EpCAM Expressed by Murine Epidermal Langerhans Cells Modulates Immunization to an Epicutaneously Applied Protein Antigen

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Langerhans cells (LCs) induce type 2 antibodies reactive with protein antigens that are applied to murine skin in the absence of adjuvant after extending their dendrites through tight junctions to acquire antigens and migrating to regional lymph nodes. In response to contact sensitizers, epithelial cell adhesion molecule (EpCAM) on LCs promotes LC dendrite mobility and LC migration. In epithelial cells, EpCAM regulates expression and distribution of selected tight junctions-associated claudins. To determine if EpCAM regulates claudins in LC and immune responses to externally applied proteins, we studied conditional knockout mice with EpCAM-deficient LCs. Although LC claudin-1 levels were dramatically reduced in the absence of EpCAM, conditional knockout mice with EpCAM-deficient LCs and control LC dendrites docked with epidermal tight junctions with equal efficiencies and ingested surface proteins. Topical immunization of conditional knockout mice with EpCAM-deficient LCs with ovalbumin led to increased induction of type 2 Ova-specific antibodies and enhanced proliferation of ovalbumin-reactive T cells associated with increased accumulation of LCs in lymph nodes. These results suggest that, in the absence of strong adjuvants, EpCAM-deficient LCs exhibit increased migration to regional lymph nodes. EpCAM appears to differentially regulate LC mobility/migration in the setting of limited inflammation as compared with the intense inflammation triggered by contact sensitizers.

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INTRODUCTION

Langerhans cells (LCs) are resident epidermal dendritic cells (DCs) that migrate to skin-draining lymph nodes (LN) during the steady state and in response to inflammatory stimuli (Jakob et al., 2001; Merad et al., 2008; Schuler and Steinman, 1985). Dogma long held that LCs were essential for the initiation of T-cell-dependent immune responses directed against foreign antigens (Ag) that were delivered onto, or into, skin (Kaplan et al., 2008), but in vivo experiments involving LC knockout (KO) mice have demonstrated that this is not invariably the case (Bennett et al., 2005; Kaplan et al., 2005; Kissenpfennig et al., 2005). Work that has been conducted in multiple laboratories has clarified the roles of LC and other skin DC subpopulations in the enhancement and attenuation of cutaneous immunity to foreign Ag and autoantigens (Allan et al., 2003; Bennett et al., 2005; Henri et al., 2010; Igyarto et al., 2011; Kaplan et al., 2005; Kautz-Neu et al., 2011; Kissenpfennig et al., 2005; Ouchi et al., 2011; Seneschal et al., 2014), but questions and controversies remain (Igyarto and Kaplan, 2013; Kaplan, 2010).

Recently, it was reported that LCs initiate Th2-type humoral immune responses (IgG1 and IgE subclasses), but not-Th1-type humoral immune responses, to protein Ag that were applied topically to murine skin (Nagao et al., 2009a; Nakajima et al., 2012; Ouchi et al., 2011). In this setting, activated LCs docked with epidermal tight junctions (TJ) in the stratum granulosum, extended their dendrites through TJ, acquired Ag that was external to TJ, and subsequently migrated to LN to initiate immune responses (Kubo et al., 2009; Ouchi et al., 2011). This process involved TJ reorganization as manifested by redistribution of TJ-associated claudins without obvious compromise of TJ function (Kubo et al., 2009). Activated human LCs also have the ability to interact with epidermal TJ, and it has been suggested that LC may initiate or facilitate immune responses to environmental Ag in atopic individuals (Yoshida et al., 2014).

EpCAM (epithelial cell adhesion molecule, CD326) is a membrane glycoprotein that is expressed on the surfaces of LCs, some developing and developed epithelia and some carcinomas (Balzar et al., 1999; Schnell et al., 2013; Trzpis et al., 2007). It has been suggested that EpCAM promotes intercellular adhesion through homophilic interactions (Litvinov et al., 1994), and that it may attenuate

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Abbreviations: Ab, antibody; Ag, antigen; DC, dendritic cell; EpCAM, epithelial cell adhesion molecule; ETA, exotoxin; KO, knockout; LC, Langerhans cell; LN, lymph node; MHCII, MHC class II antigen; Ova, ovalbumin; PKC, protein kinase C; SG, stratum granulosum; TJ, tight junction

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cadherin-mediated adhesion (Winter et al., 2003). More recent studies indicate that EpCAM may function as an outside-in signaling molecule that regulates gene transcription (Maetzel et al., 2009). We, and others, have demonstrated that EpCAM binds avidly to some claudins (Ladwein et al., 2005; Wu et al., 2013), and EpCAM regulates TJ composition and function by modulating amounts and localization of TJ-associated claudins in human colon cancer cells (Wu et al., 2013).

Among DCs, EpCAM is selectively expressed at high levels by LCs (Borkowski et al., 1996). To study EpCAM in LCs, we developed KO mice in which EpCAM is selectively deleted in LCs. We previously reported that EpCAM-deficient LCs exhibit impaired mobility and migration in, and from, murine skin that had been treated with strong contact sensitizers (Gaiser et al., 2012). Dendrite extension and retraction were also attenuated in EpCAM-deficient LCs in these experiments. Therefore, we hypothesized that EpCAM might modulate the ability of LC dendrites to interact with and penetrate epidermal TJ, and that topical immunization to protein Ag might be compromised in LC EpCAM conditional knockout (cKO) mice.

In this study, we demonstrate that EpCAM-deficient LCs did not exhibit impaired ability to dock their dendrites with epidermal TJ, or obvious defects in surface protein uptake. Moreover, topical immunization of LC EpCAM cKO mice with ovalbumin (Ova) led to enhanced formation of type 2 Ova-specific antibody (Ab) and enhanced proliferation of adoptively transferred Ova-reactive OT-II transgenic T cells accompanied by accumulation of increased (rather than decreased) numbers of LCs in regional LN. We conclude that the mobility and migration of EpCAM-deficient LCs is enhanced in this model system, whereas it was decreased in experiments involving contact sensitizers.

RESULTS

Regulation of claudin-1 expression in LCs by EpCAM

We previously reported that EpCAM modulates adhesion and TJ function by regulating intracellular localization of selected claudins in human colon cancer cells (Wu et al., 2013). EpCAM accomplishes this by associating with claudin-1 and claudin-7 and protecting these TJ-associated proteins from lysosomal degradation (Wu et al., 2013). LCs express claudin-1 (Zimmerli and Hauser, 2007), and LC claudin-1 accumulates at LC-TJ docking points as LCs extend their dendrites between keratinocytes in the stratum granulosum during Ag uptake (Kubo et al., 2009). To determine if EpCAM regulates LC claudin-1 expression, claudin-1 expression was assessed in EpCAM-deficient LCs using immunofluorescence microscopy and flow cytometry. Consistent with previous results (Kubo et al., 2009), immunofluorescence studies of unperturbed epidermis revealed that LCs expressed claudin-1 at high levels on cell surfaces while MHC class II (MHCII) molecules were detected intracellularly (Figure 1a). In control LCs, EpCAM colocalized with claudin-1, while expression of both proteins was essentially abolished in EpCAM-deficient LCs (Figure 1a). Flow cytometry confirmed that claudin-1 was downregulated in EpCAM-deficient LCs ($P < 0.01$) to almost background levels (Figure 1b). EpCAM-deficient MHCII^{high} CD11c^{+} Langerin^{+} CD103^{-} cells in skin-draining LN, representing epidermal LCs that had migrated from epidermis, also expressed reduced levels of claudin-1 ($P < 0.001$) (Figure 1b).

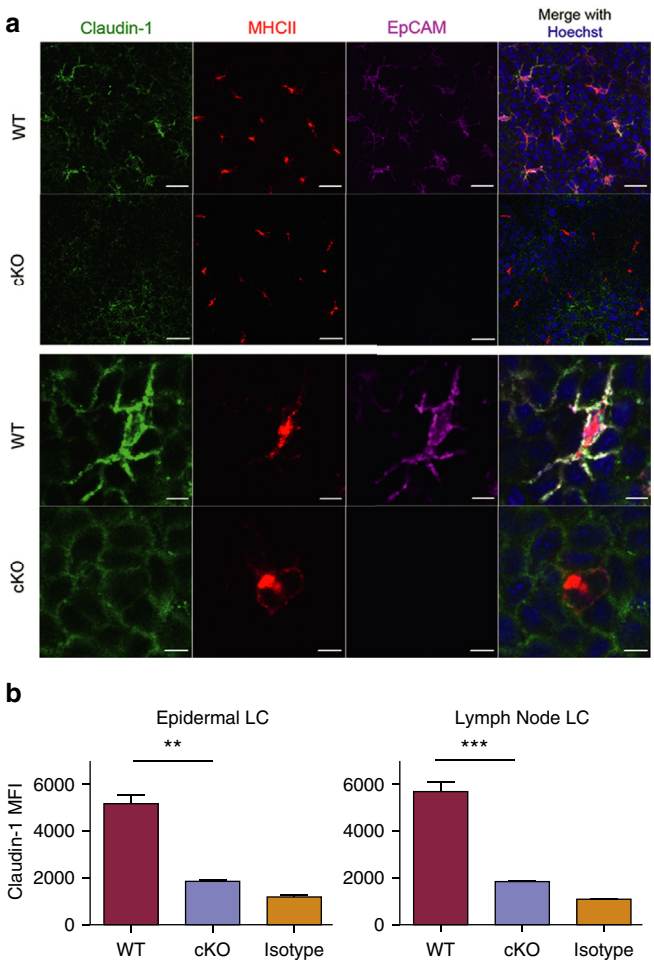

Figure 1. EpCAM regulates claudin-1 expression in LCs. (**a**) Reduction of claudin-1 expression in EpCAM-deficient LCs in unperturbed epidermis as assessed by confocal microscopy. Bars = 20 μm (upper panels); bars = 5 μm (lower panels). (**b**) Quantification of claudin-1 content of EpCAM-deficient and control LCs from unperturbed epidermis and skin draining lymph nodes using flow cytometry. Mean fluorescence intensities (MFI) of claudin-1 in MHC class II^{+} (MHCII^{+}) CD45^{+} LCs isolated from epidermis (left) or MHCII^{high} CD11c^{+} Langerin^{+} CD103^{-} cells from skin draining lymph nodes (LN) (right) are depicted. Data shown is from n = 3 experiments. $^{**}P < 0.01$ and $^{***}P < 0.001$ via Student's *t* test as indicated. cKO, conditional knockout; EpCAM, epithelial cell adhesion molecule; LC, Langerhans cell; WT, wild type/control.

We hypothesized that activated EpCAM-deficient LCs would be unable to reorganize TJ at LC-KC contacts, and that this would be reflected in reduced surface Ag uptake and immune response initiating activity. As shown previously (Kubo et al., 2009), en face images of unperturbed control epidermis revealed continuous networks of claudin-1-containing TJ located between the first and second layers of the stratum granulosum (SG1 and SG2) (Supplementary Figure S1a online). Similar networks were present in epidermis obtained from LC EpCAM cKO mice (Supplementary Figure S1a). In unperturbed epidermis, dendrites of control and EpCAM-deficient LCs did not interact with TJ and MHCII was present in an intracellular location