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Original article

Type 1 innate lymphoid cells are associated with type 2 diabetes

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ABSTRACT

Aim. – Type 1 innate lymphoid cells (ILC1s) play a major role in regulating systemic inflammatory diseases. However, the relationship between ILC1s and type 2 diabetes (T2D) remains unclear. Thus, the present study investigated the relationship between ILC1s and glucose homoeostasis in humans.
Methods. – A total of 37 newly diagnosed T2D patients and 32 subjects with normal glucose tolerance (NGT), matched for age and body mass index (BMI), were enrolled in the study. Flow cytometric analysis of ILC1s derived from peripheral blood mononuclear cells (PBMCs) and omental adipose tissue was performed.
Results. – T2D patients displayed greater numbers and frequencies of circulating and adipose tissue ILC1s ($P < 0.05$) compared with NGT subjects, and the two types of ILC1s correlated positively with each other. Circulating ILC1s were positively associated with glycated haemoglobin (HbA_{1c}), fasting plasma glucose (FPG), homoeostasis model assessment for insulin resistance (HOMA-IR), adipose tissue insulin resistance index (Adipo-IR) and serum free fatty acids (FFAs). A logistic regression model revealed that patients with higher ILC1 levels exhibited a 13.481-fold greater risk of developing T2D.
Conclusion. – This study is the first to provide evidence that ILC1 abnormalities are involved in the development of diabetes. The data also suggest a potential role of ILC1s as therapeutic indicators in the treatment of T2D.

Introduction

Type 2 diabetes (T2D) has become a major worldwide epidemic [1]. Thus, understanding the underlying pathogenic mechanisms of T2D is an urgent prerequisite for both its prevention and treatment. Chronic low-grade inflammation, triggered by an imbalance between pro- and anti-inflammatory cytokine levels in adipose tissue, is a critical characteristic of T2D [2]. Emerging evidence shows that disruption of both innate and adaptive immune systems in adipose tissue drives the progression of inflammation and subsequent insulin resistance [3]. Therefore, identification of the precise alterations of immune cells in the development of T2D is crucial.

Innate lymphoid cells (ILCs) are emerging as essential effectors of innate immunity via the rapid production of both pro-inflammatory and regulatory cytokines [4]. Mature ILCs can be further classified into types 1, 2 and 3, based on their differing expression of transcription factors and cell surface markers as well as their cytokine production [4,5]. Notably, a subset of type 1 ILCs, dubbed 'CD127 + ILC1s', has been reported to be critically involved in the pathogenesis of chronic inflammatory diseases such as

Abbreviations: ILC1s, type 1 innate lymphoid cells; T2D, type 2 diabetes; NGT, normal glucose tolerance; BMI, body mass index; PBMCs, peripheral blood mononuclear cells; SVF, stromal vascular fraction; HbA1c, glycated haemoglobin; FPG, fasting plasma glucose; HOMA-IR, homoeostasis model assessment of insulin resistance; Adipo-IR, adipose tissue insulin resistance index; FFA, free fatty acid; OGTT, oral glucose tolerance test; WHR, waist-to-hip ratio; ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transferase; eGFR, estimated glomerular filtration rate; CKD-Epi, chronic kidney disease epidemiology collaboration; CBC, complete blood count; TGs, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HOMA-β, homoeostasis model assessment of β-cell function; CPTs, cell preparation tubes; PBS, phosphate-buffered saline; FBS, fetal bovine serum; RBC, red blood cell; HFD, high-fat diet; WT, wild-type; OR, odds ratio; CI, confidence interval; NK, natural killer; Eomes, eomesodermin.

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Crohn's disease, multiple sclerosis and psoriasis [6–8]. More important, an earlier animal study demonstrated that the accumulation of adipose ILC1s can trigger local inflammation and systemic insulin resistance [9]. However, a direct relationship between ILC1s and T2D has yet to be elucidated.

In the present study, which included both subjects with normal glucose tolerance (NGT) and T2D patients, and aimed to acquire new insights into the role of ILC1s in the development of T2D, the ILC1-mediated regulation of T2D was investigated. Blood and omental adipose tissue samples were collected for evaluation of ILC1s by flow cytometry.

Materials and methods

Patient recruitment

The study was conducted between May 2017 and January 2018. A total of 37 newly diagnosed T2D patients and 32 NGT subjects, matched for age and body mass index (BMI), were recruited (Fig. 1). In addition, all participants completed questionnaires to assess their medical history, and underwent anthropometric measurement and 75-g oral glucose tolerance tests (OGTTs). Body weight was measured to the nearest 0.1 kg with an electronic digital scale, while height was assessed to the nearest 1 cm without shoes using a fixed stadiometer. BMI was calculated as weight (kg)/height (m^2). Waist and hip circumferences were measured using a flexible measuring tape, and followed by calculation of waist-to-hip ratios (WHRs). T2D was diagnosed according to 2018 American Diabetes Association criteria [fasting plasma glucose (FPG) $\geq$ 7.0 mmol/L, 2-h glucose $\geq$ 11.1 mmol/L or glycated haemoglobin (HbA_{1c}) $\geq$ 6.5%] [10]. Blood pressure was measured twice on the upper right arm and the average of the two measures used for analyses.

Study exclusion criteria were: prediabetes (FPG $\geq$ 5.6 but $<$ 7.0 mmol/L, 2-h glucose $\geq$ 7.8 but $<$ 11.1 mmol/L or HbA_{1c} $\geq$ 5.7% but $<$ 6.5%) [10]; type 1 diabetes (T1D); medical history of antihyperglycaemic, non-steroidal or steroidal anti-inflammatory drug use in the past 3 months; acute infection in the past 3 months; stroke or myocardial infarction; autoimmune disorders such as rheumatoid arthritis and systemic lupus erythematosus; malignant tumours; chronic digestive diseases such as Crohn's disease and colitis gravis; abnormal liver or kidney dysfunction with alanine transaminase (ALT) or aspartate transaminase (AST) 2.5-fold higher than the normal range or an estimated glomerular filtration rate (eGFR) $<$ 60 mL/min/1.73 m^2, according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation; pregnancy; and other endocrine system disorders such thyroid disease and Cushing's syndrome.

The study protocol was approved by the Ethics Review Committee of Drum Tower Hospital affiliated to Nanjing University Medical School. All recruited participants gave their written informed consent before enrollment in the study. The registered clinical trial number is NCT03296605.

Clinical and biological assessments

All participants underwent OGTTs and had venous blood samples taken from their antecubital vein. Blood glucose and insulin levels were measured at 0 and 120 min. Fasting blood samples were also collected for estimation of HbA1c, complete blood counts (CBCs), triglycerides (TGs), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), ALT, AST and free fatty acids (FFAs).

Plasma glucose concentration was assessed by the hexokinase method using an automated clinical chemistry analyzer (TBA-200FR, Toshiba Corporation, Tokyo, Japan). Insulin concentration was determined by chemiluminescence immunoassay (cobas 6000 e601, Roche Diagnostics, Risch-Rotkreuz, Switzerland). HbA1c was assessed using high-performance liquid chromatography (HPLC) and a haemoglobin analyzer (D-10, Bio-Rad Laboratories, Hercules, CA, USA). TGs, TC, HDL-C, LDL-C, ALT, AST and gamma-glutamyl transferase (GGT) concentrations were estimated by immunoassay (cobas 6000 e601, Roche Diagnostics), while FFAs were measured using an enzymatic method (DiaSys Diagnostic Systems GmbH, Holzheim, Germany). Homoeostasis model assessment of insulin resistance (HOMA-IR) was derived by the following formula: [fasting serum insulin (mU/L) $\times$ fasting glucose (mmol/L)]/22.5 [11], while beta-cell function (HOMA-β) was calculated by the following formula: fasting serum insulin (μU/mL) $\times$ 20/[FPG (mmol/L) $-$ 3.5] [12]. The adipose tissue insulin resistance index (Adipo-IR) was calculated as: FFAs (mmol/L) $\times$ insulin concentration (mIU/mL) [13].

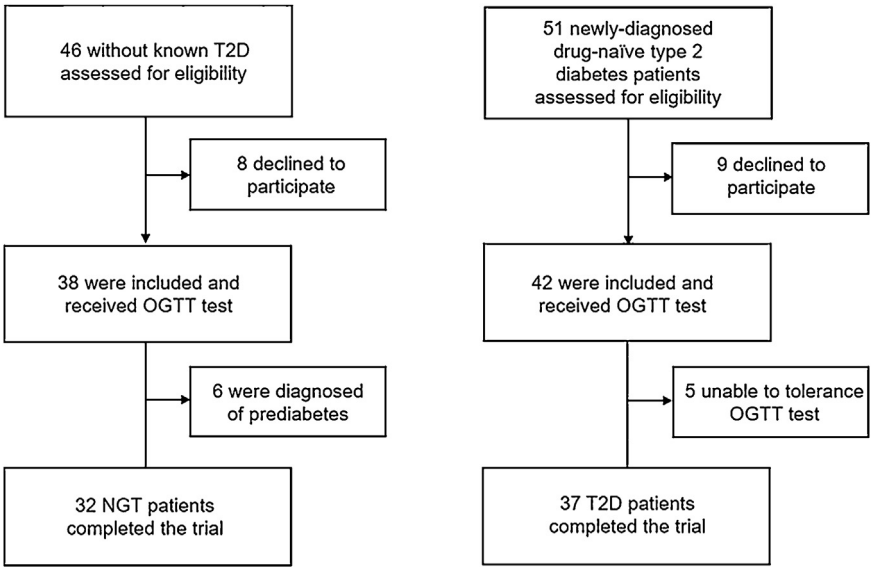

Fig. 1. Flow chart of participant selection for enrollment in the study and analyses.

Peripheral blood mononuclear cell (PBMC) isolation

PBMCs were isolated using Vacutainer cell preparation tubes (CPTs) (Becton Dickinson & Company, Franklin Lakes, NJ, USA) from fasting blood samples taken from all study participants. After centrifugation at 2700 rpm for 30 min, cells in the resultant supernatants were collected, washed with phosphate-buffered saline (PBS) two times and gradually frozen over 8 h in a freezing buffer containing 10% dimethyl sulphoxide (DMSO) and 90% fetal bovine serum (FBS). Samples were then stored in liquid nitrogen until needed for analysis of ILC1s.

Adipose tissue biopsy and stromal vascular fraction (SVF) preparation

From among all study participants, a subgroup of 22 obese subjects underwent bariatric surgery (laparoscopic Roux-en-Y gastric bypass). Their baseline characteristics are presented in Table S1 (see supplementary data associated with this article online). Omental adipose tissue samples were collected from these 22 subjects during the procedure (10 had NGT, 12 had T2D). These adipose tissue samples were put on ice and immediately transported to our laboratory.

For SVF preparation, samples were digested with 0.1% type II collagenase (Sigma-Aldrich Corporation, St. Louis, MO, USA). After agitation for 1 h at 180 rpm in a MACSmix Tube Rotator (Miltenyi Biotec, Bergisch Gladbach, Germany), all specimens were filtered through a 100-μm nylon mesh and centrifuged at 1500 rpm for 10 min. Supernatants were aspirated, and the pellets resuspended in red blood cell (RBC) lysis buffer (General Electric Company, Fairfield, CT, USA). Subsequently, the SVFs were washed with PBS and incubated with antibodies for detection of ILC1s.

Flow cytometry

All obtained PBMCs and SVFs were stained with the following antibodies for flow cytometry analysis: CD3 (UCHT1), CD19 (HIB19), CD16 (B73.1), CD45 (2D1), CD127 (HIL-7R-M21), CD117 (YB5-B8), CRTH2 (BM16) and NKP44 (p44-8) (all from BD Biosciences, Franklin Lakes, NJ, USA); and CD11c (Bu15), CD5 (L17F12), TCRαβ (IP26) and FCERIA (AER-37) (all from BioLegend, San Diego, CA, USA). ILC1s were identified as lineage-negative (Lin-) for CD3, CD19, CD16, CD11c, CD5, TCRαβ and FCERIA, and also as $CD45^+$, $CD127^+$, $CD117^-$, $NKP44^-$ and $CRTH2^-$ (Fig. 2A). Results are expressed as percentages of Lin^- peripheral blood cells, with absolute cell numbers adjusted for lymphocyte counts. Cell surface labelling was conducted as described previously [6]: in brief, cells were washed in PBS and incubated in a permeabilization solution at 37 °C for 30 min; 7-aminoactinomycin D (7-AAD, BD Biosciences) was then used to gate live cells. Absolute cell numbers were measured by a flow cytometer (LSR II Fortessa, BD Biosciences) and the data analyzed using either FACSDiva software (BD Biosciences) or FlowJo version 9.6.4 software (Tree Star, Inc., Ashland, OR, USA).

Statistical analysis

Continuous variables are presented as means ± standard error of means (SEM), whereas categorical variables are expressed as numbers and percentages. Statistical normality was assessed by Kolmogorov–Smirnov tests; group differences were evaluated by either Student's *t*-test or non-parametric Mann–Whitney *U*-test, depending on data distribution. Categorical variables were analyzed by Chi^2 test. ILC1 numbers were associated with other metabolic variables and presented as Spearman's correlation coefficients. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to determine the relationship between absolute ILC1 numbers and T2D

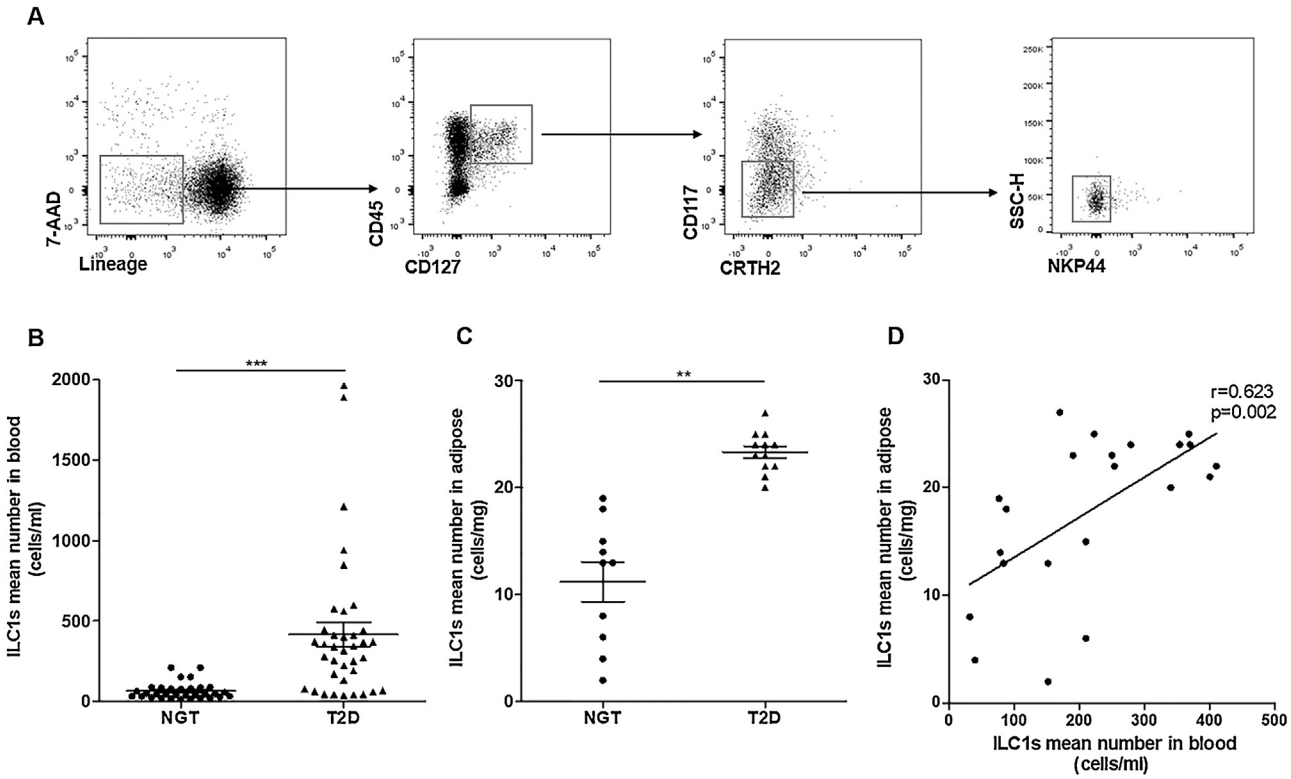

Fig. 2. Type 2 diabetes (T2D) patients had higher levels of circulating and adipose type 1 innate lymphoid cells (ILC1s). A. Analysis of ILC1s by flow cytometry. B. Absolute numbers of circulating ILC1s in normal glucose tolerance (NGT) subjects (n = 32) and T2D patients (n = 37). C. Absolute numbers of adipose ILC1s in NGT (n = 10) and T2D (n = 12). D. Correlation between adipose tissue and circulating ILC1 numbers (n = 69). $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ (Mann–Whitney *U*-test); data are means ± SEM.

after adjusting for age and gender. Differences were considered statistically significant when P was < 0.05. All statistical analyses were performed using SPSS version 19.0 software (IBM SPSS Statistics, Armonk, NY, USA).

Results

T2D patients exhibit high levels of circulating and adipose ILC1s

Clinical and biochemical characteristics of our study participants are summarized in Table 1. No differences were observed in either age or BMI between NGT and T2D groups. However, T2D patients showed significantly higher levels of HbA1c, FPG and TGs, and HOMA-IR and Adipo-IR Scores, and lower HDL-C and HOMA-β Scores compared with controls (all $P < 0.05$). Also, as shown in Fig. 2A, ILC1s were quantified in both NGT and T2D groups. However, compared with the NGT group, both the absolute numbers (cells/mL) (357 ± 78 vs. 53 ± 6; $P < 0.001$; Fig. 2B) and frequencies ($2.67 \pm 0.52\%$ vs. $0.26 \pm 0.03\%$; $P < 0.001$; Fig. S1 A; see supplementary data associated with this article online) of circulating ILC1s were markedly higher in the T2D group.

Moreover, significantly larger numbers (23 ± 1 vs. 11 ± 1; $P < 0.001$; Fig. 2C) and frequencies ($1.70 \pm 0.59\%$ vs. $0.37 \pm 0.12\%$; $P = 0.046$; Fig. S1 B) of ILC1s in omental adipose tissue (cells/mg) were observed in T2D patients. More important, both circulating and adipose ILC1 numbers were positively correlated with each other ($r = 0.623$, $P = 0.002$; Fig. 2D).

Increased circulating ILC1 levels are related to glycaemic disturbances

Based on the median number of circulating ILC1s, all participants were divided into either a low or high ILC1 group; their clinical and biological parameters are summarized in Table S2 (see supplementary data associated with this article online). Intriguingly, compared with the low-ILC1 group, those in the high-ILC1 group had higher HbA1c ($8.77 \pm 0.37\%$ vs. $6.73 \pm 0.40\%$, respectively; $P < 0.001$) and FPG (11.62 ± 0.95 vs. 7.10 ± 0.74, respectively; $P = 0.164$) levels. Moreover, patients with higher vs. lower ILC1 levels displayed higher HOMA-IR (6.85 ± 0.72 vs. 2.64 ± 0.37, respectively; $P < 0.001$) and Adipo-IR (9.45 ± 1.54 vs. 2.46 ± 0.48, respectively; $P < 0.001$) Scores, and FFA levels (0.55 ± 0.03 vs. 0.35 ± 0.04, respectively; $P < 0.001$).

Circulating ILC1 levels are markers of T2D

When correlations between circulating ILC1 numbers and various clinical parameters were analyzed, ILC1s were positively associated with HbA_{1c} ($r = 0.513$, $P < 0.001$; Fig. 3A), FPG ($r = 0.587$, $P < 0.001$; Fig. 3), HOMA-IR ($r = 0.540$, $P < 0.001$; Fig. 3C), Adipo-IR ($r = 0.439$, $P < 0.001$; Fig. 3D) and serum FFAs ($r = 0.388$, $P = 0.001$; Fig. 3E). In addition, when two patients with outlier values were removed from the analysis, ILC1s were still positively associated with HbA_{1c} ($r = 0.487$, $P < 0.001$), FPG ($r = 0.560$, $P = 0.001$), HOMA-IR ($r = 0.526$, $P < 0.001$), Adipo-IR ($r = 0.456$, $P < 0.001$) and serum FFAs ($r = 0.410$, $P = 0.001$). Moreover, there was a positive correlation between circulating ILC1s and ALT ($r = 0.288$, $P = 0.016$), but not with either AST ($r = 0.170$, $P = 0.162$) or GGT ($r = 0.189$, $P = 0.171$) (Table S3; see supplementary data associated with this article online).

On analyzing ORs (95% CIs) for developing T2D according to the median number of circulating ILC1s compared with lower numbers of ILC1s, patients with higher numbers presented with a 13.481-fold greater risk of T2D (Table 2). In addition, based on receiver operating characteristic (ROC) curve analyses, circulating ILC1 levels were good indicators of the presence of T2D (concordance or C statistic: 0.785, 95% CI: 0.672–0.897; Table 2).

Discussion

The major finding of our present study was that both circulating and adipose ILC1s were markedly increased in T2D patients, and ILC1 levels were closely associated with glycaemic disturbances and, thus, important markers of T2D.

Table 1
Clinical and biological parameters of study participants with normal glucose tolerance (NGT) and type 2 diabetes (T2D).

	NGT	T2D	P^a
Subjects, *n*	32	37	
Age, years	43.5 (39.3, 46.8)	41.0 (31.0, 51.0)	0.962
Male, %	10 (31.25)	22 (59.5)	0.019
Body mass index, kg/m^2	26.4 (22.5, 31.0)	27.1 (23.5, 36.7)	0.594
Waist circumference, cm	93.5 (82.0, 124.0)	96.0 (87.0, 116.5)	0.716
Waist-to-hip ratio	0.9 (0.8, 1.0)	1.0 (0.9, 1.0)	0.005
HbA_{1c}, %	5.6 (5.2, 5.8)	9.5 (8.4, 11.0)	0.001
Fasting glucose, mmol/L	4.9 (4.7, 5.3)	13.0 (9.9, 15.9)	0.001
Fasting insulin, mIU/mL	8.5 (4.3, 16.0)	9.1 (4.4, 20.0)	0.522
HOMA-IR	1.9 (0.9, 3.6)	5.9 (2.7, 9.7)	0.001
HOMA-β	112.9 (68.1, 204.0)	15.7 (6.6, 56.3)	0.008
Alanine transaminase, U/L	21.9 (16.7, 36.6)	35.2 (23.4, 52.8)	0.212
Aspartate transaminase, U/L	20.6 (17.6, 24.6)	21.0 (17.7, 32.9)	0.232
Gamma-glutamyl transferase, U/L	27.7 (17.9, 39.0)	38.6 (25.7, 56.3)	0.138
Triglycerides, mmol/L	1.3 (0.8, 1.7)	1.8 (1.4, 3.0)	0.002
Total cholesterol, mmol/L	4.8 (4.1, 5.2)	4.7 (4.1, 5.5)	0.393
HDL cholesterol, mmol/L	1.3 (1.2, 1.6)	1.0 (0.9, 0.1)	0.001
LDL cholesterol, mmol/L	2.8 (2.1, 3.3)	2.8 (2.4, 3.6)	0.161
Free fatty acids, mmol/L	0.5 (0.2, 0.6)	0.5 (0.3, 0.6)	0.701
Adipo-IR	2.0 (1.4, 3.9)	3.3 (1.4, 12.6)	0.047
Systolic blood pressure, mmHg	132.5 (114.8, 147.8)	132.0 (123.0, 143.0)	0.545
Diastolic blood pressure, mmHg	78.0 (71.5, 85.8)	84.0 (76.0, 90.0)	0.061
WBC, $\times 10^9$/L	5.3 (4.2, 6.6)	6.5 (5.5, 8.6)	0.030
Neutrophils, %	57.3 (51.1, 61.2)	58.9 (53.4, 66.4)	0.080
Lymphocytes, %	33.0 (30.0, 37.3)	31.7 (27.4, 37.5)	0.318

Data are medians (interquartile range) or *n* (%); HOMA-IR/-β: homoeostasis model assessment of insulin resistance/β-cell function; HDL/LDL: high-density/low-density lipoprotein; Adipo-IR: adipose tissue insulin resistance index; WBC: white blood cell.
[a] NGT vs. T2D (Student's *t*-test for continuous data, Chi^2 test for categorical variables).

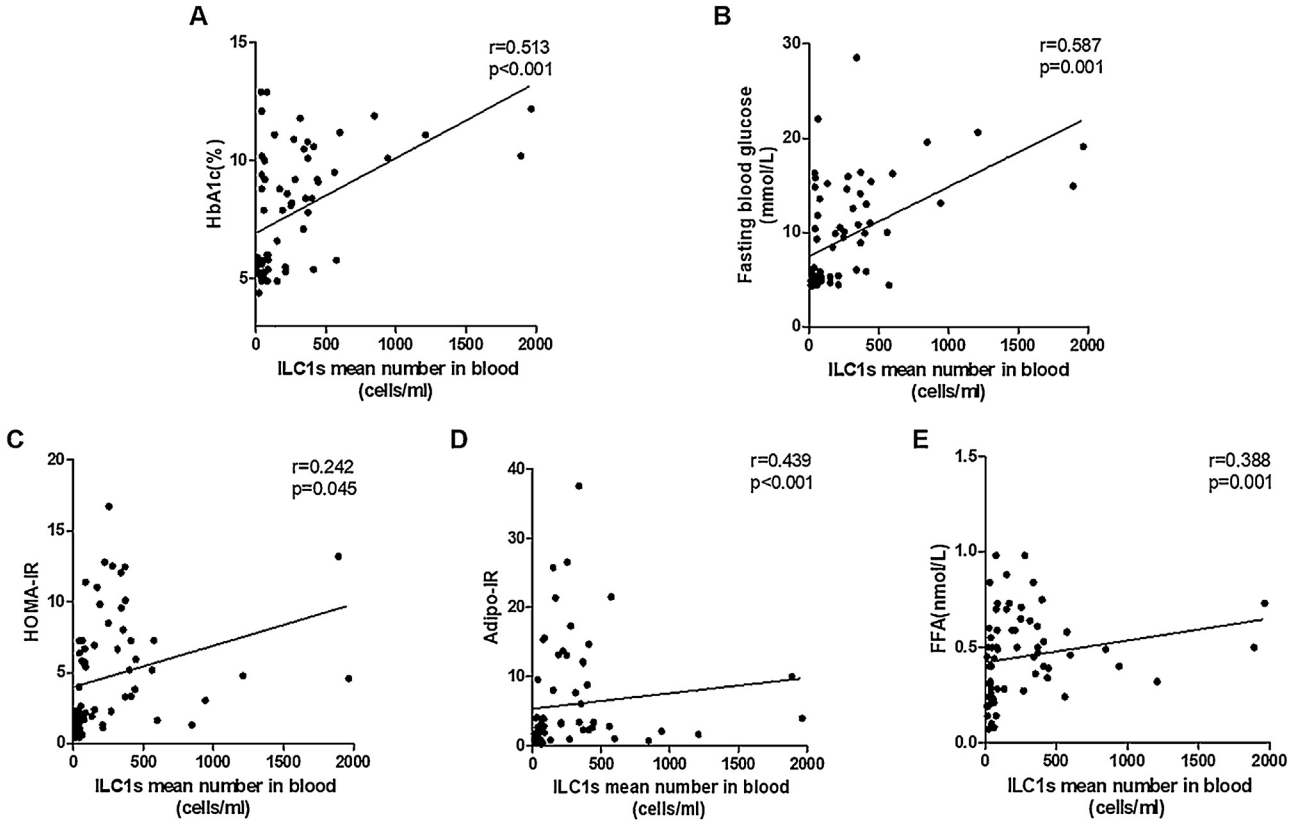

Fig. 3. Correlations between circulating type 1 innate lymphoid cells (ILC1s) and: (A) HbA_{1c} (n = 69); (B) fasting blood glucose (n = 69); (C) homoeostasis model assessment of insulin resistance (HOMA-IR; n = 69); (D) adipose tissue insulin resistance index (Adipo-IR; n = 69); and (E) free fatty acids (FFAs; n = 69). $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ (Spearman's correlation coefficient).

Table 2
Odds ratio (OR) and 95% confidential interval (CI) for developing type 2 diabetes according to median (87 cells/mg) type 1 innate lymphoid cell (ILC1) level in a binary logistic regression model.

	OR (95% CI)	*P*	C statistic (95% CI)
High ILC1 levels	13.481 (4.215–43.124)	< 0.001	0.785 (0.672–0.897)

Chronic low-grade inflammation in adipose tissue is considered the primary cause of insulin resistance [14], a state characterized not only by an increase of pro-inflammatory mediators secreted by adipocytes, but also by the presence of immune-system cells that can potentially amplify the inflammatory process. Most notably, ILC1 levels constitute a critical component of the innate immune system involved in the regulation of systemic inflammatory disorders [15]. An earlier animal study had revealed that, when purified adipose ILC1s taken from wild-type mice fed a high-fat diet were transfected into $Rag2^{-/-}$ mice [lacking T, B and natural killer T (NKT) cells], the recipient mice displayed high levels of fasting blood insulin, glucose intolerance and impaired insulin sensitivity [9]. In fact, based on previous animal studies, it may be speculated that ILC1s are key regulators of the development of T2D. In humans, visceral adipose tissue is populated by anti-inflammatory immune cells that play a major role in regulating adipose metabolic function [16]. However, changes in the number and activity of immune cells are responsible for progression of insulin resistance through the release of pro-inflammatory cytokines [17,18]. In the present study, higher levels of adipose ILC1s were observed in T2D patients than in NGT subjects (Fig. 2C), thereby indicating that adipose tissue in T2D patients is enriched with ILC1s.

In addition, on comparing circulating ILC1s in T2D patients and NGT subjects of similar age and BMI, our findings revealed that T2D patients had greater circulating numbers (Fig. 2B) and frequencies of ILC1s compared with NGT subjects. Furthermore, these circulating ILC1 numbers were positively associated with HbA_{1c} and FPG. According to a logistic regression model stratified according to the median ILC1 number, patients with higher numbers had a 13.481-fold greater risk of T2D, while ROC curve analysis revealed that circulating ILC1 levels were good indicators of T2D (Table 2). Considered together, these results highlight the link between ILC1s and blood glucose, thereby suggesting that elevated levels of ILC1s may be contributing to the development of T2D.

Moreover, our present study has also provided evidence that clarifies why ILC1 abnormalities increase blood glucose. Patients with higher circulating ILC1 levels had higher FFA, Adipo-IR and HOMA-IR values, and circulating ILC1 levels were likewise positively associated with these values. As a strongly positive correlation was also observed between circulating and adipose ILC1 levels, our data confirm that elevated levels of adipose tissue ILC1s could be contributing to systemic insulin resistance and subsequent hyperglycaemia by increasing the release of FFAs from adipose tissues, thereby potentiating adipose tissue insulin resistance. These data suggest that the pattern of ILC1 activation in adipose tissue reflects the regional pro-inflammatory environment, as blood glucose levels were also increased.

The present study has, nevertheless, several limitations. First, phenotypical and functional heterogeneity in ILC1 subsets was found in various tissues and species, making this a controversial strategy. Our type 1 ILCs included both ILC1s and NK cells [19]. However, in humans, ILC1s in contrast to NK cells lack cell surface markers such as CD56, CD16 and CD94, as well as

eomesodermin (Eomes) transcription factor [19,20]. ILC1s also do not express cytotoxic molecules such as perforin and granzyme B [21]. Thus, although our strategy worked for most markers of ILC1s, it would have been more convincing to identify ILC1s more comprehensively. Second, a previous study had described a subset of ILC1s located in the intestinal epithelium. This subset expressed NKp44 + CD103+, but lower levels of CD127 [22]. As NKp44 + CD103+ contribute to inflammatory bowel disease [23], further study is now required to clarify the distinct role of ILC1 subsets sited in peripheral blood and adipose tissue. Third, while adipose ILC1s were measured in NGT and T2D patients, for ethical reasons, adipose tissue samples could only be acquired from patients during bariatric surgery, thereby potentially restricting the diversity of our samples. Finally, as this was a cross-sectional study, no causal relationship between ILC1s and blood glucose could be determined. Further animal studies are now required to investigate the underlying mechanism(s) of the increased levels of ILC1s in T2D patients.

Conclusion

This study is the first to demonstrate the clinical association between ILC1s and T2D. In fact, the results suggest that ILC1s are closely related to elevated blood glucose levels and, thus, may constitute important markers of T2D. In addition, ILC1s also have potential implications for the treatment of T2D.

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Authors' contributions

F.C.L. conceived the study, designed and performed the experiments, interpreted the data and wrote the manuscript. H.D.W. contributed to the study design, performed experiments, and edited and revised the manuscript. X.Y. performed experiments. W.H.F., X.T.S., C.P.J., X.H.C., P.Z.Z., C.J. and Y.W. performed the surgery and collected samples from patients. Y.B. and D.L.Z. supervised the project, designed experiments, interpreted results and co-wrote the manuscript. Y.B. and D.L.Z. are the guarantors of this work and, as such, had full access to all study data, and take responsibility for the integrity of the data and accuracy of the data analyses. All authors had the opportunity to comment on the manuscript.

Disclosure of interest

The authors declare that they have no competing interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found, in the online version, at https://doi.org/10.1016/j.diabet.2018.08.005.

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