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ASSOCIATION OF IL-6 IN VESICULOBULLOUS LESIONS OF SKIN

Pathology

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ABSTRACT

Vesiculobullous lesions are autoimmune disorders characterised by autoantibodies formation directed against antigens of the intercellular substance. Autoreactive T cells produce cytokines that play a crucial role in the pathogenesis. IL-6 is a cytokine whose role in inflammatory lesions is well established, however its role in vesiculobullous lesion is not fully understood. The serum collected from thirty patients suffering from active vesicullobullous lesions was separated and levels were measured by chemiluminescence. The difference in serum levels of IL-6 were statistically significant between cases and controls (p value ≤ 0.0001). It was also observed that levels of serum IL-6 increased as we turned from non pemphigoid lesions towards pemphigoid lesions.

KEYWORDS

Pemphigus Vulgaris, Bullous Pemphigoid, Dermatitis Herpetiformis, Interleukin-6.

INTRODUCTION

Vesiculobullous lesions are autoimmune disorders characterised by autoantibodies formation directed against antigens of the intercellular substance.[1] Autoreactive T cells produce cytokines that play a crucial role in the pathogenesis. IL-6 is a cytokine whose role in inflammatory lesions is well established, however its role in vesiculobullous lesion is not fully understood. Very few studies have been documented regarding the role of different cytokines in the pathogenesis of vesiculobullous lesions and even fewer studies have been conducted which have pointed out the role of IL-6 in the pathogenesis of bullae formation. Thus this study was conducted to see if IL-6 is associated and plays a role in pathogenesis of vesiculobullous lesions.

MATERIAL AND METHODS

It was a case-control study which comprised of 30 patients who were diagnosed with vesiculobullous lesions of skin. The study was approved by institutional ethics committee. Informed consent of every patient was taken**.** Three ml of blood was collected from patients suffering from active vesiculobullous lesions who were not on treatment since preceding 3 months. A biopsy was taken from lesional and perilesional skin and sent for H&E and direct immunofluores cence studies. Thirty blood samples from age and sex matched healthy blood donors were also collected from the blood bank as controls. The blood samples were centrifuged at 2500 rpm for 10 minutes and the serum was separated and stored at – 20^{0}C. The levels of IL-6 were estimated in the serum samples by chemiluminescence using cobas e 411 analyser (ROCHE DIAGNOSTICS). The statistical details were calculated with the help of Mann Whitney Test. p value ≤.05 was considered significant.

PROCEDURE

- Blood was collected using standard sampling tubes.
- Test was done via Sandwich principle taking 18 minutes.
- 1st incubation: 200 microlitre of sample, a biotinylated monoclonal IL-6 specific antibody and a monoclonal IL-6 specific antibody labelled with a Ruthenium complex was incubated at 37^{0}C for 9 minutes. A sandwich complex was formed in which IL-6 acted as an antigen combined with the antibodies on both sides.
- 2nd Incubation: This sandwich complex was also incubated at 37^{0}C for 9 min after addition of streptavidin-coated microparticles. The complex bounded to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture was aspirated into the measuring cell (28^{0}C) where the microparticles were magnetically captured onto the surface of the electrode. Unbound substances were then removed with ProCell.
- Application of a voltage to the platinum electrode then induced chemiluminescent emission which was measured by a photomultiplier. {ECL is based on the use of a ruthenium (II) tris (bipyridyl) [Ru(bpy)^3]$^{2+}$ complex and tripropylamine (TPA)}.
- Results were determined via calibration curve.

RESULTS

Out of the 30 cases 21 were males and 9 females. The mean serum IL-6 levels were 16.99± 10.06 pg/ml with a range of 2.39-44.30 pg/ml. The distribution of cases varied from pemphigus vulgaris (n=17) to dermatitis herpetiformis (n=7) and (n=6) cases of bullous pemphigoid.

The mean serum level of IL-6 in 30 cases and controls were 16.99 ± 10.06 and 3.25±1.60 pg/ml respectively (Table I). The difference was found to be highly significant (Mann Whitney test; p-value ≤ 0.0001). Serum IL-6 levels in pemphigus vulgaris, bullous pemphigoid and dermatitis herpetiformis were 18.90±11.07 pg/ml, 14.58±7.56 pg/ml and 12.73±6.10 pg/ml respectively (Table II).

Table I. Comparison of serum IL-6 levels between cases and controls

Group	N	Range (pg/ml)	Mean (pg/ml)	p- value
Cases	30	2.39-44.30	16.99 ± 10.06	**≤0.0001**
Control	30	0.30-6.25	3.25 ± 1.60	

Table II. Serum levels of IL-6 in vesiculobullous lesions

Diagnosis	Number of cases	Mean ± SD	Range
Pemphigus vulgaris	17	18.90±11.07 pg/ml	3.85-44.30 pg/ml
Bullous pemphigoid	6	14.58±7.56 pg/ml	5.67-29.73 pg/ml
Dermatitis herpetiformis	7	12.73±6.10 pg/ml	2.39-20.93pg/ml
Total	30	16.99± 10.06 pg/ml	2.39-44.30 pg/ml

Serum IL-6 levels of the three vesiculobullous lesions were plotted on a graph (Figure1) which revealed IL-6 to be more in pemphigoid lesions (pemphigus vulgaris and bullous pemphigoid) a compared to nonpemphigoid lesions (dermatitis herpetiformis). Infact dermatitis herpetiformis had the lowest, bullous pemphigoid the intermediate and pemphigus had the highest level of IL-6. Thus the level of IL-6 increased as the vesiculobullous lesion became prognostically poorer.

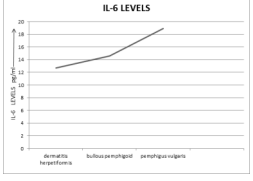

Figure 1. Levels of IL-6 in vesiculobullous lesions of skin

All seventeen cases of pemphigus vulgaris were IgG positive while seven of them were C3 positive too (41.7%) (Table III). Fourteen of these cases, including the 7 cases with both C3 and IgG positivity, had increased interleukin -6 levels. Similarly all cases of bullous pemphigoid were C3 positive. However, three of them showed dual positivity for IgG and C3. Serum IL-6 levels were raised in five (83.3%) of these cases which included the three cases with dual IgG and C3 positivity. All the 7 cases of dermatitis herpetiformis showed only IgA positivity. Five of these had increased levels of IL-6 (71.4%).

TABLE III- Serum IL-6, C3 and Immunoglobulin status in vesiculobzullous lesions.

Vesiculobullous lesions	Interleukin-6 levels (pg/ml)	Immunoglobulin status			C3 status
Pemphigus vulgaris	9.08	IgG+	IgA -	IgM-	C3-
Pemphigus vulgaris	14.83	IgG+	IgA-	IgM-	C3+
Pemphigus vulgaris	11.71	IgG+	IgA-	IgM-	C3-
Pemphigus vulgaris	44.3	IgG+	IgA-	IgM-	C3+
Pemphigus vulgaris	4.9	IgG+	IgA-	IgM-	C3-
Pemphigus vulgaris	7.34	IgG+	IgA-	IgM-	C3-
Pemphigus vulgaris	30.74	IgG+	IgA-	IgM-	C3+
Pemphigus vulgaris	22.27	IgG+	IgA-	IgM-	C3+
Pemphigus vulgaris	3.85	IgG+	IgA-	IgM-	C3-
Pemphigus vulgaris	27.4	IgG+	IgA-	IgM-	C3-
Pemphigus vulgaris	28.41	IgG+	IgA-	IgM-	C3+
Pemphigus vulgaris	35.4	IgG+	IgA-	IgM-	C3+
Pemphigus vulgaris	24.3	IgG+	IgA-	IgM-	C3-
Pemphigus vulgaris	5.5	IgG+	IgA-	IgM-	C3-
Pemphigus vulgaris	12.6	IgG+	IgA-	IgM-	C3-
Pemphigus vulgaris	21.39	IgG+	IgA-	IgM-	C3+
Pemphigus vulgaris	17.42	IgG+	IgA-	IgM-	C3-
Bullous pemphigoid	18.38	IgG+	IgA-	IgM-	C3+
Bullous pemphigoid	14.34	IgG-	IgA-	IgM-	C3+
Bullous pemphigoid	23.81	IgG+	IgA-	IgM-	C3+
Bullous pemphigoid	5.67	IgG-	IgA-	IgM-	C3+
Bullous pemphigoid	7.23	IgG-	IgA-	IgM-	C3+
Bullous pemphigoid	29.73	IgG+	IgA-	IgM-	C3+
Dermatitis herpetiformis	5.43	IgG-	IgA+	IgM-	C3-
Dermatitis herpetiformis	16.98	IgG-	IgA+	IgM-	C3-
Dermatitis herpetiformis	13.5	IgG-	IgA+	IgM-	C3-
Dermatitis herpetiformis	20.93	IgG-	IgA+	IgM-	C3-
Dermatitis herpetiformis	19.54	IgG-	IgA+	IgM-	C3-
Dermatitis herpetiformis	10.4	IgG-	IgA+	IgM-	C3-
Dermatitis herpetiformis	2.39	IgG-	IgA+	IgM-	C3-

DISCUSSION

A number of interleukins have been implicated in the genesis of vesiculobullous lesions such as IL-3, IL-4, IL-5, IL-6, IL-10, IL-13.[2] Out of this the role of IL-6 appears to be least investigated. Though its role in inflammation is well established its role in vesicle or bullous formation remains illusive as inflammation alone cannot explain bullae formation.

IL-6 is a pleiotropic cytokine that is synthesised by a wide variety of cells such as T cells and macrophages.[3] Upon activation by antigen-presenting cells, naïve T-helper precursors can differentiate into T_H1, T_H2, or T_H17 and regulatory T (T_{reg}) cells (Figure 2).[1] In the presence of IL-4, naive T cells commence differentiation into the TH2 subset which produce multiple cytokines, such as IL-3, IL-5, IL-6, IL-10, IL-13, and even IL-4. These provide positive feedback and promotes TH2 differentiation, up-regulating its own production.[1] This group of cytokines then activates B cells and induces differentiation into Dsg3specific autoreactive plasma cells.[1] The activated plasma cells produce immunoglobulins mainly IgG in pemphigus vulgaris and IgA in dermatitis herpetiformis. The immunoglobulin gets attached to the antigen (desmosome) resulting in acantholysis and blister formation[1] as was seen in our cases. We also found that all the cases of pemphigus vulgaris only expressed IgG and IgA and IgM were absent. This probably is because the B cells which produce antibodies synthesize an immunoglobulin specific to only one epitope which is the part of the antigen which binds with the antibody. The recognized epitope determines where on the protein the antibody binds. Dsg 3 is the predominant antigen against which antibodies are directed in pemphigus vulgaris. Studies have been conducted on mice to locate the exact epitope which produces IgG antibodies and it was shown that antibodies were dominantly raised against the middle to C-terminal extracellular domains of mouse Dsg3 where amino acid sequences are less conserved among desmoglein isoforms and that these antibodies may also be involved in the blister formation.[4]

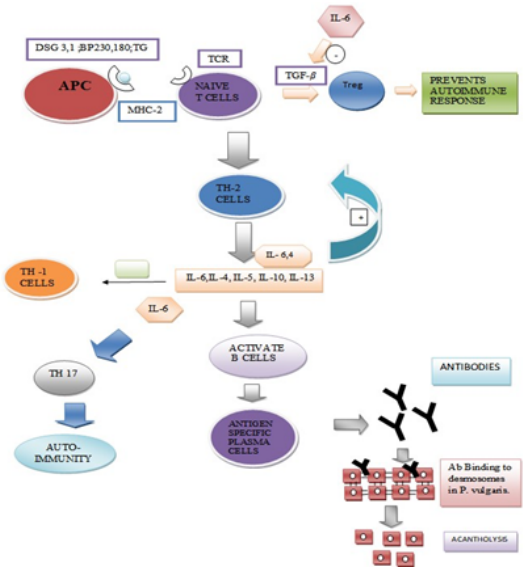

Figure 2 – Pathway depicting activation of interleukin along with interleukin 6 causing acantholysis and blister formation

Similarly in case of bullous pemphigoid BP180 and BP 230 are the predominant antigens against which antibodies are directed.[5] In bullous pemphigoid the immunodominant epitope of BP180 is NC16A, which consists of five subdomains, MCW-0 to MCW-4 (or NC16A subdomain 1 to 5). In bullous pemphigoid three regions (NC16A subdomain 1 to 3) on NC16A are targeted which produce IgG antibodies.[6] For BP230, other major autoantigen in bullous pemphigoid, epitope mapping demonstrated multiple antigenic reactive sites located predominantly within the B and C subdomains of the COOH-terminus of BP230.[7] In dermatitis herpetiformis the epitope on transglutaminase 3 antigen stimulates B cells to produce only IgA antibodies[8] and in all our cases these were negative for IgG and C3 and only expressed IgA.

Even C3 was predominantly expressed in pemphigoid lesions (pemphigus vulgaris and bullous pemphigoid) as compared to non pemphigoid lesions. Complement activation has been documented in both pemphigoid and non pemphigoid lesions.[9] It has been reported that in cases of pemphigoid lesions activation of complement occurs through both classical and alternate pathway while that of non pemphigoid by alternate pathway. In cases of pemphigus vulgaris we found C3 deposition in 41.7% of the cases as compared to bullous pemphigoid in which it was positive in all the cases. This can be due to the fact that in pemphigus vulgaris there may be a predominant activation of classical pathway of complement in which IgG antibodies activate the complement system. In bullous pemphigoid in addition to classical pathway alternate pathway may play a predominant role in the pathogenesis which explains why C3 expression was observed in 100% of the cases. It was also observed that where C3 was expressed along with IgG for example in cases of pemphigus vulgaris and bullous pemphigoid, serum interleukin -6 levels was much higher. This probably is because in cases where complement is expressed along with antibody the degree of inflammation is more severe thus causing increased interleukin -6 levels. Expression of C1q and C4 along with C3 has been well documented in cases of both pemphigus vulgaris and bullous pemphigoid while mainly C3 expression has been reported in dermatitis herpetiformis.[9] In our study, however C3 expression was

absent in dermatitis herpetiformis probably reflecting a milder and a prognostically better lesion. Perhaps there was no activation of the complement system and thus C3 expression was absent in our study group.

We also observed that as the lesion progressed from non pemphigoid to pemphigoid lesions the levels of serum IL-6 increased. Thus IL-6 probably is some way related to the severity of the disease. Dermatitis herpetiformis has a waxing and waning course.[10] Recent studies have suggested more than 40 % mortality in one year even in treated cases of bullous pemhigoid.[11] However the most severe lesion is pemphigus vulgaris where mortality can reach up to 90% if untreated.[12] This probably is reflected in higher IL-6 levels in pemphigus vulgaris compared to bullous pemphigoid. This raised production of interleukin -6 results in the formation of more antibodies and thus more severe autoimmune reaction. The increased production of IL-6 then activate B Cells to produce antibodies which may inturn bind or activate the complement system and thus cause more severe auto immune reaction in pemphigoid lesions as compared to non pemphigoid lesions.

IL-6 has also been shown to skew T cell differentiation towards T_H2 and T_H17.[1] It has been shown recently that IL-6 can strongly inhibit the TGF β-mediated differentiation of naïve $CD4^+$ T-cells into regulatory T-cells (T_{reg}), which inhibit autoimmunity and protect against tissue injury. On the other hand, the combination of TGFβ and IL-6 induced the formation of IL-17 secreting T_H17 cells, a subset of T helper cells, distinct from T_H1 and T_H2 cells that are implicated in the induction of autoimmune diseases[13,14] but the pathway is largely unknown. IL-6 also promotes B cell helper capabilities of $CD4^+$ T cells through increased IL-21 production.[15]

CONCLUSION

Thus we think that IL-6 may play a central role in the pathogenesis of vesiculobullous lesions and anti IL-6 may be an important therapeutic modality. It may even serve as an alternative to steroid therapy.

DECLARATION OF INTEREST

The author does not have any conflict of interest nor did he receive any funds for the study.

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