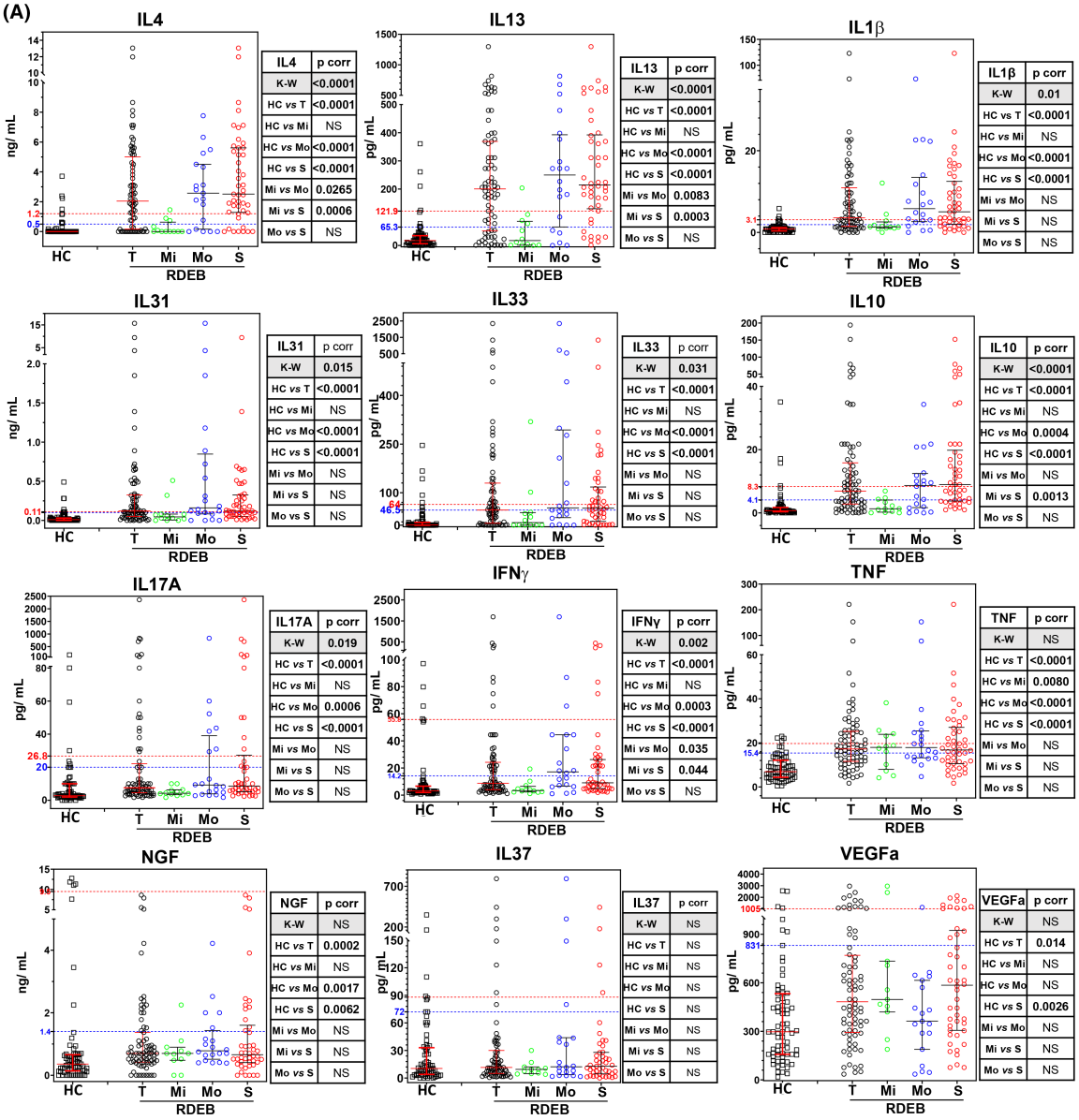


FIGURE 1 Interrelationship between CRP and IL6 circulating levels and RDEB severity. (A, B) Distribution of individual CRP and IL6 serum levels among EBDASI total categories. Median and IQR are represented by bars. Tables shown statistical analysis: K–W, Kruskal–Wallis test followed by Dunn's multiple comparison test. (C) Correlation among CRP and IL6 serum levels. (D, E) Correlation of CRP and IL6 circulating levels with numerical scores of severity markers: All EBDASI scores and the percentage of body surface area affected (BSA%). ρ = Spearman's rank-order correlation coefficient indicative of the strength (1–0.8: Very strong, 0.6–0.8: Strong, 0.4–0.6: Moderate, 0.2–0.4 weak). NS, non-significant; *p*-value >0.05.

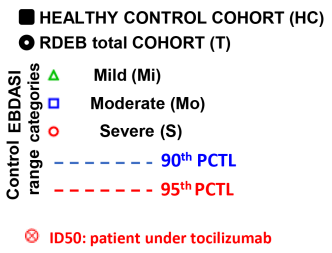
FIGURE 2 Relationship between circulating cytokines and RDEB severity. (A) Distribution of individual cytokine serum levels among EBDASI total categories. Median and IQR are represented by bars. Tables show the statistical analysis: K–W, Kruskal–Wallis test followed by Dunn's multiple comparison test. Number of patients, descriptive statistics and prevalence of high levels (above reference percentile) are shown online at supplemental Table S3. (B) Correlation of cytokine circulating levels with numerical scores of severity markers: All EBDASI scores and the percentage of body surface area affected (BSA%). Continuous lines depict semilogarithmic correlation ($p < 0.0001$). ρ = Spearman's rank-order correlation coefficient. NS, non-significant; *p*-value >0.05.

(A)



(B)

	CRP mg/L	EBDASI SCORE ACTIVITY	EBDASI SCORE DAMAGE	EBDASI SCORE TOTAL	BSA %
CRP mg/L		ρ=0.76 p<0.0001	ρ=0.83 p<0.0001	ρ=0.84 p<0.0001	ρ=0.83 p<0.0001
IL6 pg/mL	ρ=0.30 p=0.007	ρ=0.35 p=0.002	ρ=0.33 p=0.003	ρ=0.33 p=0.003	ρ=0.40 p<0.0001
IL13 pg/mL	ρ=0.45 p=0.028	ρ=0.25 p=0.026	ρ=0.27 p=0.018	ρ=0.25 p=0.029	ρ=0.29 p=0.01
IL4 ng/mL	ρ=0.28 p=0.015	ρ=0.43 p=0.001	ρ=0.41 p=0.001	ρ=0.40 p=0.002	ρ=0.43 p=0.002
IL1β pg/mL	NS	NS	NS	NS	NS
IL33 pg/mL	NS	NS	NS	NS	NS
IL31 ng/mL	NS	NS	NS	NS	NS
IL17A pg/mL	NS	NS	NS	NS	NS
IFNγ pg/mL	NS	NS	NS	NS	NS
IL10 pg/mL	NS	ρ=0.30 p=0.007	ρ=0.24 p=0.031	ρ=0.26 p=0.022	ρ=0.26 p=0.020
IL37 pg/mL	NS	NS	NS	NS	NS
NGF pg/mL	NS	NS	NS	NS	NS
TNF pg/mL	NS	NS	NS	NS	NS
VEGF-A pg/mL	NS	NS	NS	NS	NS
IL6/IL10 ratio	ρ=0.34 p=0.002	ρ=0.28 p=0.014	ρ=0.26 p=0.021	ρ=0.27 p=0.019	ρ=0.34 p=0.0025

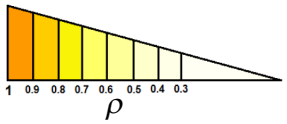


including IL10, IL33, and IL31. Concordance in the status (elevated or not) of IL4 and IL13 was significative (72/76, $p=0.014$), with both being high in 59% of patients. A similar correlation and concomitance were found between IL31 and IL33 levels (69/79, 39% both high). The interrelation involving IL17A and IFNγ was also strong, but they were raised simultaneously in only 16 patients. A more atypical, but also strong statistically relevant correlation among IL17A and IL4 was found. Finally, TNF, a cytokine with a driving role in other pathologies, showed a moderate association with the innate cytokine IL1β, but a weak correlation only with IL6, IL13 and INFγ.

The IL6/IL10 ratio over 5.6, proposed as an EB severity marker,[14] was exceeded in 80% of patients, including 33.3% with mild severity (Figure S2A). In addition, there were non-significant differences in IL6/IL10 ratio among moderate and severe EBDASI ($p=0.49$), without a clear cut-off discriminating between these categories. Accordingly, correlation of IL6/IL10 ratio with severity markers was

TABLE 1 Correlation coefficients between cytokine levels in RDEB patients.

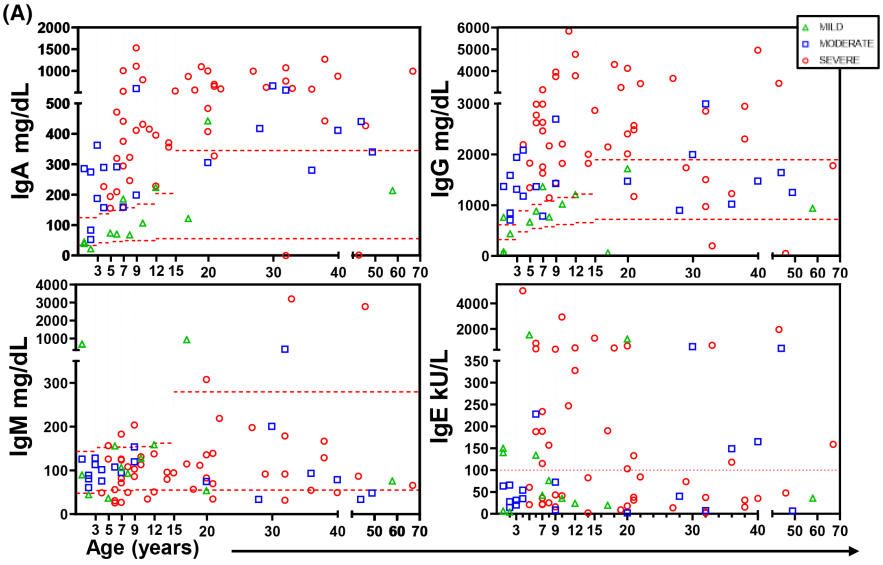
Spearman´s rank correlation coefficient		IL6	IL13	IL4	IL1β	IL33	IL31	IL17A	IFNγ	IL10	IL37	NGF	TNF	VEGF-A
IL13	ρ	0.92												
	p-value	<0.0001												
	n	78												
IL4	ρ	0.88	0.93											
	p-value	<0.0001	<0.0001											
	n	760	76											
IL1β	ρ	0.79	0.85	0.80										
	p-value	<0.0001	<0.0001	<0.0001										
	N	78	78	76										
IL33	ρ	0.72	0.80	0.77	0.83									
	p-value	<0.0001	<0.0001	<0.0001	<0.0001									
	n	78	78	76	78									
IL31	ρ	0.71	0.79	0.74	0.84	0.97								
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001								
	n	78	78	76	78	78								
IL17A	ρ	0.51	0.55	0.80	0.70	0.57	0.64							
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001							
	n	76	76	76	76	76	76							
IFNγ	ρ	0.60	0.66	0.60	0.79	0.71	0.71	0.86						
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001						
	n	78	78	76	78	78	78	76						
IL10	ρ	0.73	0.76	0.76	0.63	0.60	0.57	0.61	0.55					
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001					
	n	78	78	76	78	78	780	77	78					
IL37	ρ	0.49	0.60	0.46	0.60	0.65	0.66	0.42	0.59	0.51				
	p-value	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001				
	n	77	77	76	77	77	77	76	77	77				
NGF	ρ	0.38	0.43	0.40	0.48	0.31	0.36		0.32	0.36	0.52			
	p-value	0.001	<0.0001	0.001	<0.0001	0.006	0.001	NS	0.004	0.001	<0.0001			
	n	78	78	76	78	78	78		78	78	77			
TNF	ρ	0.32	0.24		0.41				0.26			0.31		
	p-value	0.004	0.037	NS	0.0002	NS	NS	NS	0.023	NS	NS	0.006		
	n	78	78		78				78			78		
VEGF-A	ρ													
	p-value	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
	n													
IL6/IL10 RATIO	ρ	0.48	0.39	0.29	0.37	0.31	0.31			-0.53				
	p-value	<0.0001	<0.0001	0.013	0.001	0.006	0.006	NS	NS	<0.0001	NS	NS	NS	NS
	n	76	76	74	76	76	76			76				



Note: ρ = correlation coefficient Spearman's rank test (1–0.8: very strong, 0.6–0.8: strong, 0.4–0.6: moderate, 0.2–0.4 weak). NS, non-significant; *p*-value >0.05.

FIGURE 3 Immunoglobulin circulating level abnormalities and their correlation with inflammatory factors in patients with RDEB. (A) Individual circulating levels of IgA, IgG, IgM, and IgE classified according to EBDASI total categories and their distribution in relation to age. Ig normal ranges according to age are shown by horizontal dashed lines. (B) Contingency table of the prevalence of Ig abnormalities among EBDASI categories. (C) Correlation coefficients between immunoglobulin and cytokine levels. (D) Correlation between circulating levels of immunoglobulins in RDEB patients, noting the concomitance of increased IgA and IgG levels, and instances of IgG deficiency with high IgM levels. (E) Correlation between CRP and IgA or IgG levels. (F) Contingency table of the prevalence of IgA, IgG, and IgE abnormalities among patients with elevated levels or not of both IL4 and IL13. NR, normal range. Statistical tests: χ^2 = Pearson's chi-squared test (used in B and F); ρ = Spearman's rank correlation coefficient (used in C, D, and E); NS = non-significant, *p*-value >0.05.

(A)



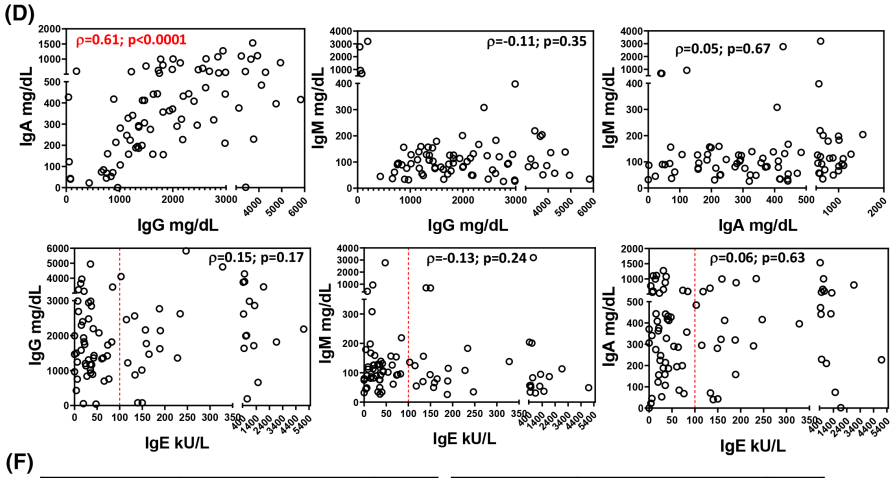
(B)

	TOTAL COHORT n=80-81			EBDASI SEVERITY CATEGORIES						Prevalence of abnormal values χ2
				MILD n=13		MODERATE n=19-20		SEVERE n=48		
	MEAN ± SD (RANGE)	<NR n (%)	>NR n (%)	<NR n (%)	>NR n (%)	<NR n (%)	>NR n (%)	<NR n (%)	>NR n (%)	
IgA mg/dL	444.4±323.1 (0-1535)	3/81 (4%)	63/81 78%)	1/13 (8%)	3/13 (23%)	0 (0%)	16/20 (80%)	2/48 (4%)	44/48 (92%)	p<0.0001
IgG mg/dL	1986.0±1190.4 (42-5827)	5/81 (6%)	55/81 (68%)	3/13 (23%)	3/13 (23%)	0 (0%)	13/20 (65%)	2/48 (4%)	39/48 (82%)	p=0.003
IgM mg/dL	199.9±468.6 (26-3197)	13 /81 (16%)	12/81 15%)	2/13 (15%)	5/13 (39%)	2/20 (10%)	1/20 (5%)	9/48 (19%)	6/48 (13%)	NS
IgE kU/L	295.9±707.9 (2-5000)	-	33/80 (41%)	-	5/13 (39%)	-	5/19 (26%)	-	23/48 (48%)	NS

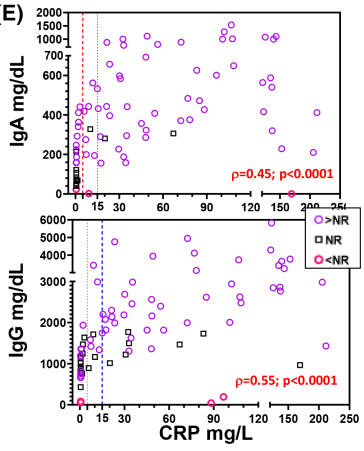
(C)

Spearman´s rank test		IgE	IgG	IgM	IgA
IL6	ρ	NS	0.45	NS	0.34
	p		0.000		0.003
	n		78		73
IL13	ρ	NS	0.46	NS	0.29
	p		0.000		0.013
	n		78		73
IL4	ρ	NS	0.41	NS	0.24
	p		0.000		0.046
	n		75		70
IL1β	ρ	NS	0.36	NS	NS
	p		0.001		
	n		78		
IL33	ρ	NS	0.278	NS	NS
	p		0.014		
	n		78		
IL31	ρ	NS	0.29	NS	NS
	p		0.010		
	n		78		
IL17A	ρ	NS	0.228	NS	NS
	p		0.048		
	n		76		
INFγ	ρ	NS	0.22	NS	NS
	p		0.05		
	n		78		
IL10	ρ	NS	0.40	NS	NS
	p		0.000		
	n		78		
IL-37	ρ	NS	NS	NS	NS
	p				
	n				
NGF	ρ	NS	NS	NS	NS
	p				
	n				
TNF	ρ	NS	NS	NS	NS
	p				
	n				
VEGF-A	ρ	NS	NS	NS	NS
	p				
	n				

(D)



(E)



(F)

↑IL4 & ↑IL13 p=0.021		IgA <NR	IgA NR	IgA >NR	Total
NO	n	2	10	19	29
	%	67%	71%	33%	41%
YES	n	1	4	39	44
	%	33%	29%	67%	59%

↑IL4 & ↑IL13 p=0.014		IgG <NR	IgG NR	IgG >NR	Total
NO	n	3	13	15	31
	%	100%	68%	29%	41%
YES	n	0	6	36	32
	%	0%	32%	71%	59%

↑IL4 & ↑IL13 p=0.15		IgE NR	IgE >NR	Total
NO	n	21	10	31
	%	67%	33%	41%
YES	n	21	22	43
	%	33%	67%	59%

lower than that of CRP or IL6 (Figure S2B). These results support that the informative value of CRP for RDEB severity is superior to that given by IL6/IL10 ratio.

3.5 | Dysregulation of circulating immunoglobulins is not only concordant with increased type 2 cytokines but also is compatible with a primary immunodeficiency disease

To gather information related to the patient's humoral status, circulating immunoglobulin (Ig) levels were quantified (Figure 3A,B). Most RDEB patients presented humoral alterations (76/81). A high prevalence of elevated IgA (78%), IgG (68%), IgE (41%), or IgM (15%) was detected (Figure 3A,B). Remarkably, 62% (50/81) of patients had polyclonal hypergammaglobulinaemia (both IgA and IgG overproduction). Extremely high IgA (>1000 mg/dL) and/or IgG (>3000 mg/dL) levels were observed in 16 patients (Figure 3A), who could be at risk of increased serum viscosity (and thus of vasculitis or microvasculopathy).[31]

IgA and IgG levels correlated and were associated with EBDASI, BSA ($\rho = 0,6$, $p < 0.0001$) and inflammatory factors (CRP, IL6, IL13, IL4) (Figure 3C,E). IgA and IgG hypergammaglobulinaemia was associated with concomitant increase of both IL13 and IL4 (Figure 3F). In this cohort, only five adult patients exhibited renal failure. Four of them had a severe phenotype and all had elevated CRP and IgA levels. Among these patients, three were diagnosed with IgA nephropathy (Table S4). However, due to this small sample size and that all patients were under treatment, drawing solid conclusions about the role of IgA in renal failure was not feasible.

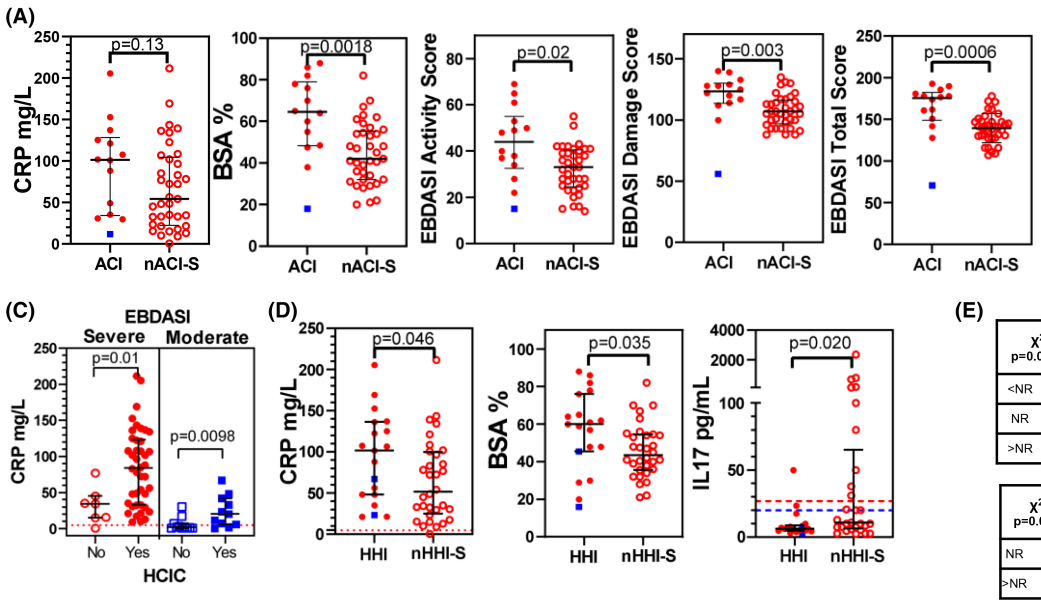
IgE levels, an important indicator of allergy and atopy, were markedly elevated (>1000 kU/L) in 6 of the 81 patients. Among them, 3 of the 6 patients had IgE near or above 2000 kU/L (hyper-IgE) (Figure 3A) which prompt further evaluation. No significant correlation between IgE levels and IL13 or IL4 levels was found (Figure 3C).

Unexpectedly, another 9% (7/81) of RDEB patients presented a humoral profile compatible with a primary immunodeficiency disease (PID) (Figure 3A,B). Of the 81 patients, 5 displayed severe IgG hypogammaglobulinaemia (42–192 mg/dL) concomitantly with very high IgM levels (Figure 3D). Additionally, 2 of the 81 had selective IgA deficiency (<2 mg/dL), one of them with very high levels of IgE (Figure 3D).

Finally, of the 81 patients, 13 had IgM below NR but above the level for IgM deficiency (Figure 3A).

3.6 | Clinical history of infection influences the inflammatory profile of RDEB patients

To assess the impact of infection on the immune system, a multiparameter study was performed by correlating inflammatory and severity markers with ACI at recruitment and with a clinical history of infection (HCIC) and HHI (Figure 4).



χ^2 p=0.025		IgM ACI n=13	IgM nACI-S n=37	IgM Total n=50
<NR	n	5	4	9
	%	**39%**	11%	18%
NR	n	5	29	34
	%	39%	78%	70%
>NR	n	3	4	7
	%	**22%**	11%	12%

χ^2 p=0.0069		IgM HHI n=18	IgM nHHI-S n=32	IgM Total n=50
<NR	n	7	2	9
	%	**39%**	6%	18%
NR	n	8	27	35
	%	44%	77%	70%
>NR	n	3	3	6
	%	**17%**	9%	12%

χ^2 p=0.048		IgE HHI n=18	IgE nHHI-S n=32	IgE Total n=50
NR	n	6	20	26
	%	33%	63%	52%
>NR	n	12	12	24
	%	**67%**	37%	48%

FIGURE 4 Influence of clinical history of infection on the inflammatory profile of RDEB patients. (A, C, D) Distribution of individual numerical values of severity markers (BSA, CRP or EBDASI) and infectious history. Median and IQR are represented by bars. *p*-values <0.05 were considered statistically significant (Mann–Whitney *U*-test). (A) Comparison between the sub-cohort of patients from all EBDASI categories with active superficial cutaneous wound infection (ACI; $n = 14$) and the sub-cohort of patients with severe EBDASI category without ACI (nACI-S, $n = 32$). (C) Comparison between patients within the same EBDASI category with a history of cutaneous infection microbiologically confirmed (HCIC; moderate EBDASI $n = 11$, severe EBDASI $n = 42$) and without HCIC (moderate EBDASI $n = 11$, severe EBDASI $n = 7$). (D) Comparison between the sub-cohort of patients with clinical history of hospitalization for infection (HHI, all EBDASI categories, $n = 18$) and the sub-cohort of patients with severe EBDASI without HHI (nHHI-S, $n = 32$). (B, E) Contingency tables of prevalence of circulating IgM or IgE abnormalities among patients with or without ACI (B) and HHI (E). χ^2 = Pearson's chi-squared test.

At recruitment, of 84 patients, 14 (17%; 13 severe and 1 moderate) presented with typical signs of wound-related ACI without evidence of cellulitis. Among them, of the 14 patients, 12 reported frequent episodes of true or low-grade fever; 4 of the 14 patients showed leukocytosis (Cohort's WBCs data: Figure S3) and 12 of the 14 patients displayed increased platelets. However, PCT, an indicator of bacterial infection, was normal in all samples. Patients with ACI had higher EBDASI and BSA than patients without ACI within the severe category (non-ACI-S patients) (Figure 4A) but also a higher prevalence of low IgM (38% vs. 11%) (Figure 4B). In contrast, no significant differences were found in CRP, prevalence of IgA, IgG or IgE abnormalities, cytokine levels, or leukocytosis between infected and non-infected severe patients. These results suggest that the inflammatory profile in patients with RDEB did not reflect the presence of ACI but rather a higher severity status and a possible immunocompromise.

Of the 84 patients, 55 had HCIC (42/49 severe, 11/22 moderate and 2/11 mild), including all the patients with frequent episodes of fever (22/53). The comparison among patients with or without HCIC within the same EBDASI category revealed a positive impact of HCIC on CRP levels (Figure 4C). In contrast, no significant impact of HCIC on EBDASI scores, BSA or cytokine levels was found.

In addition, of the 84 patients, 19 (17 severe and 2 moderate) had an HHI, caused by sepsis or a severe infection refractory to oral antibiotics. In fact, 8 of the 19 patients had ACI at recruitment and 10 of 18 patients reported recurrent true or low-grade fever. Comparing patients with HHI (all EBDASI categories) to severely affected patients without HHI (nHHI-S), we found similar severity scores, but higher BSA and CRP levels in the HHI group (Figure 4D). Remarkably, patients with HHI showed significantly lower levels of IL17A and lower prevalence of increased IL17A levels (2/18 vs. 13/29, $p=0.024$) than severe patients that had never been hospitalized for infection. No differences in other cytokines were found.

Regarding Igs, patients with HHI (vs. nHHI-S) had higher prevalence of elevated IgE (67% vs. 37%) and abnormal levels of IgM ($p=0.0069$) (Figure 4E). Strikingly, 39% of patients with HHI had IgM below NR compared with 6% in the nHHI-S group, indicating a possible post-infection immunocompromise (Figure 4E). Over the 2 years following this study, six patients with severe EBDASI died, four of whom were among the HHI group and three with low IgM.

4 | DISCUSSION

RDEB is a rare orphan genodermatosis[1] in which inflammation combined with autoimmunity appears to substantially impact disease progression and outcome.[9,13–17,19–23] Thus, immunotherapy to dampen inflammation has entered the pharmacological repertoire of RDEB, but without enough success.[12] To comprehend inflammatory and immunological phenomena involved in RDEB, we have dissected the interrelationship between a broad range of inflammatory and severity markers. Moreover, valuable clues are provided to guide the choice of rationale biological treatment in RDEB.

Our results demonstrate that CRP is an excellent inflammatory biomarker in RDEB, and performs better than IL6/IL10 ratio.[14] In this cohort, high CRP was not predictive of ACI but was associated with disease severity. CRP is a bioactive molecule with both protective and negative effects and an innate immune mediator which also has prothrombotic and profibrotic effects and may contribute to tissue damage aggravation.[32,33] Having been proposed as a prognostic indicator and therapeutic target in other diseases,[34,35] it is conceivable that chronically elevated CRP participates in RDEB severity. A longitudinal study would be necessary to validate the value of CRP as a severity biomarker and to determine whether these higher levels of CRP are a consequence of the history of infection or have prognostic value. Understanding this relationship is crucial, as persistent high CRP levels might have a direct deleterious role, highlighting its importance beyond being a mere severity biomarker.

According to our findings, IL6, IL13 and IL4 could be potential immunotherapeutic targets in RDEB. The inhibition of these three pathways by targeting a single JAK protein (tofacitinib or baricitinib) may also hinder IFNs and EPO signalling, which increases the risk of infection and anaemia.[36] On the other hand, the proinflammatory cytokine IL6, in the apex of the cytokine network described here in RDEB, also functions as a protective molecule[37] critical for wound healing[38] and pathogen elimination.[39] In fact, monoclonal antibodies against IL6 receptor (e.g. tocilizumab) inhibit all IL6 potential effects, thus attenuating inflammation and polyclonal hypergammaglobulinaemia, but increasing the risk of infection.[40,41] Strategies targeting IL6 trans-signalling and trans-presentation (e.g. olamkicept) would preserve the classical protective signalling route, but conclusive data on contraindications are still lacking.[42]

The dysregulation of type 2 inflammatory response demonstrated in RDEB would contribute to epithelial barrier disruption,[43] fibrosis initiation/perpetuation[44] and itching[44,45] and would hinder the immunoprotective activation of IL17-dependent response.[46–48] Dupilumab or tralokinumab and other developing biological drugs like lebrikizumab, specifically target IL4 and IL13.[49] Dupilumab, currently prescribed for atopic dermatitis, reduces pruritus and inflammation while improving skin barrier[49,50] and cutaneous microbiome by reducing colonization and infection by *S. aureus*.[51] The good response without side effects in patients with DEB pruriginous treated with dupilimab[52–56] suggests the inhibition of IL4/IL13 as a therapeutic option for other RDEB subtypes. Reinforcing this possibility, targeting type 2 cytokines has recently been proposed to treat itching in RDEB, based on data obtained from a paediatric cohort.[19]

IL17 response plays a non-redundant role in mucosal immunity by participating in pathogen defence, enhancing barrier function[57–60] and positively affecting wound healing.[59,61] This protective role could be reflected in this cohort by the low IL17 levels in patients with HHI or high levels in patients able to resolve ACI or who were free of infection. Thus, although IL17 has been identified as a key pathogenic cytokine in many autoimmune and inflammatory diseases,[57–60,62] there is not a clear rationale to use the inhibition of this pathway in RDEB.

IL1β is crucial for the induction of IL17 secretion.[63] The rise in IL1β could also act in the bone marrow, promoting the development of trained innate immunity[64] and emergency myelopoiesis,[65] which are necessary to renew granulocytes recruited to skin. However, because of the sustained acceleration of HSC proliferation and differentiation into the myeloid lineage, acute and chronic elevation of IL1 might damage HCS,[66] thus affecting bone marrow function in RDEB.

Recurrent infections are one of the key factors influencing the inflammatory profile and exacerbating the clinical presentation in RDEB,[24–26,67–69] with *S. aureus* the most commonly isolated bacteria from wounds and in sepsis.[19,26,67,68,70] In patients or caregivers, colonization of the nose and the intestine, even asymptomatically, are potential reservoirs of the pathogen, which can also be mother-to-child transmitted.[71] More relevant is the neonatal transmission of methicillin resistant *S. aureus* (MRSA) strains in the intensive care unit,[72] as manifestation of RDEB may require neonatal hospitalization. Consequence of this pathogen's virulence mechanisms, conditions such as IgA nephropathology, chronic wounds, or itch in the context of epithelial barrier disfunction are prevalent in patients colonized or infected with *S. aureus*.[73–76] The induction of type 2 inflammatory responses and the increase of IgE secretion have also been linked to these bacteria.[73–76] These characteristics are shared with RDEB, which suggest that this pathogen could be a major immunological disruptor in this disease. The presence in RDEB patients of genetic susceptibility variants to *S. aureus*[77] may act as modulators and could impact in disease progression.

Polyclonal hypergammaglobulinaemia (IgA + IgG) was highly prevalent among RDEB patients since childhood, at a level comparable to that found in Sjögren syndrome or liver cirrhosis.[78,79] The remarkable increase of different Ig isotypes combined with those of other acute inflammatory reactants could lead to augmented plasma viscosity, reaching levels associated with the risk of microvascular pathology.[31,80] After this observational study, a 19-year-old patient died due to stroke (IgA = 1101 mg/dL; IgG = 3230 mg/dL; CRP = 143 mg/L). Chronic elevation of IL6, IL4 and IL13, and recurrent contact with pathogens related to epithelial barrier disruption, could support polyclonal B activation and hypergammaglobulinaemia in this RDEB cohort. Determining the specificity of this excess of Igs could help to clarify this issue. In RDEB patients, van der Kooi-Pol et al.[81] described high levels of IgG against a panel of *S. aureus* antigens, which could have a protective role.

The type 2 inflammatory profile is also related with the excess of IgE secretion, typically associated to allergy and atopy. The lack of correlation between IL-4/IL-13 and IgE levels in this cohort, may result from the complex regulation of IgE production, which involves multiple cytokines and immune pathways beyond IL4 and IL13[82] and could also be associated to a primary immunodeficiency disease (PID).[83] In some of the patients with elevated IgE or hyper-IgE, epithelial barrier damage or IL17 pathway disruption might be further extended, increasing susceptibility to *S aureus* recurrent infections.[84] In a pilot study, wound healing improved in RDEB patients treated with anti-IgE (omalizumab),[85] which points to a possible mechanistic role of IgE in disease activity. Immune response to bacteria is defective in *Col7a1*-deficient mice, which suggests PID by alterations in lymph node micro-circuits.[86] The prevalence of humoral alterations, suggestive of PID diagnosis,[87] particularly those affecting the IgG isotype, was strikingly high among RDEB patients and requires further investigation. Likewise, it would be of interest to serologically assess the level of immune protection against antigens in common vaccination programs or the early response to immunization in RDEB.

This is the first study performed in an EBDASI-stratified representative RDEB cohort in which the prognostic value of CRP on disease severity has been determined in relation with a type 2 inflammatory profile. This profile is likely triggered, at least in part, by skin colonization/infection consistent with *S. aureus*, a pathogen strongly related with wound burden and disease activity in RDEB.[69] Polyclonal hypergammaglobulinaemia described here, a condition not characteristic of skin autoimmune diseases,[78] should not be neglected. Our data also support the coexistence of humoral immunodeficiency in RDEB as an aggravating mechanism worthy of further exploration as patients could benefit from targeted therapeutic interventions.

Despite the limitations of cross-sectional analysis and the possible underrepresentation of milder RDEB forms, our study makes a significant contribution to the understanding of this devastating disease. It establishes a foundation for future research and emphasizes the importance of longitudinal studies to validate our findings across diverse RDEB populations.

AUTHOR CONTRIBUTIONS

Conceptualization, supervision and project administration: M.J.E., R.S.; Methodology: L.Q.-C., S.S.-R., R.M., N.I., M.J.E., R.S.; validation: L.Q.-C., S.S.-R., R.M., N.B., M.M.-L; formal analysis: L.Q.-C., S.S.-R., P.Z., M.R., A.V., M.J.E., R.S.; investigation: L.Q.-C., S.S.-R., R.M., N.I., I.P.-C., M.M.-L, N.B., E.G.A.-S., E.M.-M., L.M.-S., M.F.-A. S.G.L., A.M., R.L.; resources: L.Q.-C., S.S.-R., R.M., I.P.-C., M.M.-L, N.B., S.G.L., A.M., R.L.; data curation: L.Q.-C., S.S.-R., M.M.-L, N.B. P.Z., M.J.E., R.S.; visualization and writing—original draft preparation: L.Q.-C., M.J.E., R.S.; writing—review and editing: all authors; funding acquisition: R.L., A.M., M.R., A.V., M.J.E.

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CONFLICT OF INTEREST STATEMENT

SSR is the president of the Spanish Immunology Society and a member of the Medical Advisory Board of the International Patients Organisation for Primary Immunodeficiency.

DATA AVAILABILITY STATEMENT

Data supporting this study are available to qualified academic researchers upon a substantiated request that aligns with the objectives specified in the participants' consent agreements. Any data release will adhere to privacy protection methods, such as deidentification, consistent with legal requirements.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. Supplementary material.

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