The Stenosis of Cerebral Arteries and Impaired Brain Glucose Uptake by Long-Lasting Inflammatory Cytokine Release from Dermatitis Is Rescued by Anti-IL-1 Therapy

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TO THE EDITOR

The skin is one of the most important immune organs, known as *skin-associated lymphoid tissues*. Once activated, it produces and releases large amounts of inflammatory cytokines, inducing prolonged cutaneous and systemic inflammation. Recently, we showed that persistent release of IL-1s from inflammatory skin lesions induces abdominal aortic remodeling, including vascular stricture, wall fragility, and deteriorated peripheral circulation mimicking arteriosclerosis in mice (Yamanaka et al., 2014). Psoriasis is an intractable inflammatory cytokine-mediated skin disorder, and the average life span of psoriasis patients is 6 years shorter than that of individuals without history of psoriasis because of the cardiac (Abuabara et al., 2010; Horreau et al., 2013; Koch et al., 2015) and cerebrovascular comorbidities (Prodanovich et al., 2009). Adults with eczema also have increased risk for cardiovascular disorders (Andersen et al., 2016; Silverberg and Greenland, 2015), leading to a concept of inflammatory skin march: the close relationship between skin disorders and cardiovascular complications (Yamanaka and Mizutani, 2015).

We next investigated the cerebrovascular system. The potential roles of systemic or local IL-1 in the development of acute brain injuries in ischemic or hemorrhagic stroke were reported (Galea and Brough, 2013); however, the risk of long-lasting systemic IL-1 for cerebrovascular involvement remains unelucidated.

We addressed this question using a skin inflammatory mouse model, keratin-14 driven caspase-1 transgenic (KCASP1Tg) mice (Yamanaka et al., 2000), that releases IL-1s from the skin lesions. In KCASP1Tg mice, the plasma levels of IL-1α and IL-1β were below the limits of detection but increased after the development of dermatitis. C57BL/6 littermate mice (wild type [WT]) were used as controls (see Supplementary Materials and Methods online). As reported previously, KCASP1Tg mice were treated with anti-IL-1α/β antibodies that ameliorate aortic vascular lesions (Yamanaka et al., 2014). Briefly, 10 μg of anti-IL-1α/β–neutralizing antibodies (BioLegend, CA, USA) were injected intraperitoneally into female KCASP1Tg mice three times a week from 2–6 months of age (n = at least 5). At 6 months old, the mice were deeply anesthetized, and India-ink angiography was performed. After gelatin solidification, the high-resolution pictures of the circle of Willis and basilar arteries were recorded with a scale. Three experienced researchers, unaware of the treatment groups, measured the smallest lumen diameter of the sphenoidal segment of the middle cerebral artery, pre-communicating segment of the anterior cerebral artery, intradural internal carotid artery, and basilar arteries using ImageJ software (National Institutes of Health, Bethesda, MD) and determined the mean value per the artery (Kawakita et al., 2017) (see Supplementary Materials and Methods). The mean diameter of the brain arteries was decreased in KCASP1Tg compared with WT mice, which was significantly prevented by anti-IL-1α/β antibody therapy (Figure 1a and b, and see Supplementary Materials).

To determine whether the vascular changes affect brain metabolism, we used positron emission tomography with 2-deoxy-2-[fluorine-18] fluoro-D-glucose (^{18}F-FDG) positron emission tomography (PET) for sensitive and noninvasive evaluation of the cerebral function. ^{18}F-FDG accumulates in the metabolically active tissues in an activity-dependent manner (Sokoloff et al., 1977). After overnight fasting, each female mouse was administered 25 MBq of ^{18}F-FDG intravenously via tail vein. After a 20-minute conscious uptake period, the mouse was scanned with a LabPET4 micro-PET scanner (see Supplementary Materials and Methods). The regions of interest were placed on the coronal images of mouse head (thickness = 1 mm), the data were expressed as standardized uptake value, and the therapeutic effects on each treated mouse were evaluated. ^{18}F-FDG uptake of KCASP1Tg mice significantly decreased in the whole brain. In clear contrast, the brain ^{18}F-FDG uptake of treated KCASP1Tg mice was successfully reserved compared with untreated KCASP1Tg mice (Figure 2a and b).

Here, we showed that sustained circulating levels of IL-1 derived from severe skin inflammation causes

Abbreviations: BBB, blood-brain barrier; ^{18}F-FDG, 2-deoxy-2-[fluorine-18]fluoro-D-glucose; KCASP1Tg, keratin 14-specifically overexpressing caspase-1 transgenic; PET, positron emission tomography; WT, wild type

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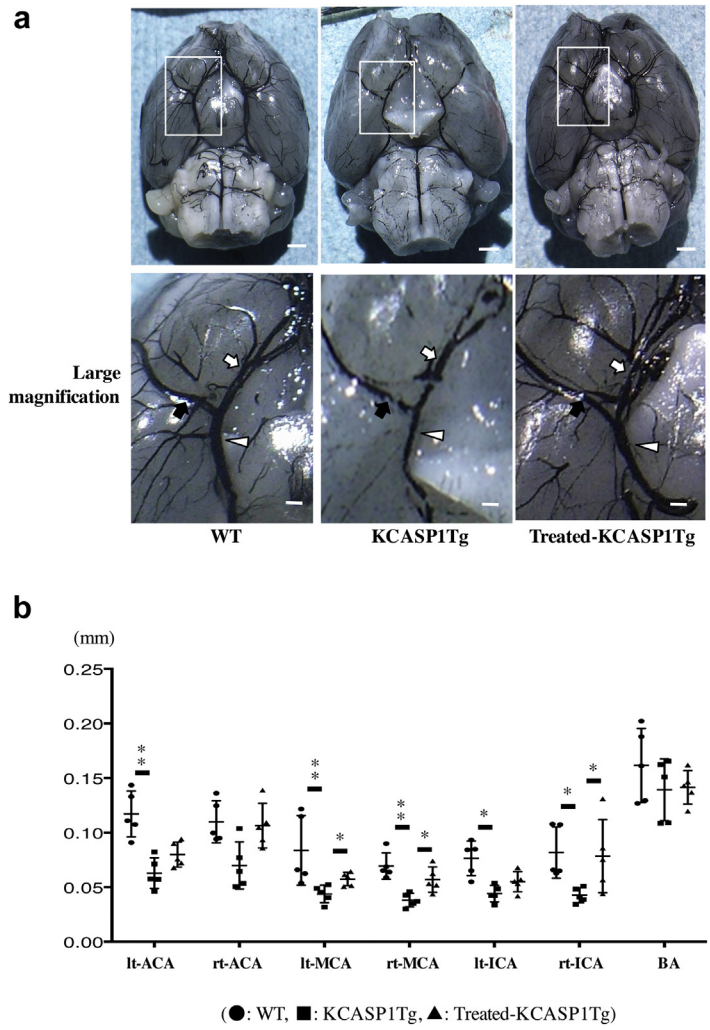

Figure 1. KCASP1Tg mice developed reduced vessel diameter of cerebral arteries. (**a**) The representative staining of brain artery in WT, KCASP1Tg, and anti-IL-1–treated KCASP1Tg (Treated-KCASP1Tg) mice. Compared with the WT, brain arteries in KCASP1Tg mice are narrow but have been recovered in treated KCASP1Tg mice. Lower panel is larger magnification of each mouse. White arrows, anterior cerebral artery (ACA); black arrows, middle cerebral artery (MCA); white arrowhead, internal carotid artery (ICA). Scale bars = 1 mm in upper panel and 200 μm in lower panel. (**b**) The diameter of brain arteries decreased in KCASP1Tg (■) compared with WT (●) mice, which was recovered by the simultaneous treatment with anti-IL-1α and -β neutralizing antibodies (▲) (n = 5 in each group). Statistical analysis was performed using the Kruskal-Wallis nonparametric analysis of variance test with post hoc analysis using Dunn's multiple comparison test. $P < 0.05$ was considered statistically significant. Error bars show mean ± standard deviation. *$P < 0.05$, **$P < 0.01$. BA, basilar artery; KCASP1Tg, keratin 14-specifically overexpressing caspase-1 transgenic; lt, left; rt, right; WT, wild type.

decreases in brain glucose uptake and anatomical changes in vasculature. Surprisingly, the pathological changes were rescued by treatment with anti-IL-1α/βantibodies. The radius is the most powerful determinant of cerebral blood flow, and even small changes in lumen diameter have significant effects on cerebral blood flow (Cipolla, 2009). Previous reports showed that greater than 50%–60% reduction in diameter is needed to reduce cerebral blood flow (Tureyen et al., 2005; Voldby et al., 1985). In this study, large-artery diameter reduction of the anterior circulation of approximately 50% was detected. Therefore, a 50% reduction in vascular diameter of major arteries rather than a direct effect of IL-1 on brain glucose metabolism is the likely explanation for the reduced glucose uptake observed by ^{18}FDG-PET. Under neuroinflammation, the injected antibody has an increased ability to cross the blood-brain barrier (BBB) because of the inflammation loosening the tight junctions of the BBB (Sas et al., 2008). Because IL-1β can cause BBB disruption (Wang et al., 2014), we speculated that IL-1 antibodies could cross the BBB in this study.

Our results suggest the importance of proper control for long-lasting skin inflammation, including psoriasis and atopic dermatitis, to prevent cerebrovascular complications, and IL-1s may be one of the critical cytokines for vascular changes. Although the pathogenesis is very different between atopic dermatitis and psoriasis, the inflamed keratinocytes release a massive amount of pro-inflammatory cytokines stored in the epidermis into the systemic circulation in both diseases. In atopic dermatitis, leakage of IL-1s from injured keratinocytes can occur if patients with severe atopic dermatitis intensely and repeatedly scratch their itchy skin (Abramovits et al., 2013; Hay et al., 2013). Psoriasis is characterized by an elevated circulating level of IL-1, and the increase of circulating IL-1 level is a risk factor for cardiovascular disorders (Yu et al., 2009). The angiography showed that amelioration of the mean diameter of brain arteries was not complete in treated KCASP1Tg mice, suggesting the involvement of other inflammatory cytokines in the mechanism of arterial stenosis. In fact, administration of neutralizing antibodies against tumor necrosis factor-α showed a marginal effect on the recovery of brain arterial diameter (data not shown). Tumor necrosis factor-α is another potential candidate, and the use of IL-1 receptor antagonist is also another possible resource.

In conclusion, anti-IL-1 therapy is a promising candidate for preventing vascular complications in severe inflammatory skin diseases. This concept is not limited to skin diseases but also may be applicable to other systemic disorders such as IL-1–associated autoinflammatory diseases.

Animal care was performed according to current ethical guidelines, and the experimental protocol was approved by the Mie University Board Committee for Animal Care and Use (#22-39).