biomarkers of PsA in PsC patients, most notably *NOTCH2NL*, *HAT1*, *CXCL10*, and *SETD2*, which were associated with PsA irrespective of clinical differences between patients. Because of the small sample size and limited power of the present study, further validation of these preliminary findings at the RNA and protein levels in larger cohorts of PsA and PsC patients is necessary.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Transglutaminase 3 Present in the IgA Aggregates in Dermatitis Herpetiformis Skin Is Enzymatically Active and Binds Soluble Fibrinogen

TO THE EDITOR

Dermatitis herpetiformis (DH), a cutaneous manifestation of celiac disease, is characterized by the deposition of granular IgA in dermal papillary tips. It has been shown that transglutaminase 3 (TG3) colocalizes with the IgA in dermal papillae (Sardy *et al.*, 2002). However, the additional roles that TG3 may have in the pathogenesis of DH remain unclear.

Transglutaminase is a family of enzymes that catalyze posttranslational modification reactions by transamidation, esterification, and hydrolysis (Iismaa *et al.*, 2009). In celiac disease, transglutaminase 2 (TG2) has been found to be crucial in the pathogenesis by deamidating gliadin and in the formation of antigenic gliadin-TG2 complexes.

Fibrin (Mustakallio *et al.*, 1970) and fibronectin (Reitamo *et al.*, 1981) have been shown to bind in a pattern similar to IgA in the initial lesions of DH. In our study of the deposits localized in the dermal papillae of DH skin, we noted not only similar localization of fibrinogen deposits with the IgA-TG3 complexes but that the stain intensity pattern of these deposits indicated a similar quantitative pattern as well. On examination of biopsies from 13 patients with DH, photographic overlays of cryosectioned skin stained by direct immunofluorescence for IgA and fibrinogen
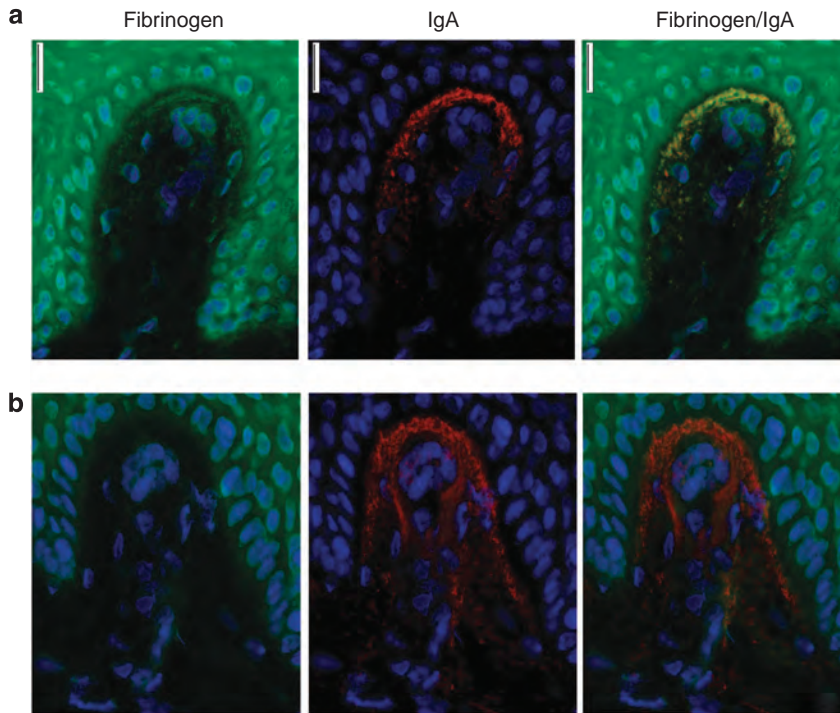
Figure 1. Fluorescein-labeled fibrinogen binds when applied in buffer to IgA aggregates in DH tissue.
Rows **a** and **b** are cryoskin sections from the same DH biopsy. Row **a**, column 1 shows fluorescein-labeled fibrinogen that has bound to DH skin in the papillary dermis when applied in 25 mM Tris/HCl, pH 8, with normal saline and 15 mM CaCl$_2$. Row **a**, column 2 demonstrates direct IgA deposits stained with rhodamine-tagged goat anti-human IgA. Row **a**, column 3 shows colocalization of the labeled fibrinogen with IgA, as denoted by the orange color. Row **b**, column 1 shows no fluorescein-labeled fibrinogen bound to DH skin when the CaCl$_2$ was replaced with 5 mM EDTA. Row **b**, column 2 shows IgA deposits in DH skin, whereas row **b**, column 3 shows no colocalization of labeled fibrinogen with IgA as the red color of the rhodamine tag on the IgA antibody is retained. Both rows were counterstained with 4′,6-diamidino-2-phenylindole. Bar = 20 μm. DH, dermatitis herpetiformis.

revealed a homogeneous color blend of the two different fluorochromes. This pattern of IgA and fibrinogen staining was not seen in five biopsies from patients with IgA vasculitis or in five biopsies from patients with linear IgA bullous dermatosis (LABD).

As TG3 colocalizes with IgA in DH skin, we hypothesized that this enzyme found in the complexes was active and responsible for the fibrinogen deposition. We tested for TGase activity by the incubation of fluorescein isothiocyanate-conjugated cadaverine (Invitrogen, Eugene, OR) in buffer containing 15 mM CaCl$_2$ with cryosections of DH skin. Cadaverine is a primary amine donor for detecting active transglutaminase in endogenous substrates (Lajemi et al., 1997). Microscopy revealed binding of the labeled cadaverine while overlays showed colocalization with IgA-TG3 complexes. This binding of cadaverine could be suppressed with either EDTA chelation of calcium or with cystamine (Jeitner et al., 2005) competition as an amine donor. All control biopsies for IgA vasculitis and LABD showed no evidence of TG3 activity by similar cadaverine binding.

To examine the potential of in silico binding of soluble fibrinogen by enzymatically active IgA-bound TG3, we incubated DH skin with fluorescein-labeled human fibrinogen (Invitrogen) in buffer containing calcium and normal saline (Figure 1). In all DH biopsies, labeled fibrinogen bound in a pattern similar to the IgA deposits. This binding could also be inhibited by the substitution of EDTA for CaCl$_2$.

All DH biopsies in addition with the monkey esophagus (ME) and human umbilical cord (HUC) tissues were stained with anti-human TG3 antibody (Zone et al., 2011) and anti-human TG2 antibody (NeoMarkers, Fremont, CA). While TG2 was detected in ME and HUC, it was not noted in DH IgA aggregates, only TG3. We then repeated the cadaverine cross-linking experiment with DH skin and ME tissue using the TG2-specific peptide-based inhibitor TAMRA-DON (N-(tetramethylrhodaminyl)-DON-Val-Pro-Leu-Ome) (Figure 2; Zedira, Darmstadt, Germany; Hausch et al., 2003). In buffer containing 5 μM TAMRA-DON, cadaverine cross-linking was completely inhibited in ME and only minimally decreased in DH skin.

The discovery that TG3 in these immune complexes retained its enzymatic activity even though it was bound by IgA antibody gave us the opportunity to test whether dapsone, a primary medication used in the treatment of this disease, had any direct effect on TG3 activity. We incubated DH tissue sections with cadaverine and various concentrations of dapsone (1–100 μg ml^{-1}; Sigma-Aldrich, St Louis, MO). Dapsone had no effect on TG3's enzymatic activity at levels up to 100 μg ml^{-1} spanning the pharmacologic range of dapsone concentration.

The presence of granular IgA deposits in the dermal papillae has long been recognized as the pathognomonic feature of DH, yet the method by which these immune deposits cause inflammation is unknown. We observed the presence of fibrinogen in clinical biopsies of DH in a pattern identical to that of the well-described IgA-TG3 immunoprecipitates. This led us to question the method of this binding, and to postulate that the TG3 might be enzymatically active and might directly lead to fibrinogen binding. We are unaware of any similar circumstance where an enzyme is antibody bound and secured to tissue, continues to be enzymatically active, and may subsequently contribute to disease pathology. We speculate that the fibrinogen deposition may have a key role in the pathogenesis of DH via the fibrinolytic system.

Several studies offer support to an active system of fibrinolysis necessary for blister formation in DH. In 1991, Cox injected autologous serum intradermally into DH patients and produced lesions. The addition of heparin, a thrombin inactivator, or epsilon-amino
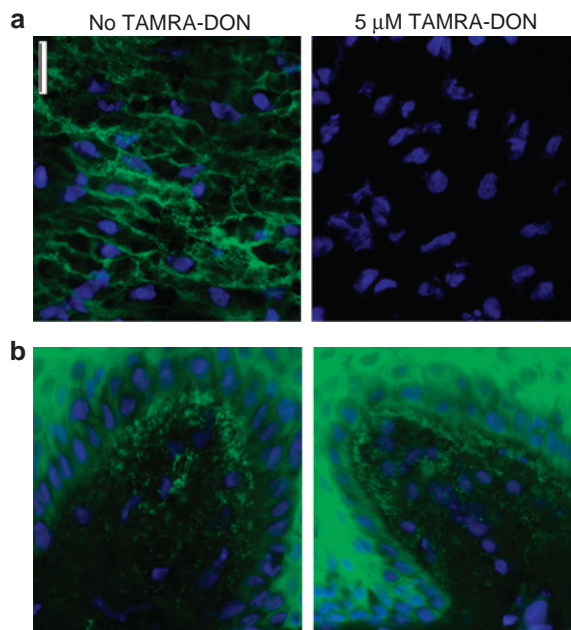
Figure 2. The TG2 inhibitor, TAMRA-DON, prevents cadaverine binding to tissue proteins in monkey esophagus but not in DH skin. Row **a** shows monkey esophagus sections and row **b** shows DH skin sections. Row **a**, column 1 shows fluorescein-labeled cadaverine that has bound when applied in buffer without TAMRA-DON. Row **a**, column 2 demonstrates no bound fluorescein-labeled cadaverine when applied in buffer with 5 μM TAMRA-DON. Row **b**, column 1 shows fluorescein-labeled cadaverine that has bound when applied in buffer without TAMRA-DON. Row **b**, column 2 demonstrates fluorescein-labeled cadaverine that has bound when applied in buffer with 5 μM TAMRA-DON. Both rows were counterstained with 4′,6-diamidino-2-phenylindole. Bar = 20 μm. DH, dermatitis herpetiformis; TG2, transglutaminase 2.

caproic acid, an inhibitor of plasmin, to the serum substantially inhibited lesion formation (Cox and Friedmann, 1991). Moreover, heparin has been reported to be an effective treatment in reported cases of DH (Tan *et al.*, 1996; Shah and Ormerod, 2000). Airola in 1995 demonstrated that in early blister formation in DH patients, urokinase-type plasminogen activator (uPA) was present in basal kertinocytes, suggesting lesion formation by way of an activated uPA-plasmin pathway (Airola *et al.*, 1995). This discovery may be of particular interest as its known wounding of the skin results in upregulation of uPA by keratinocytes (Huang *et al.*, 2002) owing to the loss of tension transmitted from the underlying dermis (Silver *et al.*, 2003). Blisters occurring in DH are typically present in extensor surfaces such as scalp, elbows, knees, and buttocks, areas of skin that are daily subjected to different types of pressure such as kneeling, sitting, and bending that may allow for similar protease production as seen in wounding.

Although the presence of extravascular fibrinogen deposition in DH is well documented, its significance is unknown. Fibrinogen besides being a clotting factor is an inflammatory protein with the ability to promote attraction of T cells, neutrophils, and macrophages (Davalos and Akassoglou, 2012). The current work demonstrates the possible mechanism of initiation of the fibrinolytic pathway in patients with DH via the fixation of fibrinogen to dermal tissue by enzymatically active TG3.

The University of Utah IRB approved this study and did not require patient consent for de-identified and otherwise discarded clinical specimens: the study was conducted according to the Declaration of Helsinki Priniples.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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