Supplementary Information

Effects of IL-1β Blocking Therapies in Type 2 Diabetes Mellitus: A Quantitative Systems Pharmacology Modeling Approach to Explore Underlying Mechanisms

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This Supplementary information contains a step-by-step description of the development of our type 2 diabetes and IL-1β disease progression model, as well as all model equations, assumptions, and the non-diseased and diseased states used in our study. All parameter values can be found summarized in Suppl. Table 2. For fitted equations, the goodness of fit is shown in Suppl. Figure 1.

Derivation of model equations and parameters

Step 1 – Use of previously published models

As a first step in the development of our model, we used previously published equations to describe the interrelationships between glucose, insulin, and HbA1c. We also extended these equations to include proinsulin dynamics and effects.

Insulin and glucose. Both plasma insulin and plasma glucose dynamics were modeled based on equations and parameters used by de Gaetano *et al.*[1], with the addition of an extra effect of proinsulin on glucose uptake:

$$Insulin(t) = \frac{B(t) \cdot TigB(t)}{Kxi} \cdot \frac{\left(\frac{Glucose(t)}{Gh}\right)^{vh}}{1 + \left(\frac{Glucose(t)}{Gh}\right)^{vh}} \quad (1)$$

$$Glucose(t) = \frac{Tgl}{Kxg + Kxgi \cdot (Insulin(t) + 0.1 \cdot Proinsulin(t))} \quad (2)$$

Here, *B* is β-cell mass, *TigB* is the maximum insulin secretion capacity per unit β-cell, *Kxi* is the insulin clearance rate, and *Gh* and *vh* define a Hill-shaped relationship between insulin secretion and glucose. Furthermore, *Tgl* describes basal insulin-independent glucose output, *Kxg* insulin-independent glucose uptake, and *Kxgi* insulin sensitivity.

While values for *Kxi*, *Ghi*, *vh*¸ and *Kxgi* were taken from[1], *Tgl* was calculated from steady-state conditions (Suppl. Table 1) and *Kxg* was set according to the value reported in[2]. Moreover, proinsulin was assumed to have 10 % the biologic activity of insulin[3].

HbA1C. The relationship between glucose and HbA1C was described using a semi-mechanistic model proposed by Hamrén *et al.*[4], and later extended in[5]. The model accounts for red blood cell (RBC) life-span using a 12 transit compartment model and includes a glucose-dependent glycosylation rate. Unlike Hamrén *et al.*[4], who estimated a RBC life-span of 135 days, we considered a shorter life-span of 100 days to be more plausible[5, 6]. For a full description of the model equations, see the original publications[4, 5].

Proinsulin. Proinsulin levels were modeled assuming proinsulin secretion to be proportional to insulin secretion and proinsulin clearance to be 10% of that of insulin[7]:

$$Proinsulin(t) = f(t) \cdot \frac{B(t) \cdot TigB}{0.1 \cdot Kxi} \cdot \frac{\left(\frac{Glucose(t)}{Gh}\right)^{vh}}{1 + \left(\frac{Glucose(t)}{Gh}\right)^{vh}} \tag{3}$$

Step 2 – Extending the model with *ex vivo* IL-1β and IL-1Ra effects

In the second stage of model development, we extended the above equations by incorporating IL-1β and IL-1Ra effects, through modulation of the IL-1 receptor, on the maximum insulin secretion capacity (*TigB*), β-cell mass (*B*), and the proinsulin-insulin secretion factor (*f*). These effects are illustrated in Figure 2 in the main text. The subfigures were produced by plotting Equation 8 minus Equation 7 (Figure 2A), Equation 5 (Figure 2B), and Equation 9 (Figure 2C). Steady state was assumed for Equation 5 and 9 when plotting. The equations were derived as follows:

IL-1R modulation. IL-1R modulation was modeled assuming competitive inhibition between IL-1β and IL-1Ra /anakinra:

$$IL1R(t) = \frac{IL1b(t)}{kmi \cdot \left(1 + \frac{IL1Ra(t) + Anakinra(t)}{kii}\right) + IL1b(t)} \tag{4}$$

The affinities for IL-1β and IL-1Ra/anakinra were set according to[8].

Insulin secretion capacity. The insulin secretion capacity per unit β-cells was modeled as a function of IL-1R modulation based on data from human[9, 10] and rodent[11] islets:

$$\frac{dTigB(t)}{dt} = \tau s \cdot \left(ks \cdot \left(1 - \frac{vs \cdot IL1R(t)}{kms + IL1R(t)}\right) - TigB(t)\right) \tag{5}$$

Studying the data from human islets, one could speculate that the relationship between IL-1R modulation and insulin secretion should be bell-shaped, and that intermediate receptor activity actually has a

stimulatory effect on insulin secretion. However, it has previously been proposed that such a stimulatory effect may in fact be time-dependent and disappear after prolonged stimulation of IL-1 signaling[11], an aspect not investigated in[9]. Therefore, we only considered an inhibitory effect of IL-1R modulation on the insulin secretion capacity.

Due to uncertainties and lack of information in the data, making anything but the simplest assumptions regarding the parameters *vs* and *kms* is difficult. In two different publications[9, 10], glucose stimulated insulin secretion was shown to be reduced by 60-70 % after exposure to high IL-1β and we thus considered a corresponding value for *vs*. Furthermore, to estimate *kms*, we ignored the stimulatory effects reported for intermediate IL-1β concentrations[9] and assumed the half-maximum negative response to be reached at an IL-1β concentration of ~100-200 pg/ml. We then translated this concentration to a corresponding value for IL-1R modulation by considering a concentration of ~140 pg/ml of endogenously produced IL-1Ra reported for the same experimental conditions and using Equation 4:

$$kms = \frac{0.2}{kmi \cdot \left(1 + \frac{0.14)}{kii}\right) + 0.2}$$

Finally, the scaling factor *ks* was calculated from steady-state conditions (Suppl. Table 1) and τs was set to 0.5 day^{-1} to capture the fact that IL-1 effects are not reached instantly, but after a few days.

β-cell rate of change. β-cell rate of change was modeled as a difference between β-cell apoptosis and replication. Any possible contribution of neogenesis to β-cell regeneration was assumed to be small[12, 13] and neglected. Both the rate of apoptosis and replication were considered to be functions of IL-1R modulation, also based on data from[9, 10]. This time, however, both beneficial and negative effects were considered, mainly because no study investigating more long-term effects could be found.

$$\frac{dB(t)}{dt} = (replication - apoptosis) \cdot B \tag{6}$$

$$apoptosis = ka \cdot \left(1 - \frac{vla \cdot IL1R(t)^{xla}}{kmla^{xla} + IL1R(t)^{xla}} + \frac{vha \cdot IL1R(t)^{xha}}{kmha^{xha} + IL1R^{xha}}\right) \tag{7}$$

$$replication = kr \cdot \left(1 + \frac{vlr \cdot IL1R(t)^{xlr}}{kmlr^{xlr} + IL1R(t)^{xlr}} - \frac{vhr \cdot IL1R(t)^{xhr}}{kmhr^{xhr} + IL1R(t)^{xhr}}\right) \tag{8}$$

All parameters except *ka* and *kr* were fitted to reported mean relative changes in apoptosis and replication rates after 4 days exposure to various IL-1β concentrations[9]. The goodness of fit is shown in Suppl. Figure 1. After fitting the parameters, *ka* and *kr* were calculated from non-diseased steady state conditions (Suppl. Table 1) and considering a basal β-cell turnover of 10 % per year. Even though the turnover and life-span of human β-cell is a question for debate and a wide range of values can be derived from current data[14, 15, 16, 17, 18], recent evidence points towards a very low or almost non-existent β-cell turnover in human adults. For instance, Perl *et al*.[15] reported an annual turnover of 25-60 % in two young adults,

while no turnover at all was observed in older subjects. Moreover, using the same reasoning as in[17] and assuming that (i) a 60 % loss in β-cell mass takes about 5 years to evolve and (ii) that this is a result of a 2-3 fold increase in β-cell apoptosis and a limited change in replication, this would imply a normal annual turnover of 6-9 %. Our assumption of a 10 % basal turnover should thus be in the range of plausible rates.

Proinsulin secretion fraction. The ratio of proinsulin to insulin secretion has been shown to be dependent on both glycemia and IL-1 signaling. While hyperglycemia seems to alter the ratio mainly through an increased β-cell secretory demand, however, increased IL-1 signaling acts by inhibiting the conversion of proinsulin into insulin, suggesting two different mechanisms[19, 20, 21]. We therefore considered a combined effect of glucose and IL-1R modulation on the proinsulin secretion factor:

$$\frac{df(t)}{dt} = \tau f \cdot \left(kf \cdot \left(1 + \frac{vf \cdot IL1R(t)}{kmf + IL1R(t)}\right) \cdot \left(1 + \frac{vfg \cdot glucose(t)^{xfg}}{kmfg^{xfg} + glucose(t)^{xfg}}\right) - f(t) \right) \tag{9}$$

The value for *vfg* was derived using data from[19], while *kmfg* and *xfg* were set equal to *Gh* and *vh* – assuming the effect of glycemia on the secretion factor to be directly related to insulin demand. Furthermore, *vf* was set based on data from human islets showing that high concentrations of IL-1β, in combination with IFN-γ, led to a 1.4-fold increase in the conversion time of proinsulin into insulin[20] and assuming that this leads to a similar effect on the secretion ratio. Finally, *kmf* and *τf* was assumed to be equal to *kms* and *τs* respectively, and *kf* was calculated from steady-state conditions (Suppl. Table 1).

Step 3 – Defining local IL-1β, IL-1Ra, and anakinra concentrations to simulate anakinra treatment

In the third and final step of model development, we first of all defined an initial state – to represent an average patient in[24, 25] – for our simulations (see section "Non-diseased and diseased states" below). To simulate treatment and placebo responses, we then defined concentration profiles for local IL-1β, IL-1Ra, and anakinra.

Anakinra pharmacokinetics. The anakinra plasma concentration was described by a one-compartment PK model with a subcutaneous administration site and with parameters estimated previously using data from[22, 23]. The local anakinra concentration at the β-cell was then assumed to be equal to the plasma concentration.

$$\frac{dAnakinrasc(t)}{dt} = -kab \cdot Anakinrasc(t) \tag{10}$$

$$\frac{dAnakinra(t)}{dt} = kab \cdot \frac{Anakinrasc(t)}{Vp} - \frac{CL}{Vp} \cdot Anakinra(t) \tag{11}$$

IL-1β. Local IL-1β was described using different equations for the treatment and placebo simulation. In the treatment simulation, local IL-1β was set to decrease to its assumed non-diseased level (*IL1bH*) during treatment and thereafter increase back towards its placebo state (Equation 13):

$$\frac{dIL1b(t)}{dt} = \begin{cases} k1 \cdot \left(IL1bH - IL1b(t)\right), & t < 91 \\ k2 \cdot \left(IL1b(0) + kplacebo \cdot t - IL1b(t)\right), & t > 91 \end{cases} \quad (12)$$

The rate of IL-1β decrease in the treatment phase, and thus the value of *k1*, was set based on a peripheral IL-1β clearance rate estimated from[24]. However, the exact value of the parameter has no significant impact on our conclusions as long as the non-diseased level is reached within a couple of weeks. Thereafter, the rate of IL-1β increase (*k2*) was estimated based on values of IL-6, CRP, PI/I ratio reported in[25, 26]. Admittedly, Larsen *et al*.[25] speculate that, while being surrogate markers for systemic IL-1 activity, IL-6 and CRP might not reflect IL-1 activity in the vicinity of the β-cells. However, the reported levels of CRP and IL-6 do demonstrate that 13 weeks of IL-1 blockade is capable of inducing a significant and partly sustained inflammatory remission, which at least suggests that there might be a sustained reduction also in local IL-1β. Such a sustained reduction in IL-1β is also supported by the partly sustained reduction seen in the PI/I ratio[25]. Therefore, we used a moderate value for *k2*, giving us an IL-1β level at 52 weeks of approximately half of its original value.

Using the same reasoning as above, IL-1β in the placebo simulation was assumed to increase slowly at a rate based on a slight increase in IL-6, CRP, and PI/I ratio[25]:

$$\frac{dIL1b(t)}{dt} = kplacebo \quad (13)$$

IL-1Ra. The local concentration of endogenous IL-1Ra was assumed constant in all simulations:

$$\frac{dIL1Ra(t)}{dt} = 0 \quad (14)$$

Non-diseased and diseased states

Non-diseased steady-state

Similar to the work of de Gaetano *et al.*[1], we considered a non-diseased steady-state corresponding to a glucose concentration of 5 mM and an insulin concentration of 50 pM. Furthermore, the steady-state proinsulin concentration was set to 6.5 pM, corresponding to a PI/I ratio of 0.13[27], and normal β-cell mass was set to 100 %. Finally, steady-state values for local IL-1β and IL-1Ra levels were determined according to the following reasoning: Average serum IL-1β and IL-1Ra concentrations in non-diabetic patients have been reported to be in the region of 0.5 pg/ml and 250 pg/ml respectively[28, 29]. Considering that both IL-1β and IL-1Ra are produced locally at the islets, however, the concentrations close to the β-cells might be much higher. For instance, Lachmann *et al*.[24] present a model of in-vivo regulation of IL-1β in patients with cryopyrin-associated periodic syndromes showing a 100-fold difference between IL-1β plasma concentrations and the concentration at the site of IL-1β production. By assuming the same difference in our case, and that the ratio of local IL-1β and IL-1Ra is similar to that in plasma – which is supported by the observed ratio of IL-1β and IL-1Ra secretion from human islets *in vitro*[30] – we arrived at local IL-1β and IL-1Ra concentrations of 50 pg/ml and 25 ng/ml respectively.

Diseased state

The diseased state was defined considering a reported average FPG concentration of 10.8 mM[25], an insulin concentration of 100 pM, and a 60 % decrease in β-cell mass[16]. This allowed us to calculate required changes in insulin sensitivity (to 22 % of normal), insulin secretion capacity (to 64 % of normal), and IL-1R modulation. Furthermore, by assuming a slightly increased level of local IL-1Ra (to 40 ng/ml) based on a reported 1.6 fold increase seen in serum at the time of T2DM diagnosis[28], we arrived at a required 100 fold increase in local IL-1β. Such a drastic increase is in line with an observed 2-200 fold increase in IL-1β mRNA expression seen in β-cells from patients with T2DM[31]. Both the non-diseased and diseased states are summarized in Supplementary Table 1.

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