Proinflammatory Cytokines and Antiskin Autoantibodies in Patients With Inherited Epidermolysis Bullosa

Giuseppina Annicchiarico, MD, Maria Grazia Morgese, PhD, Susanna Esposito, MD, Giuseppe Lopalco, MD, Michele Lattarulo, PhD, Marilina Tampoia, PhD, Domenico Bonamonte, MD, Luigia Brunetti, MD, Antonio Vitale, MD, Giovanni Lapadula, MD, Luca Cantarini, MD, and Florence Iannone, MD

Abstract: Epidermolysis bullosa (EB) is a rare disorder characterized by inherited skin adhesion defects with abnormal disruption of the epidermal–dermal junction in response to mechanical trauma. Our aim was to investigate a set of cytokine levels in serum samples from patients suffering from epidermolysis bullosa simplex (EBS), dystrophic epidermolysis bullosa (DEB), and healthy controls (HCs), exploring their potential correlations with antiskin autoantibody titers and disease activity. Forty patients afferent to the Dermatological Ward of Bari City Hospital and 9 HCs were enrolled and subdivided according to the dystrophic (DEB) and simplex forms (EBS). We found a significant increase in interleukin (IL)-1β plasmatic levels of DEB ($P = 0.0224$) and EBS ($P = 0.0465$) patients compared to HCs; IL-6 levels were significantly higher in DEB than in EBS patients ($P = 0.0004$) or HCs ($P = 0.0474$); IL-2 levels were significantly increased in DEB compared with EBS ($P = 0.0428$). Plasmatic tumor necrosis factor-β and interferon-γ were higher in DEB patients than in HCs ($P = 0.0448$ and 0.0229). Conversely, tumor necrosis factor-α was significantly decreased in DEB ($P = 0.0034$). IL-5 correlated with anti-BP180 ($r = -0.5018$, $P = 0.0338$), anti-BP230 ($r = -0.6097$, $P = 0.0122$), and anticollagen VII ($r = -0.5166$, $P = 0.0405$) autoantibodies; interferon-γ correlated with anti-BP180 ($r = 0.9633$, $P < 0.0001$), anti-BP230 ($r = -0.9071$, $P < 0.0001$), and anticollagen VII ($r = 0.8619$, $P = 0.0045$) autoantibodies. Score of disease severity was significantly correlated with IL-6 ($r = 0.6941$, $P = 0.029$) and IL-12 ($r = 0.5503$, $P = 0.0272$). The present study supports that EB might be considered a systemic inflammatory disease rather than a skin-limited disorder; clinical disease activity scores could be also integrated by laboratory data such as IL-6 and IL-12 dosage; biotherapies targeting specific cytokine networks probably represent a way to go in the future.

INTRODUCTION

Epidermolysis bullosa (EB) represents a rare group of severe inherited defects known as genodermatoses, and is characterized by abnormalities in skin adhesion. In particular, clinical phenotype is distinguished by an abnormal skin and mucosa fragility due to the disruption of epidermal–dermal junction in response to mechanical trauma, resulting in mutilating scars, local and systemic infections, syndactyly, and esophageal strictures. According to a recently introduced “onion skinning” approach to classification, EB can be distinguished taking into consideration the account type, the mode of inheritance, phenotype, immunofluorescence antigen-mapping findings, and mutations. Nevertheless, the authors did not change the previously used names for the major EB types to maintain continuity with past scientific literature and to prevent any confusion. In particular, EB types are identified according to the level in which blisters develop and also include the simplex form (EBS), with mechanical fragility and blistering confined to the epidermis; the junctional EB (JEB) consisting of all subtypes with blisters arising within the lamina lucida of the dermal–epidermal basement membrane; and the dystrophic form (DEB), including subtypes with blisters occurring within the uppermost dermis.

The simplex form derives mainly from mutations of K5 and K14 genes encoding for type I and II keratin intermediate filaments, whereas DEB derives from mutations in the COL7-A1 gene encoding for type VII collagen—the major component of anchoring fibrils at the dermal–epidermal junction. However, genotype–phenotype correlations are not always found and several different phenotypes are reported for DEB regardless of the genetic background. In this context, the Birmingham Epidermolysis Bullosa Severity (BEBS) score is currently employed to assess disease activity.
Antitype VII collagen and other antiskin autoantibodies (anti-BP180, anti-BP230) can be detected. Although their pathogenic role is unclear, their levels have proved to be significantly correlated with the BEBS score, thus suggesting that the phenotype severity may rely on autoimmune mechanisms.8–10 The potential occurrence of AA-amyloidosis and organ involvement,8–10 along with previous reported data on cytokine serum level imbalances,11–13 suggests that moderate to severe forms of EB should be regarded as systemic inflammatory disorders rather than local skin diseases.

Therefore, we investigated the levels of a core set of cytokines in serum samples from EBS and DEB patients, and healthy controls (HCs), exploring their potential correlations with antiskin autoantibody titers and disease activity.

MATERIALS AND METHODS

Patients

We collected serum samples from a cohort of 40 EB patients (15 men, 25 women) consecutively seen at the Dermatological Ward of Bari City Hospital and diagnosed by clinical assessment and immunofluorescence antigen mapping (IFM) and/or transmission electron microscopy. According to the cross-sectional design of the study, serum samples were collected once during routine follow-up visits. Patients were divided into 2 groups according to skin cleavage: intraepidermal blistering diseases (EBS) (15 patients, 7 men and 8 women, mean age 16 ± 7.3 years), and subepidermal blistering diseases (DEB) (25 patients, 8 men and 17 women, mean age 33 ± 6.3 years). Samples were also obtained from 9 HCs (1 man, 8 women, mean age 46.77 ± 5.23 years).

The primary aim of the study was to compare cytokine profiles of EBS patients, DEB patients, and HCs; the secondary aim was to investigate any potential correlation among cytokines levels, autoantibody titers, and clinical features. The BEBS score3 was used to perform clinical assessment of disease severity. Written informed consent was obtained both from patients and HCs. The study protocol was reviewed and approved by the Ethical Committee of the Medical University of Bari.

Cytokine Measurement

Serum cytokine levels of interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, along with TNF-α, TNF-β, and IFN-γ, were analyzed in serum samples from EB patients and 9 HCs. In both EBS and DEB patients, IL-1β serum levels were found significantly increased when compared to HCs (P = 0.0465 and 0.0224, respectively), whereas IL-6 levels were significantly higher in DEB than in EBS patients or HCs (P = 0.0044 and 0.0474, respectively). In addition, IL-2 levels were also significantly increased in DEB compared to EBS patients (P = 0.0428), whereas serum concentration of TNF-β (P = 0.0448) and IFN-γ (P = 0.0229) was significantly higher in DEB patients than in HCs. Conversely, TNF-α was significantly decreased in DEB patients (Figure 2C, P = 0.0034) than in HCs. For other cytokines, no difference was detected. Figure 1 graphically shows significant differences in cytokine serum levels between EBS and DEB patients, and HCs.

Clinical and Laboratory Correlations

The BEBS score, used to perform clinical assessment of disease severity, showed a positive correlation with IL-6 (r = 0.6941, P = 0.029) and IL-12 (r = 0.5503, P = 0.0272) serum levels. In addition, IL-5 serum levels inversely correlated with anti-BP180 (r = -0.5018, P = 0.0338), anti-BP230 (r = -0.6097, P = 0.0122), and anticollagen VII (r = -0.5166, P = 0.0405) autoantibody titers. Furthermore, IFN-γ levels were directly correlated with anti-BP180 (r = 0.9633, P < 0.0001), anti-BP230 (r = 0.9071, P < 0.0001), and anticollagen VII (r = 0.8619, P = 0.0045) autoantibody titers.

Finally, serum levels of IL-10 were found to be correlated with anticollagen VII (r = -0.48, P = 0.037) and with anti-BP230 titer (r = 0.45, P = 0.05). Table 1 provides more information about autoantibodies detected in the present study. Figure 2 shows the main correlations among cytokine serum levels, autoantibody titers, and BEBS score.

DISCUSSION

Our cross-sectional study was aimed at searching for a peculiar cytokine pattern in EB patients and exploring the potential associations among cytokine profiles, autoantibody serum levels, and disease activity, evaluated by the BEBS score. In particular, we found increased IL-1β serum levels in EBS and DEB patients, whereas IL-2 was higher in DEB than in EBS patients, and IL-6 was higher in DEB patients than in both EBS patients and HCs. In addition, TNF-β and IFN-γ levels were higher in patients affected by DEB, whereas TNF-α was significantly lower in such patients. Furthermore, IL-6 and IL-12 were positively correlated with the BEBS activity score, whereas IFN-γ and IL-5 were correlated with skin autoantibodies in a positive and negative manner, respectively.

These results strengthen that systemic involvement is hidden below mucocutaneous manifestations in EB patients.
Indeed, extracutaneous features previously reported in patients with a more severe phenotype suggested a potential multisystem involvement in such patients. In particular, IL-1 has been referred to be responsible for abnormal fat metabolism, cardiovascular complications, renal sclerosis, weight loss, and systemic amyloidosis in most severe EB cases. In addition, IL-1β signaling was found constitutively activated in keratinocytes from EBS patients, resulting in activation of the JNK stress pathway and overexpression of IL-1β in a positive feedback loop. Notably, our results support the role of IL-1β in inducing systemic involvement in EB patients.

According to previously reported data, we found high IL-6 serum levels in DEB patients. This cytokine, mainly produced by activated macrophages, monocytes, antigen-presenting cells, and lymphocytes, is capable of promoting Th17 cell and monocyte differentiation, and also inducing the production of acute-phase proteins, thus playing a key role in the switch from innate to acquired immunity. Noteworthy, IL-6 upregulation has been previously identified in DEB patients complaining of a more severe phenotype, thus supporting our findings showing a correlation of IL-1β with the BEBS score. Noteworthy, since BEBS score is not adjusted for patients’ age, IL-6 and IL-12 dosage could provide more details on disease severity, especially among children. However, since IL-6 levels were found significantly higher in DEB than in EBS patients, the role of IL-6 on disease activity evaluation could be more important in the former group. In addition, IL-6 has previously proved to be significantly upregulated at the mRNA level in skin obtained from K5−/− mouse models. Consequently, both the role of IL-6 in pathogenesis and the possible relation with disease activity in EBS patients should be investigated on a larger number of patients.

To date, in vitro production of IL-2 from in vitro peripheral blood mononuclear cells (PBMCs) has been previously reported to be very low in DEB patients, and a diminished number of IL-2 receptors were also found in patients with severe forms of EB. Noteworthy, in the present study, IL-2 was found to be significantly increased in DEB than in EBS patients, thus making necessary further studies to better understand the role of IL-2 in such patients. In any case, the different IL-2 expression in DEB and EBS patients and the higher IL-6 levels found in DEB than in EBS patients probably reflect diverse pathogenetic mechanisms supported by the different genetic defects.

Interestingly, we found decreased TNF-α serum levels in DEB patients compared to HCs. According to previous published data, mesenchymal stem cells preconditioning with TNF-α showed to improve transplantation functional utility in recessive DEB. In addition, collagen VII gene expression was reported to be increased by TNF-α through TGF-β signaling in HaCaT keratinocytes. In this regard, reduced TNF-α levels in EB patients could have a role in inducing a decreased or aberrant collagen VII production.

A significant increase in IFN-γ levels was also found in our DEB patients. In this context, IFN-γ proved to mightily activate the keratin K15 promoter in human epidermal keratinocytes, whereas IFN-γ blockers have been successfully proposed as possible pharmacological approach for DEB patients. Notably, in our DEB patients, IFN-γ positively correlated with anti-BP180, anti-BP230, and anticollagen VII autoantibody titers, pointing out its role in immunomodulation and regulation of adaptive immune response. Furthermore, collagen VII and BP230 autoantibody titers negatively correlated with IL-5, indicated as promoter of CD4+CD25+ T-regulatory cells that are involved in autoimmune suppression.

**FIGURE 1.** Significant differences in cytokine serum levels between patients with epidermolysis bullosa simplex (EBS), dystrophic epidermolysis bullosa (DEB), and healthy controls (HCs). Data (pg/mL) are expressed as means; since data were not homoscedastic, P values were obtained from Dunn multiple comparison test after Kruskal–Wallis test.
The altered cytokine levels highlighted in the present study could be the consequence of genetic defects and skin blistering rather than the keystone of etiopathogenetic mechanisms. Nevertheless, although further studies on larger populations are mandatory to better establish the real role for each cytokine, in the present study, IFN-γ and IL-5 were found related to antibody titers, thus suggesting their pathogenetic role in inducing deregulation of adaptive immune system in such patients. However, looking further into the future, our findings encourage the employment of biologic agents targeting specific cytokine networks. This alternative therapeutic approach could be useful to counteract both skin manifestations and systemic long-term complications such as AA-amyloidosis, thus avoiding life-threatening consequences and improving quality of life. Nevertheless, nonhealing blisters may ease infections in EB patients, and patients treated with biologic therapy are at an increased risk of developing infections. For these reasons, anti-IL-1 agents could represent an optimal opportunity because of their lower infectious risk than other biologics. Furthermore, wound healing might close potential opened doors to infections.

In conclusion, the imbalance of several cytokines highlighted in the present study confirms that EB is a systemic inflammatory disease rather than a skin-limited disorder. In addition, IL-6 and IL-12 dosage may represent a useful and not
invasively laboratory tool, in addition to the currently used severity score aimed at establishing disease activity. Whether or not some of these cytokines may be a potential target of new biotherapies needs further investigations. This might open new avenues in the treatment of a complex disease often leading to life-threatening complications.

REFERENCES

1. Tabolli S, Sampogna F, Di Pietro C, et al. Quality of life in patients with epidermolysis bullosa. Br J Dermatol. 2009;161:869–877.
2. Fine JD, Eady RA, Bauer EA, et al. The classification of inherited epidermolysis bullosa (EB): report of the Third International Consensus Meeting on Diagnosis and Classification of EB. J Am Acad Dermatol. 2008;58:931–950.
3. Bolling MC, Lemmink HH, Jansen GH, et al. Mutations in KRT5 and KRT14 cause epidermolysis bullosa simplex in 75% of the patients. Br J Dermatol. 2011;164:637–644.
4. Christiano AM, Ryynanen M, Uitto J. Dominant dystrophic epidermolysis bullosa: identification of a Gly–Ser substitution in the triple-helical domain of type VII collagen. Proc Natl Acad Sci USA. 1994;91:3549–3553.
5. Moss C, Wong A, Davies P. The Birmingham Epidermolysis Bullosa Severity score: development and validation. Br J Dermatol. 2009;160:1057–1065.
6. Tampoia M, Bonamonte D, Filoni A, et al. Prevalence of specific anti-skin autoantibodies in a cohort of patients with inherited epidermolysis bullosa. Orphanet J Rare Dis. 2013;8:132.
7. Esposito S, Guez S, Manzioni F, et al. Epidermolysis bullosa and the partnership with autoimmunity: what should we assimilate? Immunol Res. 2015;61:63–69.
8. Yamanaka K, Nakashita T, Saito H, et al. Persistent release of IL-1α from skin is associated with systemic cardiovascular disease, emaciation and systemic amyloidosis: the potential of anti-IL-1 therapy for systemic inflammatory diseases. PLoS One. 2014;9:e104479.
9. Chen CC, Isomoto H, Hayashi T. Gastrointestinal amyloidosis secondary to inherited skin disorder. Gastroenterol Res. 2012;4:9–e10.
10. Kaneko K, Kakuta M, Ohtomo Y, et al. Renal amyloidosis in recessive dystrophic epidermolysis bullosa. Dermatology. 2000;200:209–212.
11. Wally V, Lettner T, Peking P, et al. The pathogenic role of IL-1beta in severe epidermolysis bullosa simplex. J Invest Dermatol. 2013;133:1901–1903.
12. Kawakami Y, Oyama N, Ohtsuka M, et al. Increased serum levels of interleukin-6, immunoglobulin and acute phase protein in patients with the severe clinical form of inherited epidermolysis bullosa. J Dermatol. 2005;32:503–505.
13. Annicchiarico G, Morgese MG, Fiore T, et al. HLA typing in epidermolysis bullosa patients: relevance to gluten sensitivity. J Genet Syndr Gene Ther. 2013;4:182.
14. Fine JD, Hall M, Weiner M, et al. The risk of cardiomyopathy in inherited epidermolysis bullosa. Br J Dermatol. 2008;159:677–682.
15. Fine JD, Johnson LB, Weiner M, et al. Inherited epidermolysis bullosa and the risk of death from renal disease: experience of the National Epidermolysis Bullosa Registry. Am J Kidney Dis. 2004;44:651–660.
16. Annicchiarico G, Morgese MG, Brunetti L, et al. Improvement of renal function in epidermolysis bullosa patients after gluten free diet: two cases. Eur Rev Med Pharmacol Sci. 2012;6:138–141.
17. Samavedam UK, Kalies K, Scheller J, et al. Recombinant IL-6 treatment protects mice from organ specific autoimmune disease by IL-6 classical signalling-dependent IL-1ra induction. J Autoimmun. 2013;40:74–85.
18. Odorico T, Di Salvo M, Orcreekia A, et al. Monozygotic twins discordant for recessive dystrophic epidermolysis bullosa phenotype highlight the role of TGF-beta signalling in modifying disease severity. Hum Mol Genet. 2014;23:3907–3922.
19. Lu H, Chen J, Planko L, et al. Induction of inflammatory cytokines by a keratin mutation and their repression by a small molecule in a mouse model for EBS. J Invest Dermatol. 2007;127:2781–2789.
20. Chopra V, Tyring SK, Johnson L, et al. Patients with severe forms of inherited epidermolysis bullosa exhibit decreased lymphokine and monokine production. J Clin Immunol. 1990;10:321–329.
21. Chopra V, Tyring SK, Johnson L, et al. Peripheral blood mononuclear cell subsets in patients with severe inherited forms of epidermolysis bullosa. Arch Dermatol. 1992;128:201–209.
22. Perdoni C, McGrath JA, Tolar J. Preconditioning of mesenchymal stem cells for improved transplantation efficacy in recessive dystrophic epidermolysis bullosa. Stem Cell Res Ther. 2014;5:121.
23. Takeda H, Kon A, Ito N, et al. Keratinocyte-specific modulation of type VII collagen gene expression by pro-inflammatory cytokines (tumor necrosis factor-alpha and interleukin-1beta). Exp Dermatol. 2005;14:289–294.
24. Radoja N, Stojadinovic O, Waseem A, et al. Thyroid hormones and gamma interferon specifically increase K15 keratin gene transcription. Mol Cell Biol. 2004;24:3168–3179.
25. Skurkovich B, Skurkovich S. Autoimmune diseases are connected with disturbances in cytokine synthesis, and therapy with IFN-gamma blockers is their main pathogenetic treatment. Ann N Y Acad Sci. 2007;1109:167–177.
26. Tran GT, Hodgkinson SJ, Carter NM, et al. IL-5 promotes induction of antigen-specific CD4+CD25+ T regulatory cells that suppress autoimmunity. Blood. 2012;119:4441–4450.
27. Cantarini L, Lopalo G, Caso F, et al. Effectiveness and tuberculosis-related safety profile of interleukin-1 blocking agents in the management of Behcet’s disease. Autoimmun Rev. 2015;14:1–9.