Cell-based immunofluorescence test applying recombinant laminin 332 for the serological diagnosis of pemphigoid

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Introduction

A rare subgroup of patients with mucous membrane pemphigoid (MMP) exhibits autoantibodies against laminin 332 (Lam332), a glycoprotein important for the structural integrity of the dermal-epidermal junction in skin (1, 2). Lam332 is a heterotrimeric protein consisting of 3 laminin chains, α 3, β 3, and γ 2. Diagnosis of anti-Lam332 MMP is important because nearly 30% of these patients develop solid cancers (3). However, no commercial diagnostic assay for the detection of anti-Lam332 autoantibodies is widely available yet. In this study, a cell-based immunofluorescence (IF) test based on the eukaryotic expression of recombinant Lam332 (heterotrimer and single chains) was developed and validated applying patients sera.

Results

• Generation and expression of recombinant laminin α 3, β 3 and γ 2

study (numbers represent amino acids). (C) After 48h, lysates of HEK 293 cells expressing the single α 3, β 3 and γ 2 chain, were separated by SDS-PAGE and protein expression was visualized by immunoblotting using an anti-His antibody.

• Detection of anti-Lam332 autoantibodies by Western blot using recombinant full length proteins of Lam332

Reactivities	Extracellular matrix (ECM)			Recombinant laminin 332 FL proteins (HEK293 cell lysates)			
	α 3	β 3	γ 2	α 3	β 3	γ 2	pTriEx
Anti-Lam332 MMP sera	(22/24) 92%	(7/24) 29%	(3/24) 13%	(22/24) 92%	(5/24) 21%	(4/24) 17%	(0/24) 0%
NHS	(0/20) 0%	(0/20) 0%	(0/20) 0%	(0/20) 0%	(0/20) 0%	(0/20) 0%	(0/20) 0%

Correlation between ECM and recombinant proteins

α 3: 22 ECM-positive sera
22 recombinant α 3-positive sera (100%)

β 3: 7 ECM-positive sera
5 recombinant β 3-positive sera (57%)

γ 2: 3 ECM-positive sera
4 recombinant γ 2-positive sera (higher sensitivity)

• Detection of anti-Lam332 IgG4 autoantibodies using a cell-based immunofluorescence (IF) test applying recombinant Lam332 as a substrate

IgG4 reactivity of sera with Lam332 using the novel IF test						
	α 3	β 3	γ 2	α 3 β 3 γ 2	His- α 3 β 3 γ 2	Empty plasmid
Anti-Lam332 MMP sera	40/93	38/93	12/93	72/93	72/93	0/93
Sensitivity %	43	41	13	77	77	
Anti-Lam332 MMP sera (α 3 + β 3)	(65/93) 70%					
Anti-Lam332 MMP sera (α 3 + γ 2)	(47/93)					
Anti-Lam332 MMP sera (β 3 + γ 2)	(44/93) 47%					
Anti-Lam332 MMP sera (α 3 + β 3 + γ 2)	(71/93) 76%					
Anti-Lam332 MMP sera α 3 + β 3 + γ 2 + α 3 β 3 γ 2	(72/93) 77%					
Salt-split skin (SSS) positive anti-Lam332 MMP sera	39/80	37/80	11/80	70/80	70/80	0/80
Sensitivity %	49	46	14	88	88	0
SSS positive anti-Lam332 MMP sera (α 3 + β 3)	(63/80) 79%					
SSS positive anti-Lam332 MMP sera (α 3 + γ 2)	(45/80)					
SSS positive anti-Lam332 MMP sera (β 3 + γ 2)	(44/80) 55%					
Salt-split skin positive anti-Lam332 MMP sera (α 3 + β 3 + γ 2)	(69/80) 86%					
Salt-split skin positive anti-Lam332 MMP sera α 3 + β 3 + γ 2 + α 3 β 3 γ 2	(70/80) 88%					

Using the novel IF-based assay, 93 anti-Lam332 positive MMP were tested. As controls sera from patients with non-anti-Lam332 MMP (n=153), with bullous pemphigoid (n=20), pemphigus vulgaris (n=20), non-inflammatory dermatoses (n=22), and from blood donors (n=100) were included. The test revealed a **specificity of 100%**.

Figure 2: Representative pictures of immunofluorescence microscopy using membrane-bound recombinant laminin α 3, β 3, γ 2 and the co-expressed chains (α 3 β 3 γ 2) with and without His-tag. Blue box, anti-Lam332 MMP serum with α 3 reactivity; green box, anti-Lam332 MMP serum with β 3 reactivity; red box, anti-Lam332 MMP serum with γ 2 reactivity; yellow box, NHS, normal human serum

• Inclusion of anti-Lam332 MMP patients with other IgG subclasses

		α 3	β 3	γ 2	α 3 β 3 γ 2	His- α 3 β 3 γ 2	Empty plasmid
Anti-Lam332 MMP sera	IgG4	35/80	32/80	10/80	58/80	58/80	0/80
Sensitivity in %		44%	40%	13%	73%	73%	0%
Anti-Lam332 MMP sera	IgG/IgG4	39/80	30/80	9/80	64/80	64/80	0/80
Sensitivity in %		49%	38%	11%	80%	80%	0%

Using an IgG/IgG4 conjugate (Euroimmun), the IF test revealed a **specificity of 99,6%**. As controls sera from patients with non-anti-Lam332 MMP (n=10), with bullous pemphigoid (n=20), pemphigus vulgaris (n=20), anti-p200 pemphigoid (n=20), epidermolysis bullosa aquisita (n=20) and from blood donors (n=50) were included.

• Autoantibody titers correlate with disease activity

Figure 3: Statistical correlation of disease activity and circulating autoantibody levels in 5 anti-Lam332 patients sera using the new IF test. Sera were analyzed during the course of the disease. The scoring of disease activity was done retrospectively based on patient records. Score of 4, >10 lesions; score of 3, 4 to 10 lesions; score of 2, 1 to 3 lesions; score of 1, clinical remission. Spearman test was used for the calculation of the correlation.

Conclusion

The novel IF-based assay with recombinant Lam332 as a substrate will facilitate the serological diagnosis of anti-Lam332 MMP.

References

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