Delayed control of herpes simplex virus infection and impaired CD4+ T-cell migration to the skin in mouse models of DOCK8 deficiency

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DOCK8 deficiency in humans and mice leads to multiple defects in immune cell numbers and function. Patients with this immunodeficiency have a high morbidity and mortality, and are distinguished by chronic cutaneous viral infections, including those caused by herpes simplex virus (HSV). The underlying mechanism of the specific susceptibility to these chronic cutaneous viral infections is currently unknown, largely because the effect of DOCK8 deficiency has not been studied in suitable models. A better understanding of these mechanisms is required to underpin the development of more specific therapies. Here we show that DOCK8-deficient mice have poor control of primary cutaneous herpes simplex lesions and this is associated with increased virus loads. Furthermore, DOCK8-deficient mice showed a lack of CD4+ T-cell infiltration into HSV-infected skin.

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Mutations in DOCK8 as a cause of primary human immunodeficiency was first described in 2009. Patients present with recurrent sinopulmonary bacterial infections and cutaneous viral infections; most prominently human papilloma virus, herpes simplex virus (HSV) and molluscum contagiosum.1,2 Some of these patients were also found to have markedly elevated levels of IgE antibodies and had previously been described as having autosomal recessive hyper IgE syndrome.1,2 In addition, these patients would usually have eczema and also food and environmental allergies.3 Due to the poor prognosis, the current recommendations are that patients with DOCK8 immunodeficiency undergo bone marrow transplantation.4 The cutaneous viral infections found in human DOCK8 immunodeficiency are severe, extensive and treatment resistant, with one survey finding HSV, varicella zoster virus (VZV), human papilloma virus and molluscum contagiosum in 95% of patients.5 The virus infections remit with bone marrow transplantation but this procedure is associated with a high risk of morbidity and mortality particularly in the context of uncontrolled viral infection. More recently the unusual herpes simplex viral infections have been shown to respond to high doses of subcutaneous interferon-alpha therapy6,7 and this treatment has also been used for papilloma virus infection.8

Several aspects of DOCK8 deficiency have been modeled in mice. DOCK8−/− and other DOCK8-deficient mice produced by mutagenesis with N-ethyl-N-nitrosourea (ENU) have a marked decrease in naive T cells and decreased numbers of natural killer T cells and marginal zone B cells.9,10 B and T cells in these mice have cell-intrinsic defects in immunological synapse formation and there are failures in generation of long-lived antibodies and persistence of CD8+ T-cell memory.11,12 Finally, the migration efficiency of DOCK8−−/− dendritic cells was decreased in a DOCK8 knockout model12 and a striking cell death phenotype has been noted for lymphocytes migrating in confined matrices and tissues such as epidermis.13 Despite these apparently profound defects, DOCK8-deficient mouse models had normal virus control and primary anti-viral CD8+ T-cell responses to infections with influenza virus and the highly attenuated MVA strain of vaccinia virus.13 By contrast a recent report found poor control of HSV infection associated with DOCK8 deficiency and this was associated with a defect in CD8+ T cells able to migrate into the skin and become resident memory (TRM).14 However, this paper did not examine other lymphocytes nor was a direct link made between the loss of TRM and poor control of primary HSV infection. Here we confirm the poor control of HSV disease in DOCK8-deficient mice, add virological data and find a defect in migration of CD4+ T cells to skin during infection.

RESULTS AND DISCUSSION
DOCK8 deficiency in mice leads to increased disease and virus load during HSV infection

Cohorts of DOCK8−/− and wild-type mice were inoculated by tattoo with HSV-1 strain KOS on the flank of shaved and depilated mice.14 In this model of primary HSV infection, virus moves to the...
innervating dorsal root ganglia (DRG) concurrent with the initial skin infection. Replication in the peripheral nervous system leads to virus spreading back to the skin at sites within the inoculated dermatome that are distinct from the site of inoculation producing a characteristic ‘zosteriform’ lesion (Figure 1a). In immunocompetent mice, HSV is limited to the skin and innervating peripheral nervous system and acute infection is controlled within 7–8 days post infection (dpi), but loss of control can lead to central nervous system involvement or dissemination. HSV lesions in DOCK8-deficient mice initially formed at a similar rate to those in wild-type littermates, but then continued to increase in size until 8 dpi, reaching a significantly larger size (Figures 1a and b). By contrast wild-type littermates began to control lesions by 6 dpi, such that the peak size was lower and lesions resolved more quickly. Despite the difference in lesion size there was no significant difference in weight loss sustained by the DOCK8-deficient mice compared with wild-type controls and no mice succumbed to disease (Figure 1c). Qualitatively the lesions in DOCK8-deficient and wild-type mice looked similar with the exception of size, and the tumorous lesions found in DOCK8-deficient human patients were not seen. We speculate that this might be due to the mouse not recapitulating the atopic features of DOCK8 deficiency in humans, for example, DOCK8-deficient mice do not exhibit hyper IgE, even when aged (KLR, unpublished data). Although the lesions in DOCK8-deficient patients are clearly distinct from those associated with eczema herpeticum, it is possible that the superimposition of these two conditions leads to a unique lesion morphology.

The larger lesions seen in DOCK8-deficient mice may have been due to increased virus replication or immunopathology. To dissect these possible causes we examined the amounts of infectious virus in skin

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**Figure 1** Pathogenesis of HSV infection in DOCK8-deficient mice. Cohorts of DOCK8-deficient mice and matched DOCK8+/+ littermates were inoculated with HSV-1 strain KOS on the flank by tattoo. (a) Lesion morphology at 7 dpi at the peak of the infection. (b) Lesion size and (c) weight change in groups of five DOCK8+/+ and four DOCK8+/− mice. Data have been independently repeated twice. (d) Left and middle, virus titers in DRG and skin at 7 dpi, right is the amount of virus obtained from latently-infected DRG after 5 days of explant culture to induce reactivation. In all cases, data are combined from two independent experiments, each point represents a single mouse and lines indicate means. (e) Lesion size in groups of seven Dock8E1886X/E1886X and four matched wild-type mice. Statistical significance (Mann–Whitney) is noted with a P-value or not significant for P>0.05.
strain was infected with HSV, significantly larger lesions with delayed healing were observed when compared with wild-type littermates (Figure 1e). These data indicate that loss of DOCK8 function in general leads to increased HSV pathogenesis.

**HSV-infected skin in DOCK8<sup>pri/pri</sup> mice shows decreased CD4<sup>+</sup> T-cell infiltration**

The timing of the difference in lesions between DOCK8<sup>pri/pri</sup> and wild-type mice suggests poor adaptive immunity to HSV. In the spleen, CD4<sup>+</sup> and CD8<sup>+</sup> T cells were significantly reduced in DOCK8-deficient compared with wild-type mice at 7 dpi when expressed as a percent of splenocytes (Figure 2a left) or as total number per spleen (Figure 2a right). Next, looking in the infected skin at 7 dpi, the amount of infiltration was reduced, reflected by the fraction of cells recovered bearing the pan-leukocyte marker CD45.2 (Figure 2b left). More striking was the observation that CD4<sup>+</sup> T cells were reduced in the skin, both as a fraction of CD45.2<sup>+</sup> cells and in total (Figure 2b middle and right). Surprisingly, CD8<sup>+</sup> T-cell infiltration was not significantly different between DOCK8<sup>pri/pri</sup> and wild-type mice, either in total number or as a percent of CD45.2<sup>+</sup> cells (Figure 2b middle and right). Further, in spleen, peripheral blood and skin, HSV glycoprotein B (gB) dextramer<sup>+</sup> cells were at a similar frequency in total CD8<sup>+</sup> T cells (Figure 2c left) irrespective of DOCK8 genotype. Only in the spleen were total numbers of gB<sup>+</sup>, CD8<sup>+</sup> cells reduced (Figure 2c right). We also looked at the effector differentiation of antiviral CD8<sup>+</sup> T cells. No significant difference was seen between DOCK8<sup>pri/pri</sup> and wild-type mice in the ability of their CD8<sup>+</sup> T cells to make interferon gamma (IFNγ) after a brief in vitro stimulation with gB peptide, or the fraction of gB-dextramer<sup>+</sup> cells making and storing granzyme B (Figure 2d left and middle). However, the total number of cells with these functions in the spleen were reduced in DOCK8<sup>pri/pri</sup> mice (Figure 2d, right), as expected due to the reduction in total CD8<sup>+</sup> T cells noted previously. Upon resolution of infection, CD8<sup>+</sup> and CD4<sup>+</sup> T-cell numbers and percents in spleen return to values similar to the baseline shown for uninfected DOCK8-deficient and sufficient mice (for example, Supplementary Figure 1D and data not shown). Together these data suggest that despite the general lymphopenia in DOCK8<sup>pri/pri</sup> mice, CD8<sup>+</sup> T cells were primed adequately by HSV infection and access the skin at the peak of the acute response. By contrast, CD4<sup>+</sup> T-cell recruitment to the skin was very substantially reduced. The intact primary CD8<sup>+</sup> T-cell responses echo the findings for influenza virus and MVA in DOCK8-deficient mice.13 At face value our data are in contrast with other recent findings,13 but where we have looked at relatively crude preparations of cells from whole skin at the peak of infection, Zhang et al. looked for CD8<sup>+</sup> T cells in the epidermis and specifically at the formation of

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**Figure 2** Immune responses during HSV infection in DOCK8-deficient mice. Cohorts of DOCK8<sup>pri/pri</sup> mice and four DOCK8<sup>+/+</sup> littermates were inoculated with HSV-1 strain KOS on the flank by tattoo and T-cell responses were assessed at 7 dpi. (a) Percentages (left) and numbers (right) of CD8<sup>+</sup> and CD4<sup>+</sup> T cells in spleens. (b) Left, infiltration of leukocytes in the skin, indicated by number of CD45.2<sup>+</sup> cells as a fraction of all cells recovered. Middle and right, CD4<sup>+</sup> and CD8<sup>+</sup> T-cell infiltration into infected skin shown as a percent of CD45.2<sup>+</sup> cells and as total number recovered, respectively. (c) HSV-specific CD8<sup>+</sup> T cells, shown as the fraction of all CD8<sup>+</sup> T cells (left) or total number (right) marked with a HSV gB-dextramer<sup>+</sup> in spleen, skin and peripheral blood. (d) Activation of HSV-specific CD8<sup>+</sup> T cells in the spleen (bottom) shown by the percent making IFNγ in response to stimulation with gB<sub>498</sub> peptide (left) and the percentage of gB-dextramer<sup>+</sup> CD8<sup>+</sup> T-cells staining for intracellular granzyme B (middle). On the right, total numbers of granzyme B<sup>+</sup> gB-specific and IFNγ<sup>+</sup> CD8<sup>+</sup> T cells are shown. All graphs include data combined from two independent experiments, each point is a single animal and lines indicate the mean. Statistical significance (Mann–Whitney) is noted with a P-value or not significant for P>0.05.
TBM. Thus, the findings are complimentary rather than in conflict. The importance of CD4+ T cells in control of primary skin infection with HSV infection has long been known and so poor migration of these to the skin would likely contribute to loss of control of HSV in DOCK8-deficient patients.17–20,14 The defect we find might be intrinsic to CD4+ T cells or could be related to impaired migration of an antigen-presenting cell.21,12,22 It is also important to note that other players in anti-HSV immunity such as natural killer cells and natural killer T cells might be damaged by DOCK8 deficiency, but we have not examined these in this study.10,13,23 Finally, we have focused on primary HSV infection because this is most faithfully modeled in mice. We speculate that control of recurrence will be further compromised by DOCK8 deficiency owing to problems associated with poor persistence of CD8+ T-cell memory cells and in particular the profound CD8+ TRM defect.9,10,13,14,23–26

METHODS

Viruses and cell lines

HSV-1 strain KOS was kindly provided by F Carbone (The University of Melbourne, Parkville, Victoria, Australia). HSV-1 was grown and titrated by standard methods using vero cells grown in Minimal Essential Medium supplemented with 10% fetal bovine serum (FBS), 2 mM l-glutamine. Samples were homogenized, freeze thawed three times and viral infections with IFN-alpha 2b therapy.25

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Conflict of interest

The authors declare no conflict of interest.
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