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Acute pancreatic beta cell apoptosis by IL-1β is responsible for postburn hyperglycemia: Evidence from humans and mice

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ABSTRACT

Background: Acute hyperglycemia is regarded as a risk factor for critically ill patients; however, insufficient understanding of its nature and underlying mechanisms hinders widespread adoption of glycemic control in critical care units.

Methods: A single center, prospective cohort study recruiting 107 burn patients and 62 controls was conducted to characterize the early phase of acute hyperglycemia in burn patients. A total of 1643 blood samples were collected and analyzed over the entire postburn 200 h. A mouse severe burn model was used to study the underlying mechanisms of acute hyperglycemia postburn.

Results: The dynamic change of postburn blood glucose represented a distinctive pattern in amplitude and duration that was in parallel with the degree of burn injury. Multiple linear regressions revealed that serum insulin, glucagon and glucocorticoid were the major factors affecting blood glucose postburn. Particularly, extensive burns impaired capacity and responsiveness of pancreatic insulin secretion, which was associated with increased serum IL-1β in burn patients. Mechanistically, acute IL-1β elevation specifically induced pancreatic beta cell apoptosis and dampened capacity of insulin secretion, leading to postburn hyperglycemia in burned mice. More importantly, inhibition of IL-1β not only alleviated pancreatic beta cell apoptosis, but also attenuated hyperglycemia and improved survival of burned mice.

Conclusions: Our findings reveal a novel mechanism of acute hyperglycemia postburn in which impaired insulin secretory capacity mediated by IL-1β leads to acute hyperglycemia. These data suggest that targeting IL-1β to restore endogenous insulin secretory function may be a novel glycemic control strategy to improve outcomes for burn patients.

1. Introduction

Hyperglycemia often occurs in critically ill patients, especially following trauma, burn or surgery. This acute hyperglycemia, now more commonly referred to as 'critical illness diabetes', has long been believed as an adaptive stress response and was commonly undisturbed in clinics [1]. However, recent studies have suggested that acute hyperglycemia is potentially toxic and identified as an independent risk factor for adverse outcomes following critical illness, such as severe infections, multiple-organ failure and death [2–5]. Thus increasing attention has been devoted to blood glucose management in patients with critical illness.

Exogenous insulin administration with frequent monitoring of blood glucose is almost the only approach in critical care units to achieve glycemic control. Although a consensus has been reached that glycemic control in critically ill patients improves outcomes, issues regarding the

Abbreviations: ASBI, abbreviated burn severity index; FWHM, full width at half maximum; HBSS, Hank's balanced salt solution; TBSA, total body surface area; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling

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precise target blood glucose level, timing for glycemic control, optimal mode of insulin administration, and what patients are most likely to benefit remain controversial due to insufficient understanding of the nature and underlying mechanisms of acute hyperglycemia [1,6–9]. Extensive burn is one of the most serious forms of critical illness that are characterized by stress, hyperglycemia, hyperinsulinemia, inflammation, and hypermetabolic response [10,11]. It is reported that postburn hyperglycemia persisted from the early phase up to 6 months following the injury, and it was associated with adverse outcomes in clinics [12]. Although several clinical trials have suggested that early phase of postburn hyperglycemia is critical for clinic outcomes [8,13,14], the dynamics and nature of postburn hyperglycemia in the early phase of burned patients is poorly defined.

The factors that contribute to acute hyperglycemia in burned patients are largely unknown; this might occur through enhanced glucose release, insulin resistance and/or impaired insulin secretion. The evidence for enhanced glucose release and insulin resistance has been documented previously [11,12,15], whereas as the existence of hyperinsulinemia, little attention has been paid to insulin secretion capacity. This study was designed to investigate the nature and underlying mechanisms of acute hyperglycemia in the early phase of burn injury in burn patients, as well as a mouse model of severe burns. We found that impaired insulin secretory capacity resulting from IL-1β-induced pancreatic beta cell apoptosis played a causal role in the development of postburn acute hyperglycemia.

2. Materials and methods

2.1. Study population

Human study was approved by the institutional review board of Fourth Military Medical University and registered at www.chictr.org.cn (ChiCTR-OOB-15006420). Informed written consent was obtained from each patient or a family member before enrollment into the study. This was a single center, prospective cohort study recruiting 107 burn patients and 62 control patients admitted to the Department of Burns and Cutaneous Surgery, Xijing Hospital, Fourth Military Medical University, from January 2015 to September 2016. Adults, aged 18–60 yr, with a total body surface area (TBSA) ≥10% burn, admitted within 12 h after injury, were eligible for enrollment. Patients with a history of burn, obesity, diabetes, severely heart failure, hepatic dysfunction, renal failure, pancreatic diseases or cancer, or who was transferred from outside facilities were excluded. All patients received standard treatments by the same team of surgeons according to our clinical protocols, including early nutrition, systemic antibiotic therapy, and early escharectomy and grafting of the wound. Within 24 h of admission, all patients underwent total burn wound excision. All patients received the same nutritional treatment according to a standardized protocol as described previously [16]. Intake was calculated as follows: 1500 kcal/m^2 body surface + 1000 kcal/m^2 burn area. There was no specific target for glycemic control during the study. Blood glucose was monitored every day and subcutaneous insulin therapy was given for blood glucose values > 10 mM.

Once admitted, the abbreviated burn severity index (ASBI) was calculated as described previously [17] and blood samples were randomly collected within 0–200 h postburn (about 1–3 samples per day from each patient). For patients with moderate burn (ASBI ≤ 6), blood samples were randomly collected within 0–150 h postburn due to a shorter length of hospital stay. Blood samples were collected at least 2 h after any treatment or meal. Patient demographic and injury characteristics, hospital course, morbidity, and mortality were recorded. Patients admitted to the same department receiving skin plastic operation were enrolled as the control with the same inclusion and exclusion criteria except for burn injury. Blood samples were all randomly collected before surgery with 3–6 samples from each patient during their hospitalization.

2.2. Multiple linear regressions

In fitting blood glucose in burned patients, the data were grouped according to postburn time points in a temporal-resolution of 5 h. In fitting blood glucose in control subjects, the data were grouped according to blood glucose levels.

2.3. Determination of blood variables

Random blood glucose levels were measured using a glucose meter (Life-Scan, Milpitas, CA) once blood samples were collected. The blood sample was centrifuged at 1000*g* for 15 min and then the supernatant was transferred into a −80 °C refrigerator waiting for ELISA measurement. Serum insulin, glucagon, glucocorticoid levels were measured using commercial ELISA kits (R&D systems, MN). IL-1β, IL-6 and other inflammatory factors were measured using commercial ELISA kits (Beijing North Institute of Biological Technology, Beijing, China) according to the manufacturer's instructions.

2.4. Animal model

The experiments were performed according to the National Institutes of Health Guidelines for the Use of Laboratory Animals and were approved by the Fourth Military Medical University Committee on Animal Care. After being anesthetized, 30% TBSA and full-thickness burn model was conducted as previously described [18]. The dorsal hair of all C57 mice (6–8 wk. old) was shaved. Then the back skin of mice in the burn group was placed in hot water (95 °C) for 10 s. An immediate injection of balanced salt solution (40 mL/kg body weight) was administered to prevent shock. Then back wounds were treated with 1% tincture of iodine and kept dry and warm. The same skin area of the mice in the sham group was placed in water at 37 °C, and the other processes were the same as those applied in the burned mice. IL-1β antibody (200 mg/kg) (R&D systems, MN) was intraperitoneally administered immediately after burns for treatment.

The Il1b-luc-EGFP knockin mouse line was generated by homologous recombination using the CRISPR/Cas9 technology. A complementary DNA encoding luciferase and EGFP fluorescent protein was inserted in frame with the translational start codon of the IL-1β gene. The Il1b-luc-EGFP line was generated by Shanghai Model Organism Center, Inc. The guide RNA (5′-GAACTCAACTGTGAAATGCC-3′) targeting specific loci in the genome of Il1b guided Cas9 enzyme to the locus and cause DNA double strand break. Two 2 kb fragments homologous to the 5′ and the 3′ end were PCR amplified and subcloned into the pcDNA3-luciferase-2a-egfp-sv40-pA vector using an in-fusion HD Cloning kit (Takara). Homologous recombination (HR) will enable the donor vector integrate into the breaking locus. The resulting construct was injected into C57BL/6 J fertilized egg. Il1b-luc-EGFP knockin mice were identified by PrimeSTAR® GXL DNA Polymerase. Two pairs of PCR primers were designed (F1: 5′tgccctcaacgtccctatct3′, R1: 5′CTGA GCCCAGAAAGCGAAGGA3′, F2: 5′ggccagcacgttttgagga3′, R2: 5′TCGC ATTGTCTGAGTAGGTGTC3′) to test the correct targeted allele. Mice used for experiments were 6–8 wk. old.

Unless otherwise specified, 6 mice were used for each experiment/condition. For studying the survival of burned mice after neutralization of IL-1β, 45 mice were used for each group.

2.5. Immunofluorescence

To localize alpha cells and beta cells in the pancreatic islets, immunofluorescent staining was performed. Frozen sections of pancreatic tissue were warmed up at room temperature and then fixed in 4% paraformaldehyde for 10 min before permeabilization with 0.1% Triton X-100 for 2 min. After three times of washing, pancreatic tissue was blocked with 5% BSA at room temperature for 30 min. The tissue was washed and incubated at 4 °C overnight with anti-insulin antibody

(1:100, Abcam, USA) and anti-glucagon antibody (1:100, Abcam, USA). The slide were washed three times and incubated with anti-sheep IgG H &L (1:200, Alexa Fluor 647, Abcam, USA) and anti-mouse IgG H&L (1:200, Alexa Fluor 488, Abcam, USA) for 2 h at room temperature. Images were acquired using a confocal microscope (FV1000, Olympus, Japan; LSM 800, Zeiss, Jena, Germany) with a 20× objective.

2.6. Cell culture and detection of apoptosis

Mouse pancreatic alpha cells, alpha TC1 clone 6 (alpha TC1–6, GuangZhou Jennio Biotech, China) were cultured in DMEM medium containing 10% fetal bovine serum (FBS) and mouse pancreatic beta cells (Beta TC-6, BeNa Culture Collection, China) were cultured in DMEM medium containing 20% FBS. Cells at 70%–80% confluence were ready for experiments. The cells were synchronized in serum free DMEM for 8 h before treatments. For insulin stimulation, the isolated pancreatic islets or cultured beta cells were treated with different doses of IL-1β (0, 100, 1000 or 10,000 ng/L) for 6 h. The supernatant of culture medium was collected for insulin or glucagon test by ELISA, and the beta cells fixed by 4% paraformaldehyde were used for cell apoptotic index test by terminal deoxynucleotidyl nick end labeling (TUNEL) assay using an In Situ Cell Death Detection Kit (Roche Molecular Biochemical, Indianapolis, IN) according to the protocol provided by the manufacturer.

2.7. Pancreatic islet isolation

Islets were isolated from C57BL/6 J mice through collagenase digestion as described previously [19]. Collagenase V-S (3.0 mL, 1.0 mg/mL, Sigma, USA) was injected into the bile duct of the mouse and the swollen pancreas were excised. The pancreas tissue was incubated with collagenase at 38 °C for 20 min with gentle shaking. Then cold Hank's balanced salt solution (HBSS) was added to stop digestion. The digested tissue was passed through a 400-mm screen and centrifuged at 4 °C for 2 min at 1000*g*. After washing for three times, the pellet was re-suspended in 4 mL HISTOPIQUE1077 (Sigma, USA) and 2 mL HBSS was added on top of the solution. Further centrifugation was needed at 4 °C for 5 min at 1000*g*. Islets were hand-picked under a microscope at 20× to ensure pure islet preparations. After isolation, the islets were cultured overnight in DMEM medium with 10% FBS.

2.8. Statistics

Statistical analysis was performed using SPSS statistical analysis software package (International Business Machines, Chicago, IL). Categorical data were compared by Fishers exact tests, and continuous variables by Mann–Whitney U or Student's *t*-test as appropriate. Linear relationships between variables were tested by Pearson's correlation coefficient. Multiple linear regression analysis was performed to evaluate independent contributions of the studied variables to blood glucose. The survival data were estimated using Kaplan-Meier method and compared using log-rank test. The results are expressed as means ± SEM unless noted otherwise. A p value of < 0.05 was considered statistically significant.

3. Results

3.1. Study population

A total of 169 subjects were enrolled in the study, of whom 107 subjects were suffered with burn injury with a mean TBSA of 43.7 ± 2.5% and 62 subjects were control patients. Control patients and burned patients did not differ with respect to age, gender, weight and BMI (Table 1). A total of 1643 blood samples were collected, of which 1358 blood samples were from burn patients and 285 blood samples were from control patients.

3.2. Characteristics of postburn acute hyperglycemia in burn patients

To explore the nature of the postburn hyperglycemia, we initially analyzed its amplitude and temporal characteristics. The level of blood glucose over 200 h postburn displayed a 1.5-fold increase compared with control subjects (Fig. 1A). Gaussian fitting to blood glucose histogram revealed that blood glucose in control patients was centered at 5.4 mM with a full width at half maximum (FWHM) of 1.6 mM, and postburn blood glucose shifted to right by 2.4 mM with a FWHM of 4.4 mM (Fig. 1B). Remarkably, postburn blood glucose showed increased occurrences of both pronounced hyperglycemia (> 11.1 mM) and hypoglycemia (< 3.9 mM). In addition, postburn blood glucose had a positive correlation with both TBSA (Fig. 1C) and ASBI (Fig. 1D), reinforcing the notion that acute hyperglycemia is a biomarker of the degree of burn injury [3,8,18].

We then subgrouped the burn patients into moderate burns (ASBI ≤ 6), serious burns (6 < ASBI < 10) and severe burns (ASBI ≥ 10) according to ASBI. The three groups did not differ with respect to age, gender, weight, BMI and the duration of burn to admission (Table 1). As ASBI increased, the average level of postburn blood glucose was increased by 17%, 45%, and 72%, respectively (Fig. 1A). Histogram distributions of moderate, serious and severe burn were shifted to right by 1.2, 2.6, and 3.3 mM, and showed broader distributions with a FWHM of 2.7, 3.9, and 5.5 mM, respectively (Fig. 1B). The percentage of euglycemia (3.9–7.8 mM) fell to 78.7%, 41.9%, and 25.1% (89.8% in control patients), and the percentage of pronounced hyperglycemia rose to 3.3%, 10.0%, and 32.9% (1.1% in control patients) in moderate, serious and severe burns, respectively (Fig. 1E). Hypoglycemia was only observed in severe burns with a percentage of 2.5%. Time course of postburn blood glucose displayed two distinct hyperglycemia segments, 0–40 h and ~90–150 h (Fig. 1F). Evidently, acute hyperglycemia in burn patients showed broader distribution in amplitude and was highly dynamic in time.

3.3. Impaired insulin secretory capacity in burn patients

To investigate the underlying mechanism for acute hyperglycemia, we examined the major regulators of blood glucose: serum glucagon, glucocorticoid and insulin. Serum glucagon was increased by 97%, 221%, and 274% in moderate, serious and severe burns, respectively (Fig. 2A), and was temporally fluctuant (Fig. 2B). No differences in both the average levels and temporal dynamics of serum glucocorticoid were detected (Fig. 2C–D). Serum insulin was increased by 80%, 227%, and 135% in moderate, serious and severe burns, respectively (Fig. 2E). It is worth noting that serum insulin was decreased by 28% in severe burns compared with that in serious burns. Temporal levels of serum insulin were highly fluctuant, with a similar trend to blood glucose postburn (Fig. 2F).

To further explore the independent contributions of these three hormones in the regulation of blood glucose, multiple linear regressions for the level of blood glucose with that of serum insulin, glucagon and glucocorticoid as covariates were performed. The results showed that both the control and the postburn levels of blood glucose were fitted well by three hormones (Figs. 2G–I, and 1 and Tables 1–2 in Ref. [20]). The fitting results are shown as follows:

$$\mathrm{Glu} = \begin{cases} 5.909 + 0.003 \times \mathrm{I} + 0.006 \times \mathrm{G} + 0.002 \times \mathrm{C}, & \mathrm{ASBI} \le 6 \\ 5.352 - 0.042 \times \mathrm{I} + 0.010 \times \mathrm{G} + 0.017 \times \mathrm{C}, & 6 < \mathit{ASBI} < 10 \\ 7.244 - 0.097 \times \mathrm{I} + 0.017 \times \mathrm{G} + 0.010 \times \mathrm{C}, & \mathrm{ASBI} \ge 10 \end{cases}$$

where Glu indicates blood glucose, I indicates serum insulin, G indicates serum glucagon and C indicates serum glucocorticoid. As shown in Fig. 2G–I, postburn blood glucose was fitted well except for the first 3 time points (0–15 h postburn), indicating components other than the three hormones, e.g. epinephrine or norepinephrine, may contribute to the development of acute hyperglycemia at 0–15 h postburn. These

Table 1
Patient characteristics.

Characteristics	Control patients	Burned patients			
		All	ABSI ≤ 6	6 < ABSI < 10	ABSI ≥ 10
Patients no.	62	107	33	39	35
Samples no.	285	1358	395	492	471
Age (years)	39.7 ± 14.3	39.0 ± 17.1	38.8 ± 16.7	40.1 ± 15.8	38.0 ± 17.9
Male no. (%)	46 (74.2%)	83(77.6%)	26(78.8%)	32(82.1%)	25(71.4%)
Weight	65.4 ± 18.5	68.3 ± 21.1	67.1 ± 19.0	69.7 ± 18.8	67.9 ± 21.9
BMI	22.8 ± 6.4	23.3 ± 7.3	23.4 ± 6.5	22.7 ± 7.6	23.8 ± 7.5
Burn type no. (%)					
Flame	–	86(80.4%)	26(78.8%)	31(79.5%)	29(82.9%)
Scald	–	16(15.0%)	6(18.2%)	6(15.4%)	4(11.4%)
Other	–	5(4.7%)	1(3.0%)	2(5.1%)	2(5.7%)
TBSA (%)	–	40 (18–75)	13 (10–18)	35 (25–45)	80(70–92)
ASBI	–	8 (5–12)	5(4–5)	8(7–8)	12(11–14)
Inhalation injury no. (%)	–	69(64.5%)	14(42.4%)	25(64.1%)	30(85.7%)
Burn to admission (hours)	–	7.3 ± 3.1	6.8 ± 3.9	7.8 ± 3.2	7.2 ± 2.7
Length of stay in hospital (days)	–	48.3 ± 37.2	20.1 ± 7.6	42.8 ± 20.6	81.1 ± 45.4
Death no. (%)	–	6	0	1(2.6%)	5(14.3%)

Data are expressed as means ± SD, percentage of the group, or median (interquartile range), as appropriate. BMI, body mass index. ASBI, abbreviated burn severity index. TBSA, total body surface area.

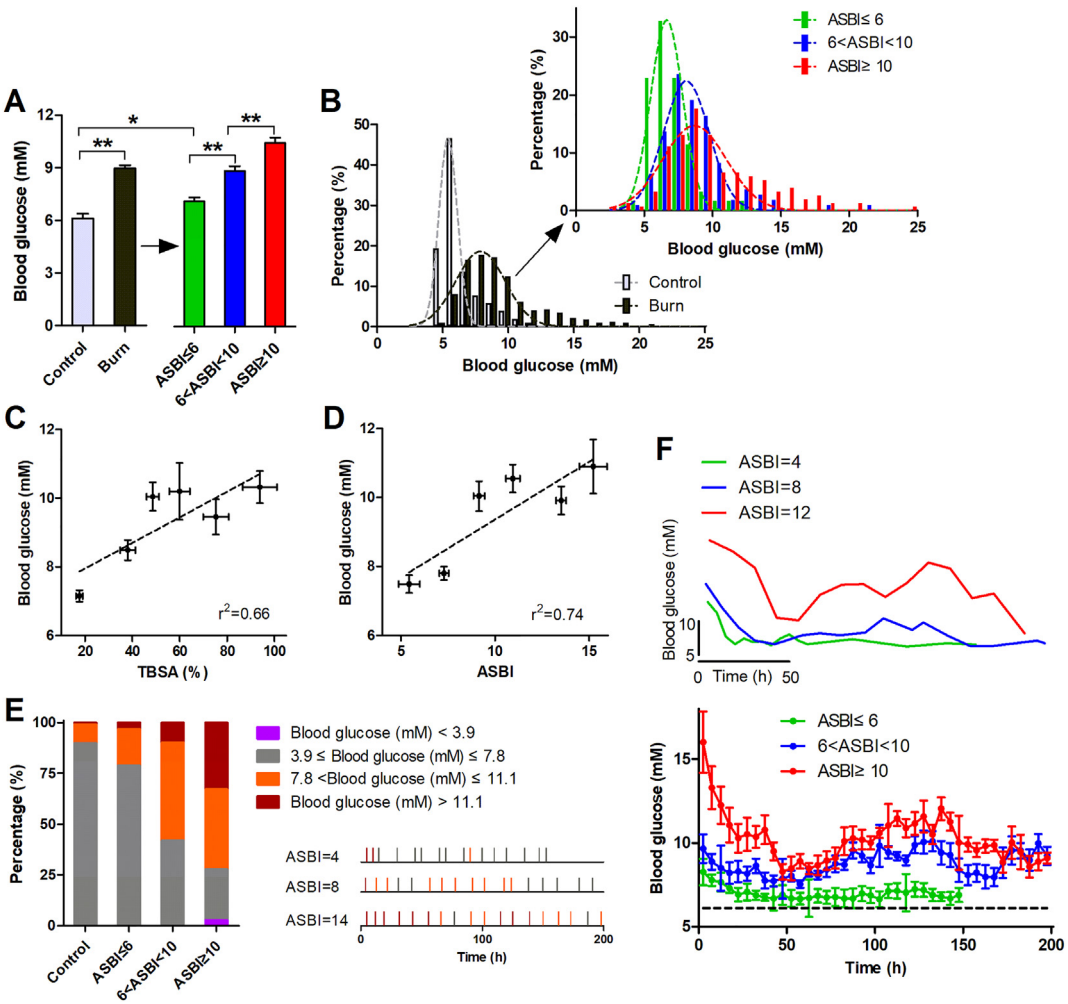

Fig. 1. Characteristics of acute hyperglycemia in burn patients. A. The level of blood glucose over 200 h postburn was increased in parallel with the degree of burn injury. B. Blood glucose histograms showed that postburn blood glucose was shifted to right and exhibited broader distributions. Dashed line shows the Gaussian regression. C–D. Proportional relation between blood glucose and TBSA (C, P = 0.05) or ASBI (D, P = 0.03). Dashed line shows the linear regression. E. Percentages of hypoglycemia (< 3.9 mM), euglycemia (3.9–7.8 mM), mild hyperglycemia (7.8–11.1 mM) and pronounced hyperglycemia (> 11.1 mM) in burn patients. Representative diaries of blood glucose of three patients with different ASBI are shown in the right panel. Each vertical tick denotes a blood glucose at the indicated time point. F. Temporal dynamics of postburn blood glucose in patients. Dashed line shows the control value. Representative traces of blood glucose of three patients with different ASBI are shown in the upper panel. ASBI, abbreviated burn severity index. TBSA, total body surface area. *, P < 0.05. **, P < 0.01.

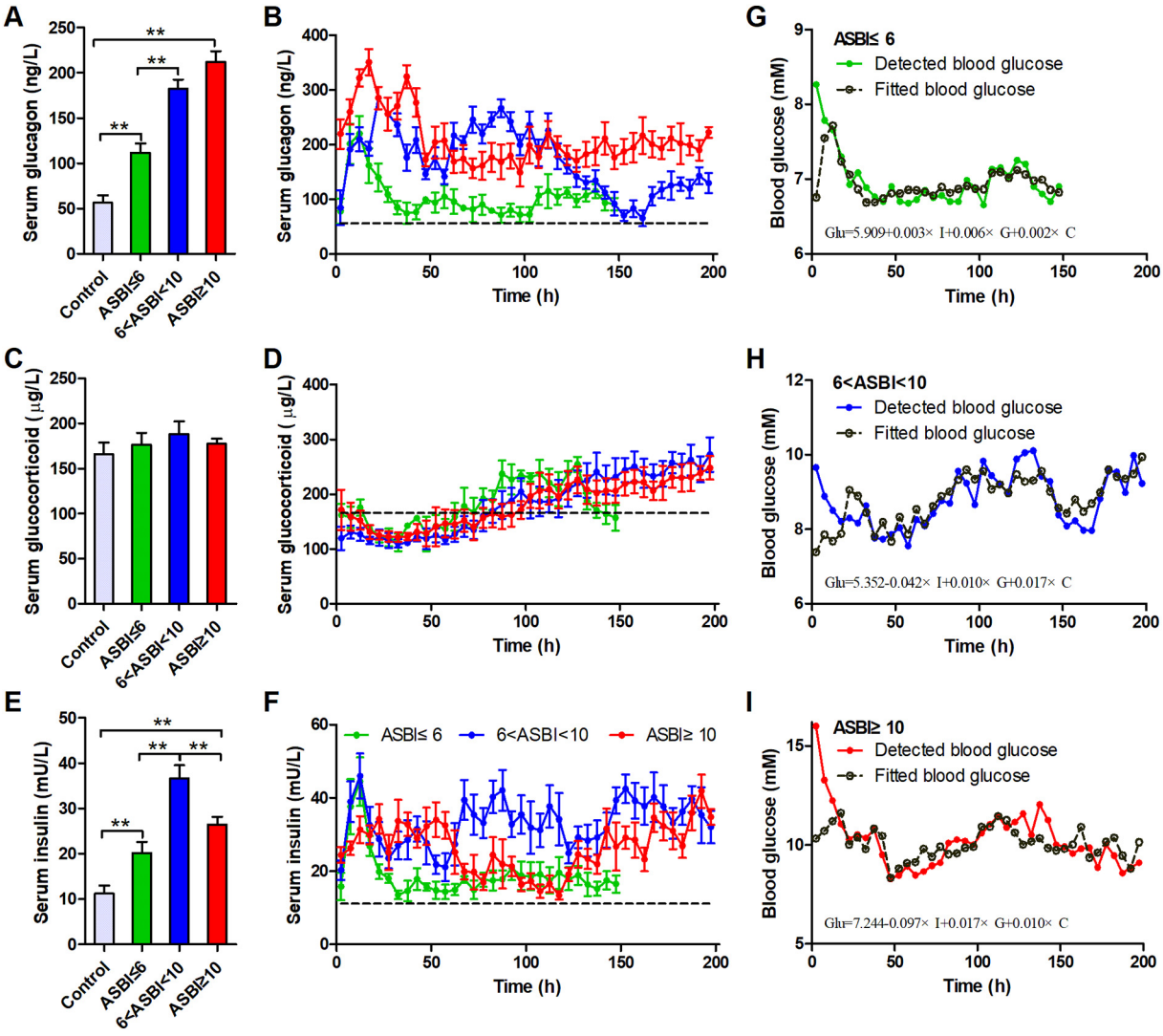

Fig. 2. Postburn blood glucose was well fitted with serum insulin, glucagon and glucocorticoid as covariates. A–B. The average serum glucagon level (A) and its temporal changes (B) at 0–200 h postburn in burn patients. Dashed line shows the control value. C–D. The average serum glucocorticoid levels (C) and its temporal changes (D) at 0–200 h postburn in burn patients. E–F. The average serum insulin levels (E) and its temporal changes (F) at 0–200 h postburn in burn patients. G–I. Linear regressions for blood glucose in patients with moderate burns (G), serious burns (H) or severe burns (I) with serum insulin, glucagon and glucocorticoid as covariates. Dashed line shows the fitted blood glucose. *, $P < 0.05$. **, $P < 0.01$.

results reinforce the notion that postburn blood glucose is largely dependent on the concentrations of serum insulin, glucagon and glucocorticoid.

Given that glucose release was increased postburn, the elevations of serum insulin and blood glucose cannot be simply interpreted as acute insulin resistance. To examine whether postburn serum insulin is sufficient, we next analyzed the correlation between blood glucose and serum insulin which exhibited a pattern of S-curve (Fig. 3A). S-curve ($y = c + \frac{d}{1+\exp(-bx-a)}$) regressions were performed, where a represents how much the curve shifts along x axis, b represents how much the curve spread out, c represents the minimum value and c + d represents the maximum value. Compared with control patients, extensive burns stretched out the curves as indicated by the decreased value of b (2.7 in control patients, 1.7 in moderate burns, 1.1 in serious burns and 0.8 in severe burns), and decreased the maximum value of serum insulin (42.6 mU/L in control patients, 41.7 mU/L in moderate burns, 38.2 mU/L in serious burns and 30.8 mU/L in severe burns), indicating that extensive burns decreased both the responsiveness and the capacity of insulin secretion (Fig. 3A). In order to quantify serum insulin with respect to postburn hyperglycemia in patients, we introduced a new index in assessing the relative level of insulin to blood glucose ($Ins_{relative}$) as: $Ins_{relative} = \frac{\sum \frac{I}{Glu}}{n}$ when Glu > 8.7 mM (serum insulin reaches 90% of the maximum value in the control group when blood glucose is 8.7 mM), where I indicates serum insulin, Glu indicates blood glucose, and n indicates number of samples. This index was designed to quantify insulin with respect to blood glucose when insulin secretion is near the maximal capacity of beta cells. The results showed that the relative insulin index was decreased by 18%, 35%, and 60% in moderate, serious and severe burns, respectively (Fig. 3B), suggesting impaired insulin secretory capacity in burned patients.

3.4. The prominent elevation of IL-1β is associated with impaired insulin secretory capacity in burn patients

Extensive burn is associated with a severe stress response resulting in a proinflammatory cascade of cytokine release [16,21]. Inflammatory factors have been reported to be involved in pancreatic beta cell dysfunction in diabetes [22–24]. Thus, the major proinflammatory cytokines were determined. Among these cytokines, IL-1α, IL-2, IL-4, IL-8, IL-10, IL-12, TNFα and MIP-1α showed little or small

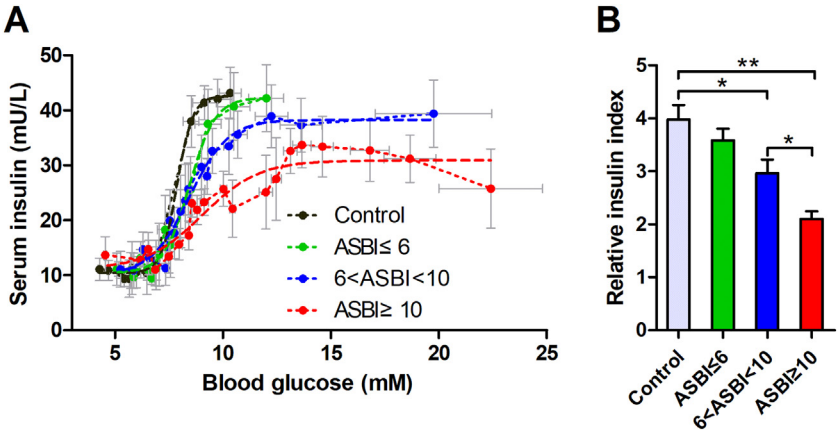

Fig. 3. Extensive burns impaired insulin secretory capacity in burn patients. A. Serum insulin levels relative to blood glucose in control and burn patients. Dashed lines show the S-curve ($y = c + \frac{d}{1+\exp(-bx-a)}$) regressions. B. Relative insulin index ($\frac{\sum \frac{I}{Glu}}{n}$, when blood glucose > 8.7 mM) in burn patients. *, $P < 0.05$. **, $P < 0.01$.

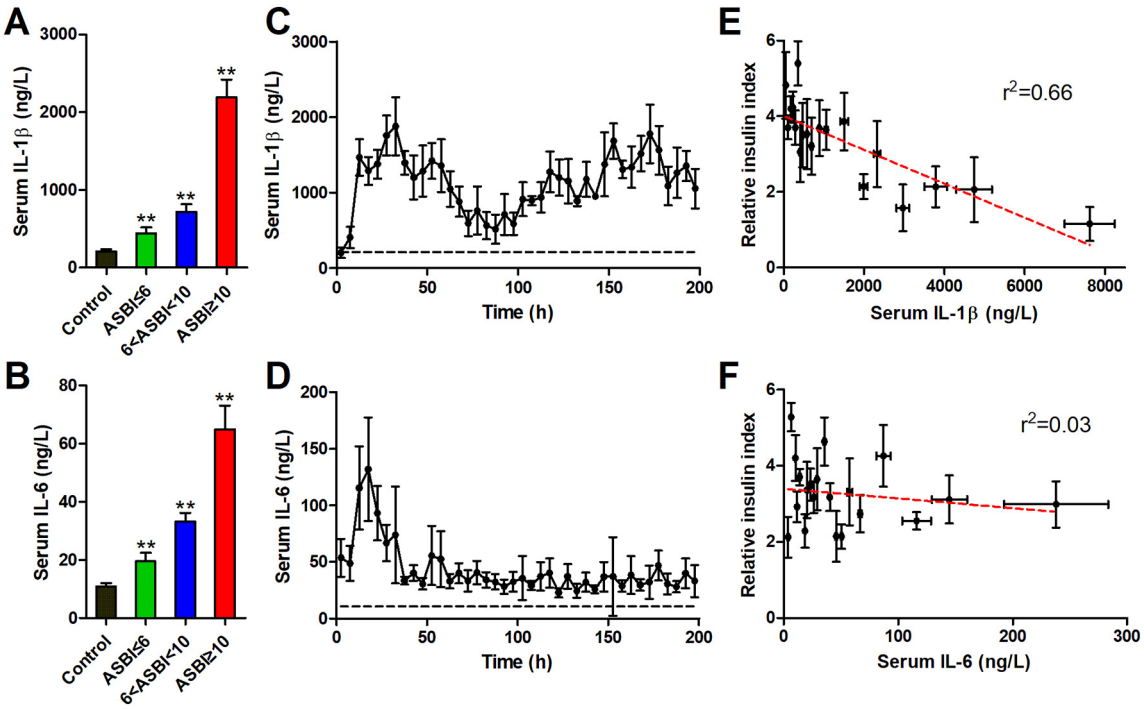

Fig. 4. IL-1β was associated with the impaired insulin secretory capacity in postburn patients. A–B. The average serum IL-1β (A) and IL-6 (B) levels at 0–200 h postburn. C–D. The temporal dynamics of serum IL-1β (C) and IL-6 (D) levels at 0–200 h postburn. Dashed line shows the control value. E. Inverse relation between relative insulin index and serum IL-1β postburn ($P < 0.01$). Dashed line shows the linear regression. F. Serum IL-6 showed no significant linear relation with relative insulin index postburn ($P = 0.47$). **, $P < 0.01$.

changes (see Fig. 2 in Ref. [20]). Particularly, extensive burns profoundly increased serum IL-1β and IL-6 levels. Serum IL-1β was increased by 108%, 240%, and 940%, and serum IL-6 was increased by 81%, 207%, and 502% in moderate, serious and severe burns, respectively (Fig. 4A–B). For temporal dynamics, serum IL-1β displayed a similar pattern to that of postburn blood glucose (Fig. 4C), and serum IL-6 displayed a transient increase, reaching peak level at 15–20 h postburn (Fig. 4D).

To test whether IL-1β and IL-6 play a role in the decreased capacity of insulin secretion, we analyzed the correlations between these variables. No linear relation between serum IL-1β and serum insulin was found ($r^2 = 0.12$) (see Fig. 3 in Ref. [20]), but there was an inverse linear relation between IL-1β and relative insulin index ($r^2 = 0.66$) (Fig. 4E). In addition, there were no linear relations between serum IL-6 and serum insulin ($r^2 = 0.00$) (see Fig. 3 in Ref. [20]), as well as serum IL-6 and relative insulin index ($r^2 = 0.03$) (Fig. 4F). These results suggested that IL-1β was associated with the impaired capacity of insulin secretion in response to blood glucose elevation.

3.5. IL-1β induces pancreatic beta cell apoptosis and impairs insulin secretory capacity in mice

To examine whether IL-1β plays a role in the induction of pancreatic beta cell dysfunction, we examined effects of IL-1β on blood glucose in mice. An expression profile of IL-1 receptor revealed that the pancreatic beta cell is a primary target of IL-1β action (see Fig. 4 in Ref. [20]). Dose-response experiments with a single bolus of 50 or 500 ng/kg IL-1β (iv) led to circulating IL-1β concentrations ranging from 355 to

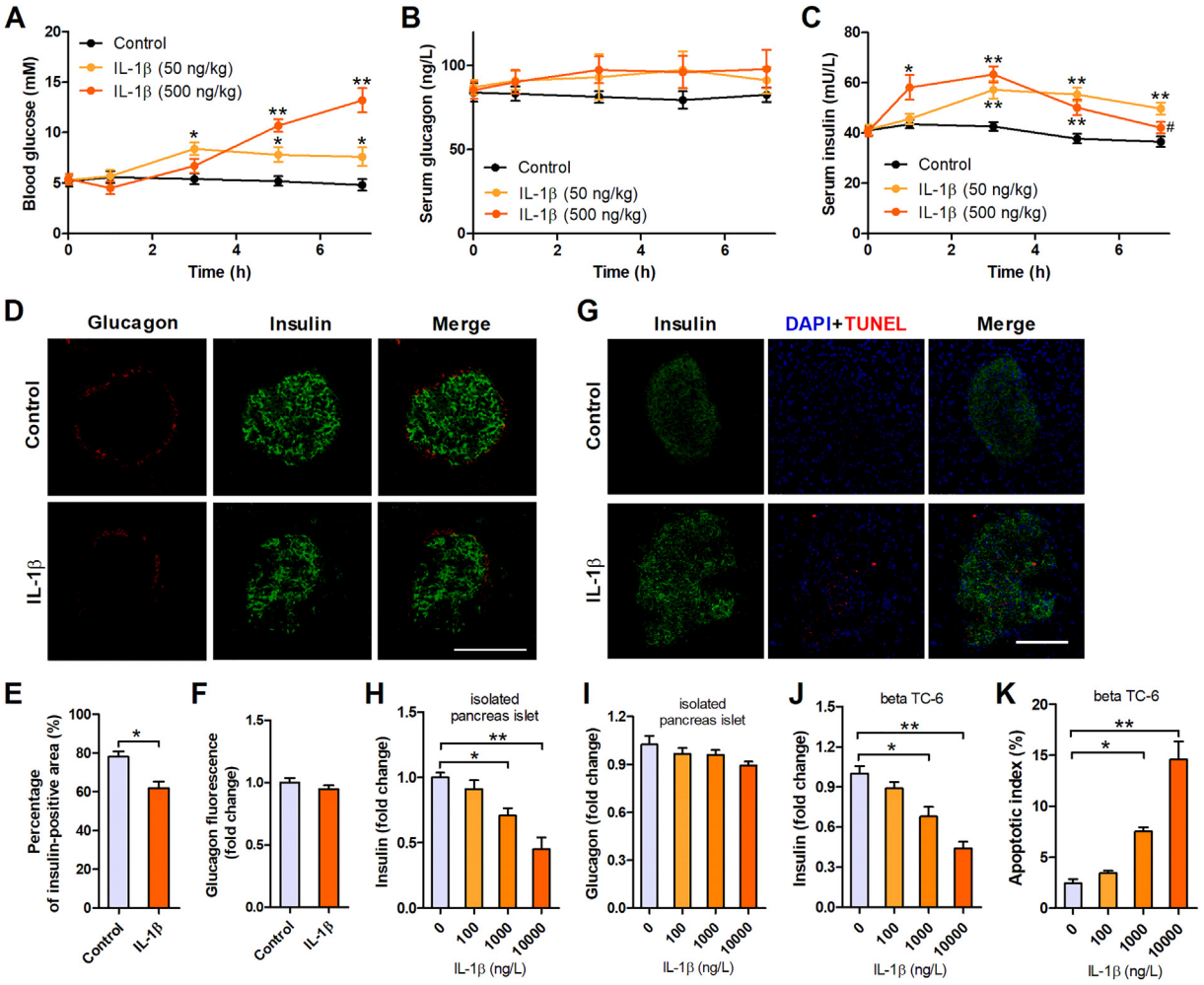

Fig. 5. IL-1β induced beta cell apoptosis. A. A single bolus of IL-1β (50 or 500 ng/kg) increased blood glucose in mice (n = 6). B–C. Serum glucagon (B) and insulin (C) in mice after IL-1β injection (n = 6). *, P < 0.05. **, P < 0.01 vs. control mice at the same time point; #, P < 0.05 vs. IL-1β (500 ng/kg) at the same time point. D. Confocal images of pancreatic islets stained with insulin (green) and glucagon (red) from the control mice and IL-1β (500 ng/kg)-injected mice. Scale bar, 200 μm. E–F. Statistical results of percentage of insulin-positive area (E) and glucagon fluorescence (F) (n = 21 from 3 mice). G. Confocal images of pancreatic islets stained with insulin (green), DAPI (blue) and TUNEL (red) from the control mice and IL-1β (500 ng/kg)-injected mice. Scale bar, 100 μm. H–I. Glucagon (H) and insulin (I) secretion of isolated pancreatic islets after IL-1β incubation (n = 6). J–K. Insulin secretion (J) and apoptotic index (K) of beta TC-6 cells after IL-1β incubation (n = 6). *, P < 0.05. **, P < 0.01.

1263 ng/L, similar to the concentrations observed in burned mice (see Fig. 5 in Ref. [20]). A single injection of IL-1β increased blood glucose in a dose-dependent manner (Fig. 5A). Low-dose IL-1β (50 ng/kg) increased blood glucose by 58%, and high-dose IL-1β (500 ng/kg) increased blood glucose by 175% at 7 h after injection (Fig. 5A). IL-1β injection did not affect serum glucagon, but increased serum insulin (Fig. 5B–C). However, compared with low-dose IL-1β-injected mice, serum insulin in high-dose IL-1β-injected mice was decreased at 7 h after injection (Fig. 5C), even though the blood glucose remained higher.

Next, we imaged the function of pancreatic islets from mice with IL-1β injection (500 ng/kg). IL-1β injection decreased insulin-positive area in pancreatic islets and showed no significant effect on glucagon fluorescence, indicating that IL-1β may induce beta cell apoptosis (Fig. 5D–F). TUNEL staining confirmed that IL-1β injection specifically induced beta cell apoptosis with mild effects on other cells (Fig. 5G). To exclude the effect of blood glucose on insulin secretion, we next incubated the isolated pancreatic islets from the control mice with IL-1β (100, 1000, or 10,000 ng/L) in the presence of 10 mM glucose for 6 h. IL-1β decreased insulin secretion in a dose-dependent manner (Fig. 5H), but with no effects on glucagon secretion, indicating a beta cell specific effect (Fig. 5I). We then confirmed the effects of IL-1β on insulin secretion and cell apoptosis in beta TC-6 cells, a pancreatic beta cell-derived cell line. Incubation with IL-1β (100, 1000, or 10,000 ng/L) for 6 h decreased insulin secretion, and increased apoptosis in a dose-dependent manner in beta TC-6 cells (Fig. 5J–K). These results indicated that IL-1β specifically induced beta cell apoptosis and the resultant impairment of insulin secretion.

3.6. Neutralization of IL-1β decreases blood glucose and improves survival in burned mice

Similar to what was observed in burned patients, blood glucose, serum glucagon, serum insulin and serum IL-1β were increased in response to burns (Fig. 6A–C, and Figs. 5–6 in Ref. [20]). To further verify whether IL-1β plays a role in the induction of acute hyperglycemia postburn, we used IL-1β antibody to neutralize IL-1β in burned mice. A single bolus of IL-1β antibody immediately after injury (iv, 200 mg/kg body weight) normalized postburn blood glucose (Fig. 6A), and increased postburn serum insulin (Fig. 6C), while no significant effect had been detected on serum glucagon (Fig. 6B). Imaging of pancreatic islets showed that severe burns increased insulin fluorescence in beta cells and decreased insulin-positive area in pancreatic islets (Fig. 6D). Neutralization of IL-1β normalized insulin-positive area in pancreatic islets

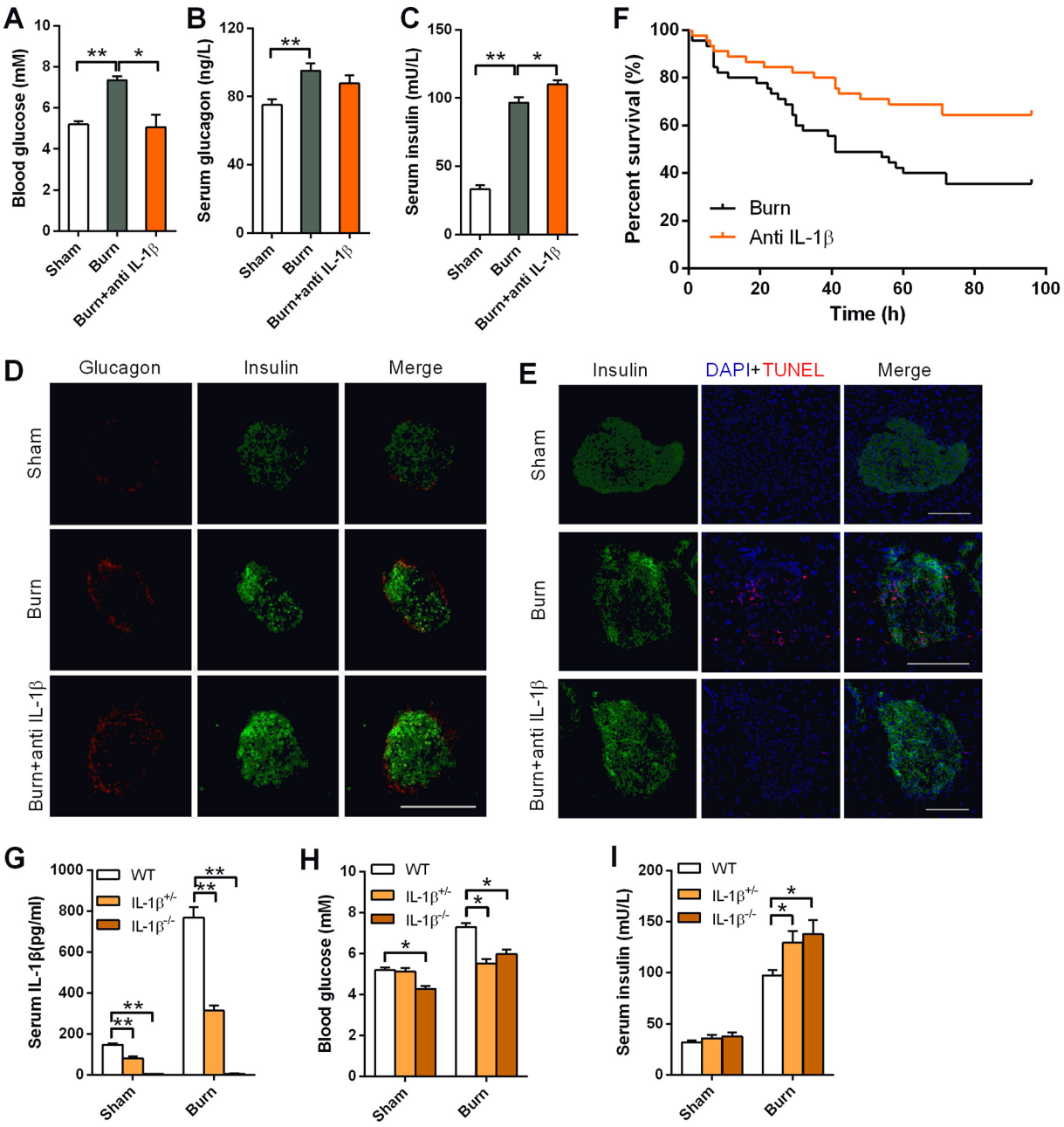

Fig. 6. Neutralization of IL-1β decreased blood glucose and improved prognosis in burned mice. A–C. Blood glucose (A), serum glucagon (B) and serum insulin (C) in IL-1β antibody-injected mice with severe burns (n = 6). D. Confocal images of pancreatic islets stained with insulin (green) and glucagon (red). Scale bar, 200 μm. E. Confocal images of pancreatic islets stained with insulin (green), DAPI (blue) and TUNEL (red) from mice with burn injury. Scale bar, 100 μm. F. Neutralization of IL-1β increased survival of burned mice ($P < 0.01$) (n = 42 mice in burn and n = 45 mice in anti-IL-1β). G. Serum IL-1β in WT, IL-1$\beta^{+/-}$ and IL-1$\beta^{-/-}$ mice with or without severe burns (n = 6). H–I. Blood glucose (H) and serum insulin (I) in WT, IL-1$\beta^{+/-}$ and IL-1$\beta^{-/-}$ mice with or without severe burns (n = 6). *, $P < 0.05$. **, $P < 0.01$.

(Fig. 6D, and Fig. 7 in Ref. [20]), suggesting that neutralization of IL-1β protected beta cells against apoptosis. This was confirmed by TUNEL staining, showing that neutralization of IL-1β alleviated beta cell apoptosis (Fig. 6E). More importantly, neutralization of IL-1β increased postburn survival by 28.8% in burned mice ($P < 0.01$) (Fig. 6F).

We further confirmed our finding in both IL-1β heterozygous (IL-1$\beta^{+/-}$) and homozygous (IL-1$\beta^{-/-}$) knockin mice. Serum IL-1β was decreased in IL-1$\beta^{+/-}$ mice and was undetectable in IL-1$\beta^{-/-}$ mice (Fig. 6G). Fasting blood glucose showed no significant difference in IL-1$\beta^{+/-}$ mice, but was decreased in IL-1$\beta^{-/-}$ mice (Fig. 6H). IL-1β deletion had no effect on serum insulin (Fig. 6I). Consistent with neutralization of IL-1β, IL-1β knockin mice displayed a decrease of blood glucose and an increase of serum insulin in response to severe burns (Fig. 6H–I). These results further confirmed that inhibition of IL-1β protected pancreatic islet function and decreased blood glucose in response to burns.

4. Discussion

Increasing evidence has shown that acute hyperglycemia is an independent risk factor for adverse outcomes in extensively burned patients and other critically ill patients because it directly or indirectly confers a predisposition to a variety of complications [7,25,26]. However, acute hyperglycemia is often undisturbed in critical care units

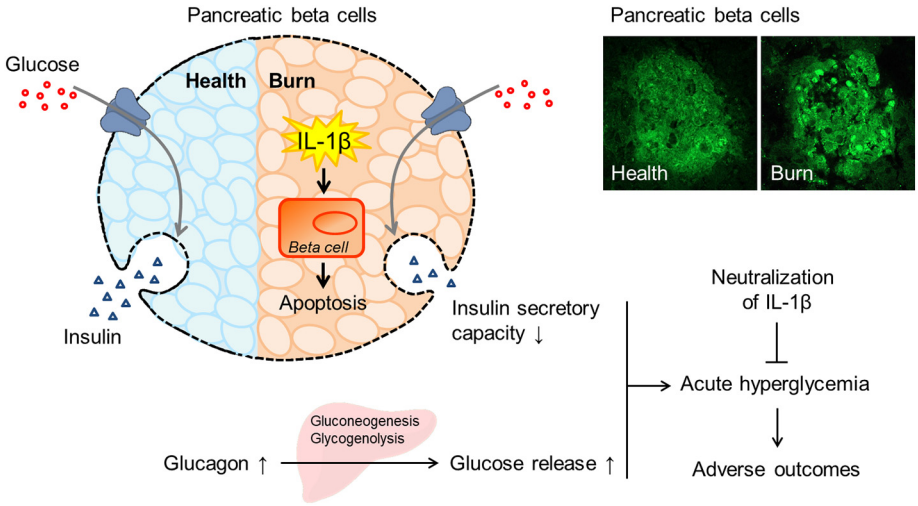

Fig. 7. Schematic figure illustrating acute hyperglycemia following extensive burns. Extensive burns induce a significant elevation of IL-1β and resultant pancreatic beta cell apoptosis, which leads to reduced insulin secretory capacity and acute hyperglycemia. Neutralization of IL-1β remarkably decreased postburn blood glucose and improved postburn survival.

because of barriers to achieve glycemic control due to insufficient understanding of its nature and underlying mechanisms. This study provides a new insight into the mechanisms responsible for the early phase of postburn hyperglycemia that impaired insulin secretory capacity resulting from IL-1β-induced pancreatic beta cell apoptosis plays a causal role in the development of acute hyperglycemia in extensive burns. This is similar to the pathophysiology of type 1 diabetes, and therefore we termed the acute hyperglycemia as “acute type 1 diabetes”. More importantly, neutralization of IL-1β produces beneficial effects on postburn glycemic control and improves survival, suggesting that targeting IL-1β or beta cell function to restore endogenous glycemic control may be a potential therapeutic approach for severe burn injuries. The schematic figure illustrating acute hyperglycemia and its underlying mechanism is shown in Fig. 7.

Although acute insulin resistance is considered to be a major cause for acute hyperglycemia, there is a lack of direct evidence on the existence of systemic insulin resistance in the early phase of critical illness. The current approaches to assessing systemic insulin resistance in the clinic require subjects under a stable metabolic state in which glucose supply, insulin secretion and other factors involved in the regulation of glucose homeostasis are relatively constant before challenge [27]. However, the metabolic state is highly fluctuant during the early phase of critical illness in which the glucose supply and insulin secretion are usually high and ever changing [10]. Thus, the available evidence may not accurately represent the existence of insulin resistance in the early phase of critical illness, in that the fluctuations of both insulin secretion and glucose supply disturb the indices used to assess insulin sensitivity. Here, we found that although serum insulin levels were decreased in severe burns compared with serious burns, the independent contribution of insulin to glucose disposal in severe burns was higher, indicating that insulin resistance may not be a major cause of acute hyperglycemia, at least in severe burns.

Based on our clinical and experimental data, we found that postburn acute hyperglycemia is attributed to the impaired capacity of insulin secretion resulting from pancreatic beta cell apoptosis. With the assistance of S-curve regressions, our data showed that insulin deficiency underlay the development of acute hyperglycemia. This may explain why administration of exogenous insulin was always effective to achieve glycemic control in the inpatient setting with critical illness and had significantly improved the prognosis of critically ill patients [2,3,8]. Reduced insulin secretion capacity as assessed by concentration of proinsulin or proinsulin/insulin ratio has also been observed in burn patients previously [15,28]. Although the absolute level of serum insulin was increased in extensive burns, it was relatively decreased when taking into account the increased requirement of glucose disposal postburn. Especially when blood glucose was above 8.7 mM, serum insulin level with respect to blood glucose level was significantly lower than that in control subjects, indicating dampened insulin secretory capacity and responsiveness in burn patients. Consistent with our findings, studies have shown that glucagon-like peptide 1 which improves pancreatic beta cell function exerts beneficial effects on glycemic control postburn [29,30].

The factors that contribute to chronic hyperglycemia in diabetes include insulin resistance and impaired insulin secretion. It is well established that chronic and low-grade inflammation is a common cause of insulin resistance [31,32]. This idea was sparked in the mid-1990s and began to gain wide acceptance at the beginning of this century [31]. Mechanistically, proinflammatory cytokines activate cellular stress signaling which impairs insulin sensitivity through phosphorylation serine residues of insulin receptor substrate, as well as dysregulation of autophagy, a conserved homeostatic process for cellular quality control [33]. Recently, proinflammatory cytokines, such as IL-1β, have also been reported to be involved in induction of beta cell dysfunction which impairs insulin secretion in diabetes and other pathological conditions [34–37]. However, whether acute elevation of proinflammatory cytokines are involved in induction of beta cell dysfunction in burns has not been reported. Here, we observed a prominent increase of serum IL-1β in burned patients, and found that IL-1β specifically induced pancreatic beta cell apoptosis but with mild effects on other cells. Importantly, neutralization of IL-1β not only improved postburn pancreatic beta cell function but also increased postburn survival in burned mice. These results suggest that IL-1β-induced beta cell apoptosis which decreases the capacity of insulin secretion plays a causal role in the development of acute hyperglycemia in extensive burns.

One of the main limitations of our study is that blood glucose of burned patients is affected by multiple factors which have not been fully excluded here. These factors include nutritional support, insulin intervention, occult diseases, circadian rhythm and diet. Although these factors are not likely to significantly confound the intrinsic dynamics of postburn blood glucose, they should be taken into consideration for data interpretation. Further, blood glucose disposal did not only regulated by insulin. It has been suggested that insulin-independent glucose disposal may play an important role in burns [10]. Thus, a more complex modeling of postburn blood glucose in which more factors are taken into consideration should be established in the further study. In addition, there is a general consensus that animal models of burn injury do not fully replicate the situation in burned patients. We only studied burn injury in mice with a standard model, while burn injury in humans is complicated with different type, duration, size, depth, location and treatments.

5. Conclusions

Our findings demonstrate that extensive burns cause a significant systemic elevation of IL-1β and resultant pancreatic beta cell apoptosis, leading to the reduced insulin secretory capacity and responsiveness. The dampened insulin secretion is an underlying cause for acute hyperglycemia in the early phase of extensive burns. More importantly, neutralization of IL-1β remarkably ameliorates postburn hyperglycemia and improves survival in extensively burned mice. This finding provides an important clue to the mechanisms responsible for acute hyperglycemia in critical illness, suggesting that targeting IL-1β to protect pancreatic beta cells and restore endogenous insulin secretion may represent a potential glycemic control strategy to preclude the side effects of exogenous insulin administration and improve outcomes of burned patients.

Transparency document

The Transparency document associated with this article can be found, in online version.

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Authors' contributions

F.G. and X.Z. conceived and supervised the study, and wrote the manuscript. Jun L., J.X., J.H., Y.J., H.Z., L.Z., D.H., X.Q. and H.Y. performed experiments. Jia L., W.X., X.Z., J.X. and F.G. analyzed data.

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