Summary of "*Keratinocyte-derived S100A9 modulates neutrophil infiltration and affects psoriasis-like skin and joint disease*"

(Mellor LF, Gago-Lopez N, Bakiri L, et al. Ann Rheum Dis 2022;**81**:1400–1408.)

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Context

Psoriasis (Ps) is an autoimmune disease that causes increased production of keratinocytes, possibly through the interplay between immune pathways in the skin. The disease usually manifests as red or purple scaley patches on the skin that can be itchy. There are several subtypes of Ps with a wide range of disease severity.

Psoriatic arthritis (PsA) is a related disease where some of the same immune pathways affect joints, particularly in the hands and feet, causing inflammation. Approximately 30% of Ps patients develop PsA. The identification of a biomarker for PsA could improve patient outcomes as early treatment is critical to controlling the disease.

S100A8 and S100A9 are small proteins with calcium binding sites that are involved in the innate immune system. These proteins are highly expressed in Ps patients and are a focal point for further elucidation of disease pathways and biomarkers. S100A9 is primarily expressed by keratinocytes, neutrophils, and macrophages when skin is inflamed. The authors have previously reported that global inactivation of S100A9 in a Ps mouse model (DKO*) decreased skin and joint inflammation.

The study summarized herein focused on the role of S100A9 in keratinocytes and its role in immunological pathways in the epidermis. Some data are provided suggesting S100A9 may be used as a marker for PsA in patients with Ps.

Key Finding

The role of epidermal S100A9 in psoriatic disease appears to differ from circulating S100A9; while globally inactivating S100A9 alleviated disease (previous study), inactivating only epidermal S100A9 worsened disease. S100A9 was also identified as a possible biomarker for PsA in patients with Ps.

Important Concepts

Information about the cell types important to understanding the study are described in Table 1.

Table 1 Cell Types Investigated in Study

Cell	Description	Role in Ps or PsA
Keratinocyte	Most abundant cell type in epidermis	Hyperproliferation causes skin lesions Attract neutrophils
Neutrophil	Part of innate immune system Makes up white blood cell count	Secretes cytokines related to Ps Found in psoriatic skin lesions

As part of the study, proteins associated with Ps and immune responses were measured in psoriatic lesions, sera, lysates, and synovial fluid. Table 2 summarizes these proteins.

Table 2 Proteins of Interest

Molecule	Function	Comments
IL-1b	Mediates inflammatory response	An increase in any of these molecules indicates an inflammatory response
IL-6	Stimulates production of neutrophils	
IL-17	Activates proinflammatory responses	
TNFα	Mediates immune response	
Ly6B	Regulatory molecule found in neutrophils	
Osm	Cytokine in IL-6 family	
S100A8 S100A9	Proteins with calcium-binding sites	Expressed in Ps patients
Calprotectin	Heterodimer of S100A8 and S100A9	Expressed in skin inflammation

Table 3 summarizes the mouse models and analytical methods used in the study.

Table 3 Methods and Models Used for the Study

Method	Use
DKO* mouse model	Established model for Ps and PsA
TKO* mouse model	S100A9 floxed mice crossed with DKO* mice to create TKO* mice
Immunofluorescence and immunohistochemistry	Analysis of mouse ears to confirm mouse models Analysis of neutrophil infiltration in mouse ears and paws Analysis of S100A8 and S100A9 in human skin
Histology	Analysis of psoriasis-like disease in mice
Quantitative RT-PCR	Analysis of gene expression in neutrophils and keratinocytes of mice
FACS analysis	Analysis of mouse cells for cytokine expression
Mass spectrometry	Proteomics of mouse ear lysates
ELISA	Human sera analysis for markers of Ps or PsA

Summary

The authors first confirmed, via immunofluorescence of mouse ear sections, that the relevant expression profiles in each mouse model met the expectations of the genotype. DKO* mice readily expressed S100A9. Dermal expression of S100A9 decreased in the TKO* mice, which had the *s100a9* gene knocked out in the skin. However, expression remained in the dermis-infiltrating immune cells.

The severity of the disease in the mice (Ps and PsA) was assessed by scoring visual observations of lesions and arthritis, measuring weight, and measuring serum levels of indicators (eg, S100A9, IL-17A). Interestingly, the TKO* mice had the most severe disease despite a decrease in S100A9 in the epidermis.

Serum levels of S100A9, S100A8, and calprotectin (CP) were similar between DKO* mice and TKO* mice with moderate to severe disease, and both groups had higher levels than wildtype (WT). Serum levels of psoriasis-indicating cytokines were higher in both groups than wildtype,

but only TNFα differed between the two test groups and was higher in TKO* mice with moderate to severe disease. Table 4 illustrates the relative results obtained from sera analyses as compared to the WT.

Table 4 Concentration in Sera of Mice with Moderate to Severe Disease

Mouse Model	S100A9	S100A8	CP	IL-17	IL-6	TNFα
DKO*	inc	inc	-	inc	inc	inc
TKO*	inc	inc	-	inc	inc	inc+

"inc" indicates increase in levels over WT
"inc+" indicates increase in levels over WT and other mouse model
"-" indicates no difference in levels from WT

The level of neutrophil infiltration found in whole ear sections was quantified, as well as levels of neutrophils expressing S100A9. In DKO* and TKO* mice, the number of neutrophils and the percentage that were S100A9+ were greater than those in the WT. TKO* mice also had levels higher than DKO* mice, indicating the absence of dermal S100A9 may allow more inflammation.

Mass spectrometry analysis showed a significant increase in proteins associated with neutrophil activation in the TKO* versus WT mice and in the TKO* versus DKO* mice, further supporting dermal S100A9's role in regulating inflammation.

FACS-sorted neutrophils and keratinocytes were analyzed for cytokine and chemokine expression. Similar amounts of *s100a9* and *s100a8* mRNA were observed in neutrophils. Levels of *il-1b*, *il-6*, and *tnfα* mRNA were increased in neutrophils of DKO* and TKO* mice when compared to the WT. mRNA expression of *osm* was increased in neutrophils isolated from TKO* mice when compared to the WT (Table 5).

In keratinocytes, levels of *s100a9* mRNA were highest in DKO* mice. In fact, DKO* mice also had levels of *s100a8*, *il-1b*, *il-6*, and *osm* greater than the WT. TKO* expression differed somewhat, with *s100a8*, *il-1b*, and *tnfα* levels greater than the WT (Table 5). Dermal S100A9 may therefore modulate TNFα production.

Table 5 Levels of Inflammation-related mRNA in Neutrophils and Keratinocytes

			Neutrophils			
Mouse Model	**s100a9**	**s100a8**	**il-1b**	**il-6**	**tnfα**	**osm**
DKO*	inc	inc	-	-	-	-
TKO*	inc	inc	-	-	inc	inc
			Keratinocytes			
DKO*	inc+	inc	inc	inc	-	inc
TKO*	inc	inc	inc	-	inc	-

"inc" indicates increase in levels over WT
"inc+" indicates increase in levels over WT and other mouse model
"-" indicates no difference in levels from WT

The expression of S100A9 and S100A8 was evaluated in lesional skin of human patients with Ps and in the serum and synovial fluid in patients with Ps, PsA, and healthy controls (HC). As expected, S100A8 and S100A9 in skin lesions were elevated compared to HC.

Serum levels of inflammation markers were compared between HC, Ps patients, and PsA patients. Table 6 shows how serum levels compared to the HC. There were no significant differences found in S100A8 levels between the three groups. Levels of all other markers increased in PsA patients compared to HC; however, only levels of S100A9 and CP could distinguish between HC, Ps patients, and PsA patients.

Table 6 Serum Levels in Ps and PsA Patients Relative to HC

	S100A9	**S100A8**	**CP**	**Il-17**	**Tnfα**	**IL-6**
Ps patients	inc	-	inc	inc	-	-
PsA patients	inc+	-	inc+	inc	inc	inc

"inc" indicates increase in levels over HC
"inc+" indicates increase in levels over HC and other mouse model
"-" indicates no difference in levels from HC