Hyperglycemia Is Associated with Psoriatic Inflammation in Both Humans and Mice

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Chronic low-grade inflammation can cause several metabolic syndromes. Patients with psoriasis, a chronic immunological skin disease, often develop diabetes. However, it is not clear to date how psoriasis leads to, or is correlated with, glucose intolerance. Here, we investigate whether psoriasis itself is correlated with hyperglycemia in humans and mice. In patients, the severity of psoriasis was correlated with high blood glucose levels, and treatment of psoriasis by phototherapy improved insulin secretion. Imiquimod-induced systemic and cutaneous inflammation in mice, with features of human psoriasis, also resulted in hyperglycemia. Although it should be determined if psoriasis-like cutaneous inflammation alone can induce hyperglycemia, imiquimod-treated mice showed impairment of insulin secretion without significant islet inflammation. Administration of anti-IL-17A monoclonal antibody improved hyperglycemia in patients with psoriasis and imiquimod-treated mice with psoriasiform features. These results suggest that hyperglycemia is highly associated with psoriasis, mainly through IL-17.


INTRODUCTION

Psoriasis is a chronic immunological skin disease involving the IL-23/T helper type 17 cell axis responses (Hawkes et al., 2017; Lowes et al., 2014). Psoriatic inflammation is not restricted to the skin and accounts for systemic inflammation such as systemic cytokine production and association with metabolic syndromes (Davidovici et al., 2010; Hawkes et al., 2017). In these metabolic syndromes, psoriasis is frequently accompanied by diabetes (Armstrong et al., 2013; Azfar et al., 2012; Wan et al., 2018). Numerous epidemiological studies have described the relationship between these syndromes and psoriasis. Several mouse models of inflammation have supported the involvement of sustained inflammation in hyperglycemia and insulin resistance. Consecutive injection of a toll-like receptor (TLR)-4 ligand, lipopolysaccharide (LPS), or viral infection can induce hyperglycemia and insulin resistance (Okin and Medzhitov, 2016; Sestan et al., 2018). Blocking TLR-7 and TLR-9 signaling improves obesity-related insulin resistance (Revelo et al., 2016). Thus, systemic psoriatic inflammation itself may play a key role in glucose metabolism. However, it is not clear whether psoriasis causes diabetes or vice versa.

To determine whether inflammation associated with psoriasis influences glucose metabolism, we took advantage of an imiquimod (IMQ)-induced mouse model. Although there are some differences between the model mice and patients, the IMQ-induced model is widely used (Herbert et al., 2018; Shimoura et al., 2018; Stockenhuber et al., 2018). IMQ is a TLR-7/8 ligand, and its application to the skin induces both psoriasis-like skin lesions and systemic inflammation, such as production of cytokines (Grine et al., 2015; Roller et al., 2012; van der Fits et al., 2009). Thus, systemic and cutaneous inflammation with psoriasiform features induced by IMQ application may contribute to hyperglycemia, similar to LPS injection or viral infection.

In this study, we analyzed the glucose levels of patients with psoriasis and in the IMQ-induced psoriatic mouse model. In humans, hyperglycemia was associated with psoriasis. In mice, hyperglycemia and impaired insulin release were observed after IMQ application in the absence of high-fat diet and obesity. Furthermore, administration of anti-IL-17A monoclonal antibody (mAb) improved hyperglycemia in patients. Blockade of IL-17 improved fasting glucose levels in IMQ-treated mice without complete clearance of the skin lesions. Thus, our results showed a clear correlation between psoriasis and hyperglycemia.

RESULTS

Severity of psoriasis is correlated with high blood glucose levels, which is improved by treatment of psoriasis in patients

We first analyzed whether hyperglycemia was observed in patients with psoriasis or was correlated with psoriatic...
inflammation. Hemoglobin A1c (HbA1c) represents mean blood glucose levels over a 1–2-month period. Patients with HbA1c levels less than 5.7% were considered normative, and those with levels of 6.5% or greater were considered diabetic (the American Diabetes Association). We used HbA1c levels instead of random blood glucose levels because HbA1c level is not influenced by the timing of the last meal. Psoriasis severity can be evaluated by the Psoriasis Area and Severity Index (PASI) score, which is measured by evaluation of erythema, induration, scaling, and percentage of body surface area affected. PASI scores range from 0 (no disease) to 72 (maximum disease). During the observational period from January through December 2015, 39 of 153 patients with psoriasis had available data regarding HbA1c levels and PASI scores at their first visit. The remaining patients were excluded because of data unavailability. All first-visit patients with available data regarding HbA1c levels and PASI scores were chosen for the analysis, regardless of their treatment for diabetes (see Supplementary Table S1 online and the Supplementary Materials and Methods online). Out of 39 patients, 5 were treated with oral antidiabetic medication. There were no insulin users in the group. Clinical records showed a significant correlation between PASI scores and HbA1c levels (Figure 1a, left). HbA1c levels were also significantly correlated with the erythema component of the PASI, which mainly indicates inflammation (Figure 1a, right). HbA1c levels were significantly higher in patients with very severe psoriasis when patients were divided into two groups of very severe psoriasis (PASI ≥ 20, n = 12) and severe to moderate psoriasis (PASI < 20, n = 27) (Figure 1a, bottom). When patients treated with antidiabetic medication were excluded, there was a more significant correlation between PASI score or the erythema component of the PASI and HbA1c levels (see Supplementary Figure S1 online). Thus, severity was correlated with blood glucose levels in patients with psoriasis.

These results led us to question whether effective psoriasis treatment influenced blood insulin levels. It was extremely difficult to obtain blood from several patients to measure insulin at each time point throughout the day. However, among patients with psoriasis hospitalized for phototherapy between February and April 2017, there were two patients who were monitored for their blood insulin levels before and after phototherapy with bath psoralen and UVA (bath-PUVA) therapy. The data from these patients is presented as case reports. After therapy, improvement was seen not only in their skin lesions but also in their serum insulin concentrations after breakfast following the treatment (Figure 1b). Patients’ serum levels of proinflammatory cytokines, including IL-6 and IL-17A, were also decreased after the bath-PUVA therapy (Table 1).

Next, we investigated patients with psoriasis treated with anti-IL-17A monoclonal antibody (mAb). Anti-IL-17A mAb is an effective and approved treatment for moderate to severe psoriasis (Gordon et al., 2016; Hawkes et al., 2017; Langley et al., 2014). All registered patients (see Supplementary Table S1) who underwent anti-IL-17A mAb therapy had improved skin lesions (Figure 1c, left). Anti-IL-17A mAb therapy also reduced HbA1c levels significantly in these patients (Figure 1c, right). There was no significant correlation between delta PASI score and delta HbA1c levels (see Supplementary Figure S2 online). Compared with the results shown in Figure 1a, the correlation between HbA1c levels and PASI score before anti-IL-17A mAb therapy was weaker (see Supplementary Figure S3 online), probably because diabetic patients were treated properly before biologic treatment (see Supplementary Table S1). These results indicate that successful treatment of psoriatic inflammation improves hyperglycemia in patients with psoriasis.

**IMQ application induces high blood glucose levels and impairs insulin secretion in mice fed with a non–high-fat diet**

To further investigate whether or how psoriatic inflammation contributes to hyperglycemia, we used a psoriatic mouse model induced by topical application of a TLR7/8 ligand, IMQ (van der Fits et al., 2009) (Figure 2a). Evaluating the mouse model is useful to confirm the findings seen in patients, because mice can be kept on the same diet, in contrast to patients. Topical treatment of the ears and the back skin with IMQ for 7 days induced altered skin morphology including erythema and scaling, which are typical of psoriatic skin lesions. Furthermore, the ears were thinned, and PASI scores indicated that disease progression was evident after IMQ treatment (Figure 2b and c). Typical psoriasis pathological findings, including epidermal hyperproliferation and parakeratosis in skin histology, were also observed (Figure 2d). All mice received regular food without a high-fat diet. They never grew obese and actually decreased in weight during the study (Figure 2e). The transient weight loss was detected in B6 mice applied with IMQ (Jin et al., 2018; van der Fits et al., 2009; Vinter et al., 2016), but this could easily be rescued by injection of phosphate buffered saline (van der Fits et al., 2009). In our experiments, without further treatment, IMQ-treated mice also regained the weight by day 7 (Figure 2e).

To investigate whether IMQ-treated mice exhibited glucose intolerance, we performed glucose tolerance tests on day 7 after starting IMQ treatment. Fasting glucose levels were significantly higher in IMQ-treated mice than control mice (Figure 2f), whereas blood insulin levels were similar between the control and IMQ-treated mice (Figure 2g). Upon injection of glucose, blood glucose levels were significantly higher and insulin levels were significantly lower in IMQ-treated mice than control mice (Figure 2h and i). In contrast, skin inflammation induced by DNFB did not induce hyperglycemia (see Supplementary Figures S4 and S5 online). To test whether IMQ-treated mice with hyperglycemia were insulin resistant, we also performed insulin tolerance tests. IMQ-treated mice responded to administered insulin similarly to control mice (Figure 2j), indicating that IMQ-treated mice were not insulin resistant. Thus, IMQ-treated mice with psoriasiform features showed hyperglycemia and impaired insulin secretion even without a high-fat diet or weight gain.

**Islets from IMQ-treated mice with psoriasiform features have the capacity to release insulin ex vivo**

To analyze possible pancreatic inflammation in IMQ-treated mice, we histologically investigated pancreatic islets (Figure 3a). Because islets were detectable in hematoxylin...
and eosin staining (Figure 3b, and see Supplementary Figure S6a online), we investigated the histology of all hematoxylin and eosin-stained pancreatic islets from IMQ-treated mice (n = 14) and control vehicle-treated mice (n = 16) in a blinded manner. No significant changes in pancreatic islets were observed between IMQ-treated mice and control vehicle-treated mice (Figure 3b, and see Supplementary Figure S6a). We further stained the pancreas with anti-insulin antibody (Ab). Islets were intact, and comparable numbers of insulin granules were detected in both control and IMQ-treated mice (Figure 3c). There were no significant differences in the numbers or proportions of insulin-positive islets per total section of the pancreas between control and IMQ-treated mice (Figure 3d). We also investigated the histology of the liver, because the liver is involved in glucose metabolism. There was no significant inflammation or necrosis in the livers of IMQ-treated mice compared with those of control vehicle-treated mice (see Supplementary Figure S7 online). Aspartate aminotransferase and alanine aminotransferase, which are released after liver damage, and lipase, which is increased during pancreatitis, were not increased in the serum of IMQ-treated mice at day 9 (see Supplementary Figure S8 online). These results indicate that the liver and pancreas were not significantly damaged in IMQ-treated mice. Thus, in IMQ-treated mice, no prominent inflammation was observed in the pancreas, and pancreatic islets were structurally normal.

To examine the function of islet cells isolated from IMQ-treated mice, we purified islet cells and measured their insulin-secreting ability ex vivo. Purified pancreatic cells contained islets, as determined by morphology and anti-insulin Ab staining (see Supplementary Figure S6b). Islets from IMQ-treated mice produced insulin at levels similar to those from control mice in response to increased glucose concentrations (Figure 3e), indicating that the ability of islets to secrete insulin was intact in IMQ-treated mice. These results also indicate that IMQ application did not damage islet cells because of its toxicity. Thus, insulin secretion from islets was not impaired ex vivo in IMQ-treated mice, indicating that islet function can be reversible in psoriasis.

Table 1. Serum inflammatory cytokine levels in pg/ml from patients with psoriasis before and after phototherapy

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Case 1 Before</th>
<th>Case 1 After</th>
<th>Case 2 Before</th>
<th>Case 2 After</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-17A</td>
<td>9.22</td>
<td>ND</td>
<td>5.26</td>
<td>1.83</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>25.73</td>
<td>5.00</td>
<td>9.83</td>
<td>9.83</td>
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<tr>
<td>IL-10</td>
<td>4.18</td>
<td>ND</td>
<td>1.30</td>
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<tr>
<td>IL-6</td>
<td>11.51</td>
<td>16.23</td>
<td>4.94</td>
<td>2.16</td>
</tr>
<tr>
<td>IL-4</td>
<td>ND</td>
<td>3.36</td>
<td>7.19</td>
<td>4.85</td>
</tr>
<tr>
<td>IL-2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>3.20</td>
</tr>
</tbody>
</table>

Abbreviation: ND, Not detected.

1 Serum inflammatory cytokine levels from patients with psoriasis before and after (day 14) phototherapy were measured. For case 1, the time point “before” was before infliximab treatment in 2010. For case 2, the time point “before” was defined as day 0 of phototherapy in 2017.

Anti-IL-17 Ab treatment improves hyperglycemia in IMQ-treated mice with psoriasiform features

To determine if control of IMQ-induced systemic and cutaneous inflammation with features of human psoriasis can improve hyperglycemia in vivo, we administered anti-IL-17A mAb to the mice (Figure 4a). We chose anti-IL-17A mAb for mice because secukinumab and ixekizumab used in patients (Figure 1c) are also anti-IL-17A mAbs. We were interested in evaluating the effect of anti-IL-17A, rather than anti-TNF-α, because IL-17A plays a role in the development of psoriasis in more peripheral parts than TNF-α (Hawkes et al., 2017). Anti-IL-17A mAb treatment decreased the fasting glucose levels (Figure 4b). Analysis of a gene database (Han et al., 2011) showed that pancreatic islets expressed inflammatory cytokine receptors including the IL-17 receptor family (Figure 4c), indicating that pancreatic islets can respond to IL-17 directly. In skin lesions (Figure 4d), there were significant differences among three groups (vehicle, IMQ + isotype IgG, and IMQ + anti-IL-17 mAb) by Kruskal-Wallis test. However, the post hoc Dunn multiple comparison test did not show a significant difference between IMQ + isotype IgG and IMQ + anti-IL-17 mAb. Together with the finding that islet function of insulin secretion was functionally attenuated in IMQ-treated mice, these results indicate that IL-17 blockade can improve hyperglycemia by restoring islet functions.

DISCUSSION

Our results highlight the importance of IL-17 in controlling glucose homeostasis in patients and mice with psoriasis by showing the correlation between psoriasis and hyperglycemia. Our data show that IMQ-treated mice had impaired insulin secretion with preserved islet function ex vivo. Whether psoriasis-like cutaneous inflammation alone can induce hyperglycemia or not was unclear. Continuous skin and systemic psoriatic inflammation without proper treatment might damage islet function of insulin secretion permanently and lead to insulin resistance in patients with psoriasis, as generally reported (Boehncke et al., 2007; Gyldenlove et al., 2015). Our studies indicate that early control of psoriasis by blockade of IL-17 may prevent permanent damage of islet cell function and further development of severe type 2 diabetes.

The mechanisms of hyperglycemia in IMQ-treated mice are different from those in mice with LPS injection or viral infection (Okin and Medzhitov, 2016; Sestan et al., 2018). First, hyperglycemia induced in IMQ-treated mice was not insulin resistant, as was reported in LPS-injected mice or virus-infected mice. Second, hyperglycemia in mice with IMQ application was observed in nonobese, lean mice that were not fed a high-fat diet, whereas glucose intolerance in viral infection was detected in obese mice on a high-fat diet. Third, we found that blockade of IL-17 alone reduced hyperglycemia induced in IMQ-treated mice. As also seen in patients, we found that anti-IL-17A mAb treatment improved the skin lesions of psoriasis, as well as HbA1c levels. It was reported that IL-17A induces the activation of inflammatory and proapoptotic responses and chemokine production from islet cells in vitro (Grieco et al., 2014; Honkanen et al., 2010). T helper type 17 is reported to be involved in type 1 diabetes.
Figure 1. Severity of psoriasis correlates with hyperglycemia, and treatment of psoriasis reverses islet function in patients. (a) Blood HbA1c levels and PASI score (upper left) or the erythema component of the PASI (upper right) score were measured in patients with psoriasis. Patients were divided into two groups defined as PASI score < 20 or ≥ 20, and HbA1c levels of each individual were plotted (bottom right). (b) PASI scores and blood insulin levels of case 1 and case 2 before and after treatment. (c) Anti-IL-17A mAb (n = 14) showed a significant decrease in PASI score and HbA1c levels after treatment.
diabetes, in which islet β cells are destroyed by autoimmune responses (Shao et al., 2012). However, we did not detect lymphocytic infiltration and destruction in the pancreas of IMQ-treated mice by histology. IL-17A— or IL-17—related proinflammatory cytokines in psoriasis may create a suppressive microenvironment to inhibit insulin secretion in the pancreas and act directly on islets. How IL-17A or other proinflammatory cytokines contribute to insulin secretion requires further analysis.

Our data show that treatment with anti-IL-17 Ab in IMQ-treated mice was not as effective as this treatment in patients. This is consistent with former reports (Dallenbach et al., 2015; Mangan et al., 2015). Although anti-IL-17 Ab alone did not have any effect on the skin lesions in IMQ-treated mice but the combination of anti-IL-17 Ab (at a higher dose than ours) and anti-IL-23 Ab did (Mangan et al., 2015). Ear swelling by IMQ application was improved only by about half with anti-IL-17 Ab treatment at a dose similar to ours or in IL-17—knockout mice (Dallenbach et al., 2015).

Moreover, type-I IFN-driven CD8+ T cells play an important role in psoriasiform inflammation of IMQ-treated mice (Stockenhuber et al., 2018). Thus, in IMQ-induced psoriasiform skin lesions, contributions of other cytokines, such as IL-23 and type I IFN, play more important roles. In contrast, administration of anti-IL-17A mAb alone improved hyperglycemia in mice, indicating that IL-17 may have more powerful effects on glucose metabolism than skin lesions in IMQ-treated mice. IMQ is a TLR ligand, so it is reasonable that the IMQ-model represents aspects different from what those seen in human psoriasis. IMQ application is also important as the model of sustained inflammation compared with LPS injection (Okin and Medzhitov, 2016).

Blood glucose levels are regulated by many factors, including hepatic glucose production; glucose uptake into the skeletal muscle and adipose tissue; and other many factors such as glucagon, corticosteroid, adrenaline, and stress levels. It is thus conceivable that several underlying mechanisms, including stress, are involved in the association between psoriasiform inflammation and glucose metabolism (Kotas and Medzhitov, 2015; Rorsman and Braun, 2013). A high-fat diet and/or endoplasmic reticulum stress induced by diabetic obese conditions exacerbated psoriasiform skin lesions in the IMQ-treated mouse model (Herbert et al., 2018; Nakamizo et al., 2017; Shimoura et al., 2018). We found that inducing psoriasiform features by IMQ application resulted in hyperglycemia in mice without a high-fat diet or obesity. IMQ-treated mice injected with anti-IL-17 Ab or isotype were under a similar degree of stress regarding skin conditions because their skin scores were similar. Under this condition, IL-17 neutralization improved fasting blood glucose levels significantly. Thus, stress due to skin lesion severity in IMQ-treated mice is not likely to be related to hyperglycemia, although we cannot deny the possibility of its contribution.

Our results indicate that glucose intolerance associated with psoriasis can be reversed with proper treatment, especially by targeting IL-17. Using mice, we showed that IMQ-induced psoriasiform features are related to hyperglycemia without obesity or a high-fat diet. Although psoriatic inflammation is known to involve many inflammatory cytokines, administration of anti-IL-17A mAb alone improved hyperglycemia in mice, suggesting an important role of IL-17 in controlling glucose metabolism in psoriasis. Diabetes associated with psoriasis tends to require systemic therapy, and many patients develop microvascular and macrovascular diabetic complications compared with diabetic patients without psoriasis (Armstrong et al., 2015; Azfar et al., 2012; Takeshita et al., 2017). Thus, psoriasis should be controlled in an early phase to prevent islet dysfunction accompanied by systemic inflammation. IL-17 could be a suitable therapeutic target for this purpose.

MATERIALS AND METHODS

Human study

All experiments with human samples were conducted in accordance with the ethical principles of the Declaration of Helsinki. The institutional review board of Nagoya City University Graduate School of Medical Sciences approved our protocols (approval number 60-18-0030). All participants provided written informed consent for the use of serum. A single-center, retrospective, cross-sectional observational study was conducted at Nagoya City University Hospital (Nagoya, Japan). Detailed human studies and cytokine measurements in human serum are described in the Supplementary Materials and Methods.

Mice

C57BL/6 (B6, 6 weeks of age, male) mice were obtained from CLEA Japan, Inc. (Tokyo, Japan) or SLC (Shizuoka, Japan). Mice were maintained at the Nagoya City University Animal Facility. All mice were kept under specific pathogen-free conditions. All animal studies were approved by the Institutional Animal Care and Use Committee of Nagoya City University according to Guideline for the Care and Use of Laboratory Animals of Nagoya City University. All procedures were performed under anesthesia with isoflurane (Wako Pure Chemical industries, Osaka, Japan).

IMQ treatment

Mice were treated with IMQ cream (5%; Beselna, Mochida Pharmaceutical Company, Tokyo, Japan) (van der Fits et al., 2009) and assessed by using a clinical scoring system based on the PASI and ear thickness, as previously reported (Shibata et al., 2015). Detailed methods are presented in the Supplementary Materials and Methods.

DNFB application

Details are presented in the Supplementary Materials and Methods.

Glucose tolerance test and insulin tolerance test for mice

Glucose and insulin tolerance tests were performed as previously reported (Bapat et al., 2015; Kubota et al., 2004; Okin and Medzhitov, 2016). Details are presented in the Supplementary Materials and Methods.
**Figure 2.** IMQ-treated mice show hyperglycemia and impairment in insulin secretion. (a) The experimental protocol in developing the psoriatic mouse model. (b) Ear thickness summary from four independent experiments. (c) Clinical scores for disease severity from one experiment. (d) Skin stained with H&E. Scale bar = 100 μm. Data are representative of five independent experiments. (e) Body weight summary from two independent experiments. (f) Summary of fasting glucose levels. (g) Summary of fasting insulin levels. (h) Glucose tolerance test results. (i) Insulin tolerance test results. (j) % Glucose after insulin injection.
Purification of islets from mice and glucose stimulation assay

Islet cells were isolated from the pancreas, as previously described (Nolan and O’Dowd, 2009; Szot et al., 2007) with collagenase P (Sigma-Aldrich, St. Louis, MO) according to the manufacturer’s protocol. Insulin secretion from islet cells was monitored, as previously described (Carter et al., 2009) with some modifications. Detailed methods are presented in the Supplementary Materials and Methods.
Histology and quantification of pancreatic islets

The skin, pancreas, and liver from mice were fixed in 20% formalin (Wako) and embedded in paraffin. Quantification of pancreatic islet cells in Figure 3 was analyzed as previously reported (Kubota et al., 2004; Terauchi et al., 1997). Details are presented in the Supplementary Materials and Methods.

Anti-mouse IL-17A Ab treatment in mice

Anti-mouse IL-17A mAb (clone no. 17F3) was purchased from BioXcell (Lebanon, NH). For isotype control, mouse IgG was purchased from Sigma-Aldrich. Antibody (80 μg [Shibata et al., 2015] or 160 μg/mouse) was administered intraperitoneally every other day during the study period (Figure 4a). Both doses of Ab showed similar results.

Gene expression of cytokine receptors in islet cells from the database

A heatmap of gene expression from the database was generated using available Gene Expression Omnibus data sets (GSE27547 [Han et al., 2011] and Supplementary Table S2 [online]). Details are presented in the Supplementary Materials and Methods.
Statistical analyses

All statistical analyses were performed using Prism (GraphPad Software, San Diego, CA). Before applying parametric analysis methods, we examined normality of the data using the D’Agostino-Pearson normality test. If data significantly deviated from the normal distribution, nonparametric methods were used. P-values were determined using the Student t test unless indicated in each figure. P-values less than 0.05 were considered statistically significant. All numerical data are summarized as mean ± standard deviation.

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CONFLICT OF INTEREST

Akimichi Morita has received research grants, consulting fees, and/or speaker’s fees from AbbVie, Boehringer Ingelheim, Celgene, Eli Lilly, Eisai, Janssen, Kyowa Hakko Kirin, Leo Pharma, Maruhou, Mitsubishi Tanabe, Nichi-Iko, Nippon Kayaku, Novartis, Sun Pharmaceutical Industries Taiho Pharmaceutical, Torii Pharmaceutical, and Ushio. The other authors state no conflict of interest.

ACKNOWLEDGMENTS

We thank Akiko Nishioka and Saori Kasuya for technical assistance, Anthony Bonito and Guido Ferlazzo for critical reading of this manuscript, and Noboru Mizushima for advice.

This work was funded by Grants-in-Aid for Challenging Exploratory Research 26670192, 16K15259, 16K15376 (to MO), and 17K19568 (to TK); Grants-in-Aid for Scientific Research B 16H05177, 17H04088 (to TK) and 17H04242 (to AM) from the Japan Society for the Promotion of Science; a Grant-in-Aid for Scientific Research on Innovative Area 17H05798 from the MEXT; Grants-in-Aid for Research in Nagoya City University; the Uehara Memorial Foundation (TK); the Japanese Diabetes Foundation; the Ichihara International Scholarship Foundation; the Minako Shiookawa Young Investigator’s Award for Collagen Disease Research Japan Rheumatism Foundation; the Toyko Scholarship Foundation the Daido Foundation; and the Kobayashi International Scholarship Foundation. These funding bodies played no role in the study design, data collection or analysis, decision to publish, or preparation of the manuscript.

AUTHOR CONTRIBUTIONS

TK, AM, and SY designed the study. KI, MO, HS, TK, AM, and SY wrote the manuscript. KI, MO, HS, MI, OT, and SY performed the experiments. KI, MO, HS, TK, AM, and SY wrote the preparation of the manuscript.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2019.01.029.

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SUPPLEMENTARY MATERIALS AND METHODS

Human study design

All experiments with human samples were conducted in accordance with the ethical principles of the Declaration of Helsinki. The institutional review board of Nagoya City University Graduate School of Medical Sciences approved our protocols (approval number: 60-18-0030). All participants provided written informed consent for the use of serum. A single-center, retrospective, cross-sectional observational study was conducted at Nagoya City University Hospital (Nagoya, Japan).

Figure 1a shows blood HbA1c levels and PASI scores of patients in the study. During the observational period from January to December 2015, 153 patients with psoriasis visited our hospital for the first time. Almost all first-visit patients were referred from other clinical practitioners. Of the 153 patients, 39 had available data regarding HbA1c levels and PASI scores at their first visit (see Supplementary Table S1). The remaining patients were excluded because of data unavailability. The patients diagnosed or treated with diabetes were included among the 39 patients. First, we analyzed the Figure 1a data from the 39 patients. Then, when we checked who received treatment among the 39 patients, and there were 5 patients taking oral antidiabetic medications but no insulin users. Supplementary Figure S1 shows the data of the 34 remaining patients after those receiving oral antidiabetic medications were excluded.

Two hospitalized psoriasis patients were monitored for their blood insulin concentrations before and during the bath-PUVA therapy during the period from February to April 2017 (Figure 1b). For bath PUVA therapy, patients were treated with 0.0001% psoralen bath (Taisho Pharmaceutical Company, Tokyo, Japan) for 15 minutes, preceding treatment with UVA radiation 5 times weekly. The UVA dose started at 0.5 J/cm² and increased by 0.5 J/cm² every therapeutic day up to 4.0 J/cm². During hospitalization for PUVA therapy, patients were served regular food by the hospital.

All 55 patients who received anti-IL-17A Ab therapy (secukinumab or ixekizumab) during the observational period from March 2015 to December 2017 were registered for the study as shown in Figure 1d, and 14 of them had available data on HbA1c levels and PASI scores before and after therapy (Figure 1c, and see Supplementary Table S1). The time point of “before” was defined as day 0 of anti-IL-17A Ab therapy. The time point of “under treatment” was defined as 4 or more months after the first injection of anti-IL-17A mAb, except for one patient who was observed 2 weeks after injection because of availability. The PASI score of the patient scored at 2 weeks after the first injection declined from 27.8 to 12.6.

Cytokine measurements in human serum

Cytometric Bead Array Human Th1/Th2/Th17 and inflammatory cytokine kits were purchased from BD Biosciences (Franklin Lakes, NJ), and cytokine concentrations were measured according to the manufacturer’s protocol.

IMQ treatment

IMQ treatment was performed as previously reported (van der Fits et al., 2009). Briefly, male B6 mice at 6 weeks of age received a daily topical dose of 62.5 mg IMQ cream (5%; Beselna, Mochida Pharmachemical Company) on shaved back skin and 12.5 mg on the bilateral ears, equaling a daily dose of 3.75 mg of the active compound per mouse, for 8 consecutive days. Control mice were similarly treated with control vehicle cream (hydrophilic cream; Nikko Pharmaceutical, Gifu, Japan). For long-term experiments, IMQ or control vehicle cream was applied on mice similarly, except at 3 times a week for 4 weeks.

Psoriatic disease score for mice

Disease severity was assessed by using a clinical scoring system based on the PASI and ear thickness as previously reported (Shibata et al., 2015). Erythema, induration, and scaling were scored from 0 to 4 (0, none; 1, slight; 2, moderate; 3, marked; 4, very marked), and the cumulative score was used for the total score (0–12). Scoring was performed in a blinded manner by one or two dermatologists, and the mean score was used. Ear thickness of both ears was measured with a Flat Anvil Dial Thickness Gage (Mitutoyo Co., Kawasaki, Japan), and the means of both ears were used.

DNFB application

Mice were applied with 50 μl of 0.5% DNFB (Sigma-Aldrich) in acetone/olive oil (4/1, volume/volume) on day 0 (25 μl on shaved back skin and 12.5 μl on each ear) (Schwarz et al., 2011; Soonthapa et al., 2011). For vehicle control, mice were applied with acetone/olive oil alone. On day 6, glucose tolerance tests were performed. Skin inflammation was confirmed by ear swelling and hematoxylin and eosin histology of back skin.

For the second experiment, mice were applied with 25 μl of 0.5% DNFB in acetone/olive oil (4/1, vol/vol) on shaved ventral skin on day 0 and challenged by application of 0.2% DNFB (25 μl on shaved back skin and 10 μl on each ear on day 7 (Schwarz et al., 2011; Soonthapa et al., 2011). On day 8, glucose tolerance tests were performed, and skin inflammation was confirmed by ear swelling. Nontreated mice were used as control.

Glucose tolerance test and insulin tolerance test for mice

A glucose tolerance test and an insulin tolerance test were performed as previously reported (Bapat et al., 2015; Kubota et al., 2004; Okin and Medzhitov, 2016). Briefly, for the glucose tolerance test, mice were fasted overnight (21 hours), and fasting blood glucose was measured with the Accu-Chek Aviva Nano (Roche DC Japan, Tokyo, Japan) immediately before starting the test. A 1.5-g/kg dose of glucose was then intraperitoneally injected (glucose solution 45%, Sigma-Aldrich). Time of injection was set at time 0 minutes, and blood glucose was measured at 15, 30, 60 and 120 minutes after injection. Blood for measuring insulin concentrations was also obtained from the tail vein. Serum insulin concentration was determined using an Ultra-Sensitive Mouse Insulin ELISA Kit (Morinaga Institute of Biological Science, Yokohama, Japan). For insulin tolerance tests, mice were fasted for 1 hour and then intraperitoneally injected with 1,000 U/kg insulin (Humulin R; Eli Lilly Japan, Hyogo, Japan). After insulin injection, blood glucose was measured as described earlier.
Purification of islets from mice

Islets were isolated from the pancreas, as previously described (Gotoh et al., 1986; Nolan and O’Dowd, 2009; Szot et al., 2007), with collagenase P (Sigma-Aldrich) according to the manufacturer’s protocol. Briefly, the pancreas was injected through the common bile duct with 1.25 mg/ml of collagenase solution and incubated for 40 minutes at 37 °C; it was then passed through a filter (mesh size = 435 μm). The digested pancreas was washed with Hank’s Balanced Salt Solution (HBSS) containing 10 mmol/L N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES) and 5% fetal calf serum, and islet cells were separated by density gradient between 1.069–1.096 in Ficoll 400 (Sigma-Aldrich). After washing several times, cells were used for experiments.

To confirm if there were islet cells within the purified cells, samples were stained with anti-insulin Ab and observed under an immunofluorescent microscope. Cells were fixed in 4% paraformaldehyde (Wako, Osaka, Japan) and permeabilized by 0.1% Triton X-100. After blocking with 10% normal goat serum (Nichirei, Tokyo, Japan), samples were incubated with rabbit anti-insulin Ab (Abcam, Cambridge, UK) or isotype Ab (rabbit IgG, Abcam) and Alexa Fluor 488-goat anti-rabbit IgG (H+L) highly cross-absorbed Ab (Invitrogen, Carlsbad, CA). Nuclei were stained with DAPI (Vector Laboratories, Burlingame, CA). We used an Olympus BX51 microscope or a FV1000 confocal microscope (Olympus, Laboratories, Burlingame, CA). We used an Olympus BX51 microscope with ×4 or ×20 objective lenses and DP Manager and Controller software (Olympus). Whole pancreases and insulin+ islets from IMQ- (n = 3) or vehicle control— (n = 3) treated mice were traced manually, and their dimensions were analyzed by ImageJ 1.51m9 software (National Institutes of Health, Bethesda, MD), using pancreatic sections stained with anti-insulin Ab. The relative volume of islets in a given pancreas was calculated as the proportion of total area of islets in that pancreas.

Glucose-stimulated insulin secretion assay

Insulin secretion from islets was monitored as previously described (Carter et al., 2009) with some modifications. Briefly, after washing several times, purified islet cells were suspended in RPMI containing 3 mmol/L glucose solution and transferred to a 24-well Transwell plate (Corning, Corning, NY). Cells were preincubated with RPMI containing 3 mmol/L glucose solution for 30 minutes. After preincubation, the islets with Transwells were moved into a new well and stimulated with 3 mmol/L glucose in RPMI. After 60 minutes, the supernatants were collected to measure insulin at the basal state. The same islets within Transwells were again moved into another well and further stimulated with 20 mmol/L glucose in RPMI. After 60 minutes, the supernatants were collected to measure insulin at the basal state. Insulin concentrations were measured with ELISA, as described earlier. The stimulation index was considered as the ratio of the insulin content of the medium containing 20 mmol/L glucose over that of the medium containing 3 mmol/L glucose for the same batch of islets. Data are the summary of three independent experiments.

Histology

Skin sections were fixed in 20% formalin (Wako) and embedded in paraffin. Fixed sections were stained with hematoxylin and eosin (Wako and Muto Pure Chemicals). Pancreas and liver samples were fixed in 20% formalin and embedded in paraffin. Fixed sections were stained with rabbit anti-insulin mAb (EPR17539, Abcam) or rabbit IsoType IgG (Abcam) and horseradish peroxidase—anti-rabbit IgG antibody (Vectastain Elite ABC-HRP kit; Vector Laboratories). We used an Olympus BX51 microscope with ×4 or ×20 objective lenses and DP Manager and Controller software (Olympus). Pancreas and liver sections stained by hematoxylin and eosin were scored in a blinded manner by one or two dermatologists.

Mouse serum biochemistry measurements

Blood was collected from the vena cava when mice were killed under anesthesia. Serum was collected after centrifuge and sent to the Oriental Yeast Company (Tokyo, Japan) to measure alanine aminotransferase, aspartate aminotransferase, and lipase levels.

Quantification of pancreatic islets

Quantification of pancreatic islets in Figure 3 were analyzed as previously reported (Kubota et al., 2004; Terauchi et al., 1997). Briefly, pancreatic sections were cut vertical to the pancreatic duct, fixed and stained as described, and imaged on an Olympus AX80 microscope with ×2 objective lenses and DP Manager and Controller software (Olympus). Whole pancreases and insulin+ islets from IMQ- (n = 3) or vehicle control— (n = 3) treated mice were traced manually, and their dimensions were analyzed by ImageJ 1.51m9 software (National Institutes of Health, Bethesda, MD), using pancreatic sections stained with anti-insulin Ab. The relative volume of islets in a given pancreas was calculated as the proportion of total area of islets in that pancreas.

Gene expression of cytokine receptors in islet cells from the database

A heatmap of gene expression from the database was generated with available Gene Expression Omnibus datasets (GSE27547 [Han et al., 2011]). Gene expression levels of proinflammatory cytokine receptors in freshly isolated pancreatic islets from mice (day 0) were used (Han et al., 2011). The Affymetrix Probe Set identifications of Il17ra, Il17rc, Il1r2, Tnfrsf1a, Il2ra, Il1r2, and Insr from platforms (GPL1261) listed in Supplementary Table S2 were used to choose data, and their GenBank accession numbers are also presented in Supplementary Table S2. The heat map was generated based on already processed log2 GC content—adjusted robust multarray averaging signals from samples GSM680266, GSM680267, GSM680268, GSM680269, and GSM680270 (Han et al., 2011).

SUPPLEMENTARY REFERENCES


Supplementary Figure S1. Severity of psoriasis correlates with hyperglycemia in patients without antidiabetic medication. As in Figure 1a, but patients treated with antidiabetic medication were excluded, and the correlation between HbA1c levels and PASI (left) or erythema component of PASI (middle) scores was similarly analyzed. The patients were divided into two groups defined as PASI score < 20 or ≥ 20, and HbA1c levels of each individual were plotted. (right).

Supplementary Figure S2. There was no correlation between delta PASI score and delta HbA1c level in patients with psoriasis who underwent anti-IL-17 mAb therapy. As in Figure 1c, but the correlation between delta PASI score and delta HbA1c level was analyzed before and under treatment with anti-IL-17A mAb.

Supplementary Figure S3. The correlation between severity of psoriasis and HbA1c level in patients with psoriasis who underwent anti-IL-17A mAb therapy. As in Figure 1c, the correlation between PASI scores and HbA1c levels in patients with psoriasis was analyzed before (left) and under treatment with anti-IL-17A mAb (right).
Supplementary Figure S4. Skin inflammation induced by hapten-application does not cause hyperglycemia. (a) The experimental protocol in developing the hapten-induced skin inflammation is shown. Back skin and ears were applied with 0.5% DNFB to induce skin inflammation (n = 3). For control, mice were applied with vehicle alone (n = 3). Details are described in the Supplementary Methods. (b) To confirm inflammation, the thickness of each ear was measured on day 6. (c) Glucose tolerance test was performed as in Figure 2h on day 6. Statistical analysis was performed by two-way ANOVA and Tukey’s multiple comparisons post-hoc test. There was no significant difference. Data are from one experiment. (d) H&E staining on back skin. Scale bar = 50 μm.
Supplementary Figure S5. Skin inflammation induced by contact hypersensitivity (CHS) does not cause hyperglycemia. (a) The experimental protocol in CHS-induced skin inflammation is shown. Mice were applied with 0.5% DNFB in acetone/olive oil on shaved ventral skin. They were challenged with 0.2% DNFB at day 7 (25 µL on shaved back and 10 µL on each ear). On day 8, glucose tolerance test was performed after overnight fasting. The swelling of each was measured on day 8. Non-treated mice were used as control. Details are described in the Supplementary Methods. (b) To confirm inflammation, the thickness of each ear was measured on day 8. (c) Glucose tolerance test was performed as in Figure 2h on day 8. Data are from one experiment.

Supplementary Figure S6. H&E and immunofluorescent staining of pancreatic islets from mice treated as described in Figure 3a. (a) H&E staining of pancreas in psoriatic mice. Images were taken at low power field. Scale bar = 200 µm. Islets are indicated by arrows. Data are representative of five independent experiments (n = 14). (b) Immunofluorescence staining for insulin (Alexa Fluor 488, green) in islets isolated from psoriatic mice. Scale bar = 20 µm. (Insulin n = 2, isotype n = 2).
Supplementary Figure S7. Histology of the liver from IMQ-treated or control mice. Mice treated with IMQ (n = 3) or control vehicle (n = 4) as in Figure 2a were sacrificed on day 9, and liver samples were stained with H&E. Pictures taken at lower power (x40, scale bar = 200 μm, left) or higher power (x200, scale bar = 50 μm, right) were shown for the individual mouse. Data are from one experiment.

Supplementary Figure S8. Serum aminotransferase and lipase levels of IMQ-treated or control mice. Mice treated with IMQ (n = 3) or control vehicle (n = 4) as if Figure 2a were sacrificed on day 9, and serum samples obtained from the inferior venous cava were analyzed for AST, ALT, and lipase (Lip). Data are from one experiment.
**Supplementary Table S1. Clinical information about patients**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Figure 1a (n = 39)</th>
<th>Figure 1c (n = 14)</th>
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<tr>
<td>Age median, years</td>
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<tr>
<td>Male sex, n (%)</td>
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<td>13 (92.9)</td>
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<tr>
<td>BMI median, kg/m²</td>
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<tr>
<td>HbA1c median, %</td>
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<td>PASI median score</td>
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<td>19.3 (before)</td>
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<td>Oral antidiabetic medication, n (%)</td>
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<td>3 (21.4)</td>
</tr>
<tr>
<td>Insulin treatment, n</td>
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<td>0</td>
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</table>

Abbreviations: BMI, body mass index; HbA1c, hemoglobin A1c; PASI, Psoriasis Area and Severity Index.

**Supplementary Table S2. Affimextrix Probe set ID used to choose data from GSE27547 and their GenBank accession numbers**

<table>
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<th>Gene Symbol</th>
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<th>GenBank Accession Number</th>
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<td>Il17ra</td>
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1Han et al. (2011).