Identification of a Serum Induced Transcriptional Signature Associated with Type 1 Diabetes in the BioBreeding Rat

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Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournals.org

Submitted 17 March 2010 and accepted 12 July 2010.

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**Objective:** Inflammatory mediators associated with type 1 diabetes (T1D) are dilute and difficult to measure in the periphery, necessitating development of more sensitive and informative biomarkers for studying diabetogenic mechanisms, assessing pre-onset risk, and monitoring therapeutic interventions.

**Research Design and Methods:** We previously utilized a novel bioassay where human T1D sera were used to induce a disease-specific transcriptional signature in unrelated, healthy peripheral blood mononuclear cells (PBMC). Here we apply this strategy to investigate the inflammatory state associated with T1D in Biobreeding (BB) rats.

**Results:** Consistent with their common susceptibility, sera of both spontaneously diabetic BB DR<sup>lyp/lyp</sup> and diabetes inducible BB DR<sup>+/+</sup> rats induced transcription of cytokines, immune receptors and signaling molecules in PBMC of healthy donor rats compared to control sera. Like the human T1D signature, the DR<sup>lyp/lyp</sup> signature, which is associated with progression to diabetes, was differentiated from that of the DR<sup>+/+</sup> by induction of many interleukin 1 (IL-1) regulated genes. Supplementing cultures with interleukin 1 receptor antagonist (IL-1Ra) modulated the DR<sup>lyp/lyp</sup> signature (<i>p</i>&lt;10<sup>-6</sup>), while administration of IL-1Ra to DR<sup>lyp/lyp</sup> rats delayed onset (<i>p</i>=0.007) and sera of treated animals did not induce the characteristic signature. Consistent with the presence of immuno-regulatory cells in DR<sup>+/+</sup> rats was induction of a signature possessing negative regulators of transcription and inflammation.

**Conclusions:** Paralleling our human studies, serum signatures in BB rats reflect processes associated with progression to T1D. Furthermore, these studies support the potential utility of this approach to detect changes in the inflammatory state during therapeutic intervention.

Type 1 diabetes (T1D) is characterized by immune infiltration of the pancreatic islets (insulitis) and destruction of the insulin-producing β cells. It is modeled by the BioBreeding (BB) rat, where disease is associated with insulitis, hyperglycemia, and exogenous insulin dependency (1; 2). Like humans and the non obese diabetic (NOD) mouse, the major histocompatibility complex (MHC, insulin dependent diabetes mellitus locus 1, <i>Iddm1</i>) contributes the largest genetic risk for T1D in BB rats (3; 4).

The DR<sup>lyp/lyp</sup> and DR<sup>+/+</sup> congenic BB rat lines differ only by the <i>Iddm2</i> locus on chromosome 4 (5). <i>Iddm2</i> has been cloned and the lymphopenia in DR<sup>lyp/lyp</sup> rats arises from a mutation in the <i>Gimap5</i> gene, that encodes a mitochondrial protein necessary for post-thymic T cell survival (6; 7). The spontaneously diabetic phenotype, which occurs in 100% of DR<sup>lyp/lyp</sup> rats during adolescence (65.3+/−6.3 days), is elicited through deficiency in regulatory T (T<sub>REG</sub>) cells, as diabetes can be rescued through adoptive transfer of this population (8).

T1D in the non-lymphopenic BB DR<sup>+/+</sup> rat, which possesses a wild-type <i>Gimap5</i>, is inducible through depletion of T<sub>REG</sub> cells (9; 10). Thus, in all BB rats, there is predisposition for T1D that is manifest upon loss of immune regulation. This predisposition is absent in Wistar-Furth (WF) rats, which share the RT1<sup>u</sup> MHC haplotype, since depletion of T<sub>REG</sub> cells does not induce disease. This susceptibility is also absent in Fischer rats, as introgression of RT1<sup>uu</sup> and/or
Gimap5-/ is insufficient for T1D development (11).

In addition to T cell responses, cytokines are important in diabetogenesis (12), as they are associated with β-cell destruction and disease status in both humans and spontaneous rodent models. Previously, we applied a sensitive genomics-based bioassay to investigate the presence of proinflammatory factors in human T1D. The approach used sera of recent onset T1D patients or healthy controls to induce transcription in unrelated healthy peripheral blood mononuclear cells (PBMC) (13). Recent onset T1D sera induced a transcriptional profile that included genes related to innate immunity and genes regulated by interleukin 1 (IL-1). The signature was distinct from that induced by sera of healthy controls or long-standing T1D patients and analysis of a limited number of pre-onset samples showed that it preceded disease and the development of autoantibodies. Our findings in T1D, and those reported for systemic onset juvenile idiopathic arthritis (14), support that expression signatures induced by serum factors associated with different inflammatory states are disease specific, are reflective of active disease, and are mechanistically informative.

Here we examine the serum induced transcriptional signatures of DR lyp/lyp and DR+/+ rats in an attempt to bridge the divide between invasive studies allowed in animal models and the peripheral blood sampling possible in humans. These studies identify an innate immune signature associated with progression to T1D in DR/lyp/lyp rats that is modulated by the addition of interleukin 1 receptor antagonist (IL-1Ra) in vitro, and find that administration of IL-1Ra to prediabetic animals modulates the inflammatory signature and delays disease onset.

**MATERIALS AND METHODS**

**Animals.** Brown Norway (BN) and BB rats were propagated as described (5; 6). Before euthanization, animals were fasted for 16 hours and those possessing blood glucose levels above 130 mg/dl were disqualified from studies of pre-diabetes. Rats were anesthetized under isoflurane and blood was collected by heart puncture. Serum was separated by centrifugation then stored at -80°C until use.

Human recombinant IL-1Ra (hIL-1Ra, 350 µg/kg/day) or vehicle (saline) was delivered to DR/lyp/lyp rats by intraperitoneal (i.p.) injection. Dosage was based upon previous hIL-1Ra administration to rodents (15; 16). Treatment was initiated by day 30 and continued either through day 40 or through diabetes onset. Diabetes onset was defined as the first of two consecutive blood glucose measurements >250 mg/dl. Survival was analyzed with the Kaplan-Meier method. All procedures were approved by the Medical College of Wisconsin Institutional Animal Care and Use Committee.

**PBMC cultures.** Fresh PBMCs of healthy Brown Norway (BN) rats (~180 days old males, to avoid variation introduced by estrous or pubertal status) were isolated by density gradient centrifugation. As described (13), transcription was induced by culturing PBMCs for 6 h at 37°C in 5% CO₂ with 20% autologous BN (self-baseline control), allogeneic BN (healthy-unrelated control), DR/lyp/lyp, or DR+/+ serum. Indicated cultures were supplemented with 1 ng/ml rat IL-1β or 1µg/ml rat IL-1Ra. RNA was extracted using TRIzol reagent, amplified/labeled using the Express Kit (Affymetrix, Santa Clara, CA), and hybridized to the Affymetrix Rat Genome 230 2.0 Array. RNA from each culture was independently analyzed. Image data were quantified with Affymetrix Expression Console Software and normalized with Robust Multichip Analysis (RMA; www.bioconductor.org/) to determine signal
log ratios. Analysis of variation (ANOVA) was conducted and false discovery rates (FDR) were determined using Partek Genomics Suite 6.2. To capture the most reliable data, limit the length of gene lists, and facilitate focused pathway analyses, differentially expressed probe sets were defined as those possessing FDR<10% and \( \log_2 \) ratio >0.5 between the compared groups. Ontological analysis was performed with the Database for Annotation, Visualization, and Integrated Discovery (DAVID) (17). Hierarchical clustering was conducted with Genesis (18). Orthologous rat and human probe sets were mapped using the Affymetrix support document: HG-U133_Plus_2.na29.orthologue.csv.zip. All raw data files have been deposited in The National Center for Biotechnology Information Gene Expression Omnibus (Accession Number GSE19537).

**Direct Detection of Inflammatory Mediators.** Sera of day 60 BB rats and day 180 BN rats were assayed with the BeadLyte cytokine assay kit (Millipore, Billerica, MA) per the manufacturer's protocol and a Bio-Plex Luminex 100 XYP instrument. Concentrations were calculated with the Bio-Plex Manager 4.1 software and a 5 parameter curve fitting algorithm applied for standard curve calculations. IL-33 levels in sera and IL-1β/α levels in conditioned culture medium were measured with Quantikine ELISAs (R&D Systems, Minneapolis, MN) and gram negative bacterial endotoxins (lipopolysaccarides, LPS) were measured by the Limulus amoebocyte lysate assay (Associates of Cape Cod, Inc; Falmouth, MA).

**RESULTS**

**Induction of Transcriptional Signatures.** To determine whether inflammatory mediators related to diabetogenesis could be detected through their ability to induce transcription, PBMCs of healthy day 180 BN rats were cultured with day 60 DR\(^{lyp/lyp}\) (representing the period immediately before onset), day 60 DR\(^{+/+}\), allogeneic BN, or autologous BN serum. To ensure induced transcription was not related to elevated blood glucose levels, only sera of normoglycemic, pre-onset rats were used. In preliminary studies, we observed that day 60 DR\(^{lyp/lyp}\) serum, like human recent onset sera, induced many genes regulated by IL-1. Therefore, PBMCs were also cultured with day 60 DR\(^{lyp/lyp}\) serum supplemented with IL-1Ra as well as autologous BN serum supplemented with IL-1β, to respectively block or induce IL-1 mediated gene expression. Culture of BN PBMCs with DR\(^{lyp/lyp}\) or DR\(^{+/+}\) sera regulated, within the threshold values, 1979 and 2904 probe sets, respectively, relative to culture with allogeneic BN sera.

**Analysis of genes commonly regulated by day 60 DR\(^{+/+}\) and DR\(^{lyp/lyp}\) sera.** As reflected in the Venn diagram illustrated in Figure 1A, culture of BN PBMCs with either DR\(^{lyp/lyp}\) or DR\(^{+/+}\) sera regulated a total of 3971 probe sets (Figure 1A, Supplement A in the online appendix available at [http://diabetes.diabetesjournals.org](http://diabetes.diabetesjournals.org)). Consistent with T1D susceptibility in both
strains, the signatures share a significantly nonrandom \( p<10^{-6}, \chi^2 \) test), commonly regulated intersection of 912 probe sets, that represents 46.1% and 31.4% of the probe sets induced by either DR\text{lyp/lyp} or DR+/+ serum, respectively. Hierarchical clustering (Figure 1B) shows the relatedness of the 5 experimental conditions for this subset. These 912 shared probe sets were annotated with DAVID, which identified significantly regulated Gene Ontology (GO) Biological Processes related to immunological activation, antigen presentation and intracellular signaling, in particular through the NFkB pathway (Table 1 and Supplement B).

NFkB is a transcriptional regulator of innate and adaptive immunity (19) that is activated through events such as binding of IL-1 to IL1R1 or LPS to toll-like receptor 4 (TLR4). These events converge at a set of I-kB kinases which phosphorylate the inhibitory I-kB proteins (NFKBIA and NFKBIB) leading to their degradation, allowing release and nuclear translocation of cytoplasmic NFkB where it facilitates target gene transcription. Both DR\text{lyp/lyp} and DR+/+ sera induced Nfk\text{b2} which encodes the p100 precursor that is cleaved to produce the NFkB p52 protein, the receptor-interacting serine-threonine kinase 2 (Ripk2), a potent activator of NFkB, as well as tumor necrosis factor receptor superfamily, member 25 (Tnfrsf25) which stimulates NFkB activity (20). Sera of either BB rat induced transcription of genes related to intracellular signaling, including signal transducer and activator of transcription 2 (Stat2), and mitogen-activated protein kinase-activated protein kinase 5 (Mapkapk5) which is activated by MAP kinases in response to exposure to proinflammatory cytokines. DR\text{lyp/lyp} and DR+/+ sera also induced transcription of genes encoding chemokines (Ccl2, Cxcl16) and receptors including Tlr4, Tlr7, Ccr1, and Cd14, important in TLR4 signaling. Other genes related to immune function included interleukin-1 receptor-associated kinase 3 (Irk3) and intercellular adhesion molecule 1 (Icam1). Both DR\text{lyp/lyp} and DR+/+ sera induced the transcription factor Kruppel-like factor 2 (Klf2), an inflammatory inhibitor that functions through the binding of coactivators necessary for optimal NFkB activity (21), and B-cell CLL/lymphoma 3 (Bcl3), an IL-10-inducible gene that impairs binding of NFkB complexes to DNA (19; 22). Importantly, all 912 probe sets of the intersection exhibited directionally concordant inductions by either DR\text{lyp/lyp} or DR+/+ sera relative to allogeneic BN sera. Furthermore, when directly comparing expression levels between the DR\text{lyp/lyp} and DR+/+, only two probes sets (2/912, 0.2%) were differentially expressed (Klf4 and Map4k2, Supplement A).

Overall, analysis of the intersection revealed that culturing BN PBMC with either DR+/+ or DR\text{lyp/lyp} sera, compared to allogeneic BN sera, induced transcription consistent with immunological activation (Figure 1C).

**Independent Analysis of genes regulated by DR\text{lyp/lyp} and DR+/+ sera.** To reveal processes associated with progression to T1D, the DR\text{lyp/lyp} and DR+/+ serum signatures were independently analyzed. The mean expression from each of the 5 experimental conditions was subjected to hierarchical clustering using the 1979 or 2904 probe sets respectively regulated by DR\text{lyp/lyp} sera or DR+/+ sera relative to the BN allogeneic induction (Figures 2A and 2C). Ontological analysis of the DR\text{lyp/lyp} signature identified Biological Processes related to proinflammatory processes possessing higher numbers of associated genes with greater significance compared to the analyses of the DR\text{lyp/lyp}:DR+/+ intersection or the DR+/+ signature (Table 1, Supplement B). Specifically, DR\text{lyp/lyp} sera regulated Biological Processes related to antigen presentation, inflammation, and leukocyte migration. Annotated genes related to these
functions included induction of \(I_{18b}, ~Tlr2, ~I_{18}, ~Icam2,\) proteasome subunit beta type 1, \((Psmb1), ~Stat1,\) and numerous MHC class II genes. Among the 1979 probe sets regulated by DR\_lyp/lyp sera were central components of the NF\(\kappa\)B cascade, including \(Nfkbia,\) and \(Nfkbib,\) as well as positive regulators of NF\(\kappa\)B activity including: casein kinase 2, beta subunit \((Csnk2b),\) a potent NF\(\kappa\)B activator that directly phosphorylates NFKBIA \((23),\) baculoviral IAP repeat-containing 2 \((Birc2)\) \((20),\) and \(Cd40\) (Figure 3A, Supplement A).

When examining the 1067 probe sets uniquely regulated by DR\_lyp/lyp sera (ie those excluding the intersection, Figure 2B), a role for IL-1 in induction of the signature became evident, as blocking IL-1R1 by adding IL-1Ra to cultures modulated the signature, yielding one more similar to that induced by DR\_+/+ sera. Specifically, after adding IL-1Ra to the culture, expression of 83.3% \((889/1067)\) of the DR\_lyp/lyp-specific probe sets no longer met the threshold values \((p<10^{-6}, ~X^2 \text{ test}).\) Among the 912 genes of the intersection only 40% \((374)\) were influenced by the addition of IL-1Ra \((p<10^{-6}, ~X^2 \text{ test}).\)

When examining the 1992 probe sets uniquely regulated by DR\_+/+ sera (Figure 2D), addition of IL-1Ra to the DR\_lyp/lyp culture resulted in induction of a signature more similar to that of the DR\_+/+ culture in terms of fold of change. However, the overall expression levels were less influenced, in that among the 1992 genes regulated in the DR\_+/+ culture, only 26.2% \((521/1992)\) of the probe sets in the DR\_lyp/lyp + IL-1Ra culture were regulated to meet the threshold values. These statistical findings are reflected in Figures 2B and 2D and show gene expression arising through IL-1R1 signaling accounts for a large part of the difference between the DR\_lyp/lyp and DR\_+/+ signatures. Furthermore, addition of IL-1Ra to DR\_lyp/lyp cultures reduced gene expression annotated as pro inflammatory (Figure 2B) and enhanced gene expression annotated as being regulatory (Figure 2D).

Addition of IL-1\(\beta\) to PBMC cultured with autologous BN sera induced a modest signature, more similar to that induced by that of BB rat sera than cultures possessing BN allogeneic sera (Figure 2), regulating 209 of the 3,971 probe sets regulated by DR\_+/+ or DR\_lyp/lyp serum relative to BN allogeneic serum.

Overall the ontological analysis of the 2904 probe sets regulated by DR\_+/+ sera revealed the greatest number of highly significant Biological Processes and these annotations were primarily related to regulation of gene expression (Table 1, Supplement B). Numerous genes related to negative regulation of inflammation and NF\(\kappa\)B signaling were regulated by DR\_+/+ sera, including \(Nfkbie,\) an inhibitor of NF\(\kappa\)B \((24),\) the transcriptional repressors zinc finger E-box binding homeobox 1 \((Zeb1),\) zinc finger and BTB domain containing 7a \((Zbtb7a),\) interferon regulatory factor 2 \((Irf2),\) ETS domain-containing protein Elk-3, the inhibitor of activated STAT 2 \((Pias2),\) suppressor of cytokine signaling-5 \((Socs5),\) the cell cycle inhibitor, myeloid/lymphoid or mixed-lineage leukemia 5 \((Mll5)\) and others (Figure 3B).

While the sera of either BB sub-strain induced gene expression consistent with immune activation, DR\_lyp/lyp sera induced an inflammatory signature consistent with the presence of IL-1, whereas that of the DR\_+/+ sera can be characterized as largely immuno-regulatory. Among the genes regulated by DR\_lyp/lyp and DR\_+/+ sera were 87 orthologues previously identified among the 587 genes regulated by human T1D sera (Figures 1 and 3, Supplement A) \((13).\)

**Direct Detection of Inflammatory Mediators.** In an effort to account for the induced signatures, cytokine levels were measured by ELISA in the DR\_lyp/lyp, DR\_+/+, and BN sera used in the expression studies (Table 2). Measurable differences in IL-1\(\alpha\) or IL-1\(\beta\) levels between DR\_lyp/lyp, DR\_+/+ or BN rat sera were not detected, raising the question
whether the induced transcription measured after 6 hours of culture was a primary effect of IL-1 on the PBMCs or a secondary effect of IL-1 produced by the PBMCs in response to other dilute mediators in DR/lyp/lyp sera. Conditioned medium of cultures supplemented with the various sera were assayed for IL-1α and IL-1β between at 0, 1, 3, 6, 12, and 24 hours. While IL-1α was not detected at all, significant detectable levels of IL-1β were measured in only DR/lyp/lyp cultures at 24 hours, but not at or prior to 6 hours (Table 3). This supports the hypothesis that the 6 hour transcriptional response is primary and is due in part to IL-1 itself, as lower IL-1β protein was not significantly detected in the DR+/+ and DR/lyp/lyp+IL-1Ra cultures. This parallels the significant detection of Il1b transcript only in cultures supplemented with DR/lyp/lyp sera. Significant differences in serum IL-10 were not observed, however, consistent with the transcriptional signatures, levels in DR+/+ and DR/lyp/lyp+IL-1Ra cultures. Recent studies have identified increased levels of LPS in human T1D patients (25). Given that TLR4 and IL-1R1 signaling both are mediated through the adaptor protein MyD88 to activate NFκB, we investigated LPS serum levels. On average, significantly higher LPS levels were detected in the DR/lyp/lyp and DR/+ rat serum compared to that of BN rats (Table 2), consistent with the significant induction of Il1b transcript in cultures possessing with DR/lyp/lyp sera even when supplemented with IL-1Ra (Supplement A).

**Delay of BB rat T1D through IL-1 receptor blockade.** Given the parallels between the signatures induced by human T1D and DR/lyp/lyp sera, in terms of over-representation of transcripts regulated by IL-1 and modulation of the DR/lyp/lyp signature upon addition of IL-1Ra to cultures, we tested the effectiveness of IL-1Ra in delaying T1D onset in BB rats. The treatment of BB rats with rat IL-1Ra is cost prohibitive while hIL-1Ra shares >70% amino acid homology with rat IL-1Ra and is bioactive in rat (15; 26). Thus, we reasoned that if IL-1 mediated inflammation was relevant to diabetogenesis in DR/lyp/lyp rats, treatment with hIL-1Ra should delay onset for approximately the amount of time required to mount a robust antibody response (~10-14 days). Treatment was initiated by day 30 and continued through T1D onset. Treated rats survived 71+/11 days while controls survived 61+/6 days (Figure 4A, p=0.007). Significant differences in weight or growth rate were not observed between treated and control rats (p>0.05). As expected, the animals possessed antibody titer to hIL-1Ra (Figure 4B).

**Use of transcriptional signature to monitor effect of IL-1 receptor blockade.** To determine if hIL-1Ra-treatment of DR/lyp/lyp rats would result in modulation of the serum signature, additional rats were treated with hIL-1Ra or saline for 10 days, beginning at d30. Serum was collected at day 40 and assayed to determine 1) if an inflammatory signature was present at this time prior to insulitis (27); 2) whether day 40 and day 60 signatures were similar; and 3) whether a day 40 signature was modulated in hIL-1Ra-treated rats. Furthermore, after only 10 days of treatment, the effect of hIL-1Ra treatment would be less likely masked by the high concentrations of neutralizing antibodies observed at onset (Figure 4B, lower panels).

Regulated probe sets between cultures possessing serum of saline- or hIL-1Ra-treated DR/lyp/lyp rats compared to allogeneic BN serum were identified and subjected to hierarchical clustering (Figures 4C and 4D). In the PBS-treated DR/lyp/lyp versus BN allogeneic comparison, 1526 probe sets met these criteria, and among these, a significant percentage (638/1526, 41.8%; p<10^{-6} \chi^2 test)
were also regulated by day 60 DR/lyp/lyp sera. Ontological analysis again identified Biological Processes related to antigen presentation, inflammation, leukocyte migration, and activation of the NFκB cascade (Table 1, Supplement 2). Selected genes related to these pathways are shown (Figure 4E, Supplement 1). Compared to BN sera, sera of day 40 DR/lyp/lyp rats treated with hIL-1Ra regulated only 5 probe sets. The 2 probe sets of the intersection exhibited directionally concordant inductions by either saline-treated DR/lyp/lyp or IL-1Ra-treated DR/lyp/lyp sera relative to allogeneic BN sera. As reflected by the fold changes illustrated in Figure 4D, treatment reduced induction of the proinflammatory signature observed in PBS-treated rats. Overall these results show that, like human T1D (13), a serum-induced signature with identity to that observed at onset is detected prior to onset in the DR/lyp/lyp rat. Moreover, this signature is modulated by treating rats with hIL-1Ra, suggesting that this approach may prove useful in monitoring the effect of therapeutic interventions in human T1D.

DISCUSSION

Previously, we defined a transcriptional signature induced by sera of human T1D patients (13). Here parallel studies were conducted that defined unique signatures for diabetes inducible DR+/+ and spontaneously diabetic DR/lyp/lyp rats. Like human T1D, the signature associated with disease progression in DR/lyp/lyp rats includes many genes regulated by IL-1 and differential regulation of NFκB signaling is a key feature distinguishing the signatures induced by sera of the DR/lyp/lyp and DR+/+ substrains.

Identity is observed between signatures induced by day 60 serum of DR/lyp/lyp and DR+/+ rats, in particular the 912 probe sets of the DR/lyp/lyp:DR+/+ intersection. Identity is also evident in the 1992 probe sets regulated to threshold levels by DR+/+ serum. These probe sets, annotated as immuno-regulatory, are regulated by DR/lyp/lyp serum to a lesser degree that does not meet threshold values. This suggests the presence of endogenous, albeit insufficient, immuno-regulatory activity in DR/lyp/lyp rats and may explain why relatively few (10^6) adoptively transferred DR+/+ T REG cells prevent T1D in DR/lyp/lyp rats (8). Conversely, from the perspective of the probe sets regulated by DR/lyp/lyp sera, the DR/lyp/lyp and DR+/+ signatures are distinct, with the DR/lyp/lyp signature possessing an IL-1 driven component that is down-regulated by adding IL-1Ra to the culture.

Among human T1D patients, a significant percentage will develop autoimmune thyroid disease (28). Likewise, lymphopenic BB rats develop autoimmune thyroiditis. Since the method measures the sum of the inflammatory factors present in serum, we cannot exclude that thyroid autoimmunity has not contributed to the signature defined for DR/lyp/lyp rats. In terms of other models, we have examined serum signatures associated with T1D in LEW.1WR1 rats (29) and find the inflammatory states distinct but sharing partial identity (Supplement C, (30)).

Despite the challenges of directly measuring peripheral cytokine levels in human T1D, studies have established that a complex cytokine milieu exists. This includes elevated IL-1 family members in patient cohorts before and after disease onset (31-35). Here ELISA analysis was unable to detect differences in IL-1β/α levels between DR/lyp/lyp, DR+/+ or BN rat sera. This may be related to assay sensitivity, the limited number of rats analyzed, or presence of soluble IL-1 receptors which impairs detection of free bioactive IL-1 and highlights the need for new, more sensitive biomarkers. Given that the amount of IL-1β (1 ng/ml) spiked into the autologous BN cultures exceeded the sensitivity of the multiplex cytokine analysis for IL-1α (>27 pg/ml) and
IL-1β (>27 pg/ml), the signatures induced by DR/lyp/lyp and DR+/+ sera (in particular genes of the intersection) are not likely an effect of IL-1 concentrations exclusively, but involve the contribution of other inflammatory mediators. Binding of IL-1 and LPS to their respective receptors initiate transcriptional programs similar to those observed. We found LPS levels in BB rats nearly twice that of BN rats, consistent with reported intestinal hyper-permeability in BB rats (36) which may lead to the translocation of bacteria and/or endotoxin and a heightened systemic inflammatory state. IL-13 was elevated in DR/lyp/lyp compared to DR+/+ and BN serum. This is consistent with elevated IgE levels and eosinophilia prior to onset in DR/lyp/lyp rats (37), as IL-13 induces immunoglobulin isotype switching to IgE in B cells and regulates eosinophilic inflammation.

*In vitro*, human and rodent pancreatic β cells are highly susceptible to the actions of IL-1 (38) and IL-1Ra can protect β cells from the downstream consequences of IL-1 exposure (39). While IL-1 transcript and protein are detected within immune infiltrated pancreatic islets of BB rats and NOD mice (40-42), *in vivo* modulation of either ligand or receptor in these models has yielded mixed results. Administration of high doses of IL-1 to diabetic prone BB rats induced higher blood glucose concentrations before and at T1D onset and accelerated onset (43). In contrast, administration of IL-1 to the NOD mouse delayed onset and reduced incidence (44; 45). IL-1 receptor deficiency in the NOD mouse slowed progression to T1D, but did not prevent it (46), and treatment of NOD mice with IL1-RA inhibited recurrence of T1D after islet transplantation (47). IL-1 has been shown to exacerbate autoimmunity by promoting expansion of effector T cells and attenuating T<sub>REG</sub> cell function (48), raising the possibility of IL-1 mediated impaired regulatory function of T<sub>REG</sub> cells in DR/lyp/lyp relative to DR+/+ rats. Treatment of DR/lyp/lyp rats with hIL-1Ra delayed onset for the time required to mount a neutralizing humoral immune response. These results are consistent with a previous study where hIL-1Ra delayed BB rat T1D onset but did not affect growth or modify lymphocyte counts (16). As reported here, anti-hIL-1Ra antibodies developed to which the short-term protection (2-3 weeks) was attributed. Importantly, with delayed onset we observe modulation of the serum induced transcriptional profile in hIL-1Ra-treated rats.

These findings support that this bioassay may not only have utility as an early mechanistically-informative inflammatory biomarker of T1D, but may prove useful in monitoring changes in inflammatory state during therapeutic interventions, including those targeting IL-1 (49).

**Author Contributions:** M.L.K., R.G., J.B. S.P. and S.K. conducted all laboratory studies. S.J. and X.W. conducted all bioinformatic and statistical analyses. Comparative studies of serum signatures between BB and LEW.1WR1 rats were done in collaboration with J.P.M. M.L.K., S.P., S.K., X.W. and Å.L. reviewed/edited manuscript. M.J.H designed/directed the study and wrote/reviewed/edited the manuscript.

**ACKNOWLEDGEMENTS**

This work was supported by National Institute of Allergy and Infectious Diseases Grants R01AI078713 (M.J.H.) and P01AI42380 (Å.L.); National Institute of Diabetes and Digestive and Kidney Diseases Grant R01DK080100 (X.W.); Juvenile Diabetes Research Foundation International Grant 1-2008-1026 (M.J.H.); Advancing a Healthier Wisconsin Initiative Grant 5520065 (M.J.H.); and The Children’s Hospital of Wisconsin Foundation. The authors are grateful to Sanja Glisic, Ph.D. and Parthav Jailwala, M.S. at The Medical College of Wisconsin for critical
review of this manuscript. We thank Shouguo Gao, Ph.D. at the University of Alabama-Birmingham for helpful discussions on statistical issues. Multiplex cytokine assays were conducted at the Baylor Institute for Immunology Research NIAID Cooperative Centers Luminex Core Facility under the direction of John Connolly, Ph.D.

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**Figure Legends:**

**Figure 1.** Analysis of genes commonly regulated by day 60 DR+/- and DR/lyp/lyp sera relative to allogeneic BN sera. PBMCs of 4 BN rats each were cultured under 6 different conditions: 1) autologous serum (n=4 cultures), 2) autologous serum spiked with IL-1β (1 ng/ml, n=4 cultures), 3) allogeneic BN serum (n=15 cultures) 4) a DR/lyp/lyp serum pool (n=4 cultures), 5) a DR/lyp/lyp serum pool supplemented with IL-1Ra (1 µg/ml, n=4 cultures), 6) DR+/- serum pool (n=4 cultures). The serum pools were created from an equal contribution of 6 individual male rats. Gene expression was measured independently in each culture and all data were normalized with that of the autologous induction to account for gene expression induced by placing the PBMCs into culture. (A) A Venn diagram illustrating relationship between the gene expression induced between the DR/lyp/lyp versus BN allogeneic and DR+/- versus BN allogeneic inductions (\(|\log_2\) ratio \(> 0.5, \pm 1.4\)-fold; ANOVA FDR<0.10). (B) The mean expression of the 5 experimental conditions were examined for relatedness by hierarchical clustering using the commonly regulated probe sets (n=912) of the DR/lyp/lyp versus BN allogeneic and DR+/- versus BN allogeneic intersection. Note the similarity between the DR/lyp/lyp and DR+/- signatures and the failure of IL-1Ra to highly influence the DR/lyp/lyp signature for this subset of genes. (C) Well annotated genes regulated in BN PBMC when cultured with either DR/lyp/lyp or DR+/- sera related to immune activation. Orthologues regulated by human T1D sera (13) are denoted by an asterisk (*). The scale represents fold of change between the serum tested relative to autologous serum (-4-fold to +4-fold).

**Figure 2.** Independent analysis of genes regulated by DR/lyp/lyp and DR+/- sera. In all cases, replicates were averaged and the relatedness of the 5 conditions was analyzed by hierarchical clustering. Panel A illustrates all probe sets regulated between the DR/lyp/lyp versus BN allogeneic inductions including probe sets of the DR/lyp/lyp:DR+/- intersection (n=1979). Panel B illustrates all probe sets regulated between the DR/lyp/lyp versus BN allogeneic inductions excluding probe sets of the DR/lyp/lyp:DR+/- intersection (n=1067). Note the distinctiveness of the DR/lyp/lyp signature relative to the DR+/- and the influence of IL-1Ra in impairing induction of the DR/lyp/lyp signature in induction of in panels A and B. Panel C illustrates all probe sets regulated between the DR+/- versus BN allogeneic inductions including probe sets of the DR/lyp/lyp:DR+/- intersection (n=2904). Panel D illustrates all probe sets regulated between the DR+/- versus BN allogeneic inductions excluding probe sets of the DR/lyp/lyp:DR+/- intersection (n=1992). Note similarity between DR/lyp/lyp and DR+/- signatures and how addition of IL-1Ra to DR/lyp/lyp cultures enhances expression in Panels C and D. The scale represents fold of change between the serum tested relative to autologous serum (-4-fold to +4-fold).

**Figure 3.** Well annotated regulated probe sets uniquely identified in (A) the DR/lyp/lyp versus BN allogeneic induction or (B) the DR+/- versus BN allogeneic induction. Orthologues
regulated by human T1D sera (13) are denoted by an asterisk (*). The scale represents fold of change between the serum tested relative to autologous serum (-4-fold to +4-fold).

**Figure 4.** Treatment of DR<sup>lyp/lyp</sup> rats with hIL-1Ra. (A) Longitudinal monitoring of DR<sup>lyp/lyp</sup> rats treated with 350 µg/kg/day human recombinant IL-1Ra (n=16, dashed line) or saline (n=23, solid line). Agents were delivered i.p. in saline. Treatment was initiated by d30 (prior to insulitis). Fasting blood glucose was measured three times per week and T1D onset was defined as the first of two consecutive fasting blood glucose measurements >250mg/dl. hIL-1Ra-treated rats survived 71 +/- 11 days (range 53–100) while saline-treated controls survived 61 +/- 6 days (range 53–75) (p=0.007, log-rank test). (B) Detection of anti-hIL-1Ra antibodies in IL-1Ra treated DR<sup>lyp/lyp</sup> rats. Indicated amounts of hIL-1Ra (17kD) were loaded onto polyacrylamide gels, electrophoresed, and blotted. Membranes were probed with a 1:2000 dilution of onset sera from hIL-1Ra-treated (top left blots), saline-treated (top right blot), day 40 hIL-1Ra-treated (bottom left blot), or saline-treated (bottom right blot) DR<sup>lyp/lyp</sup> rats. (C) A Venn diagram illustrating relationship between the gene expression induced between the PBS-treated DR<sup>lyp/lyp</sup> versus BN allogeneic and hIL-1Ra-treated DR<sup>lyp/lyp</sup> versus BN allogeneic inductions (log<sub>2</sub> ratio >0.5, ±1.4-fold; FDR<0.10). PBMCs of 6 BN rats each were cultured with a serum pool generated from 6 hIL-1Ra-treated DR<sup>lyp/lyp</sup> rats or a serum pool generated from 6 PBS-treated DR<sup>lyp/lyp</sup> rats (n=12 cultures). For PBMC of each donor BN rat (n=6), a culture possessing autologous sera was prepared. 15 cultures possessing allogeneic BN serum were prepared. Global gene expression was measured in each culture and all data were normalized with that of the autologous induction to account for gene expression induced by placing the PBMCs into culture. (D) Regulated probes were identified between the PBS-treated DR<sup>lyp/lyp</sup> versus BN allogeneic and hIL-1Ra-treated DR<sup>lyp/lyp</sup> versus BN allogeneic inductions, replicates were averaged, and the relatedness of the 3 conditions were examined by hierarchical clustering. (E) Well annotated, regulated probe sets regulated by sera of PBS-treated DR<sup>lyp/lyp</sup> rats versus the BN allogeneic induction. Orthologues regulated by human T1D sera (13) are denoted by an asterisk (*). The scale represents fold of change between the serum tested relative to autologous serum (-4-fold to +4-fold).
Table 1. Significantly Regulated Gene Ontology (GO) Biological Processes

| Biological Process                                         | Count | Percentage | P-value       |
|------------------------------------------------------------|-------|------------|---------------|
| **Day 60 DR\textit{lyp}/\textit{lyp}:DR+/+ intersection (n=912 probe sets)** |       |            |               |
| antigen processing and presentation of exogenous antigen    | 7     | 0.76%      | 2.55 X 10^-4 |
| immune system process                                      | 49    | 5.31%      | 2.28 X 10^-3 |
| antigen processing and presentation                        | 10    | 1.08%      | 4.57 X 10^-3 |
| antigen processing and presentation of peptide antigen      | 8     | 0.87%      | 8.62 X 10^-3 |
| immune response                                            | 33    | 3.58%      | 1.21 X 10^-2 |
| regulation of transcription                                | 87    | 9.44%      | 1.89 X 10^-2 |
| regulation of signal transduction                          | 33    | 3.58%      | 2.68 X 10^-2 |
| regulation of transcription, DNA-dependent                 | 79    | 8.57%      | 2.71 X 10^-2 |
| regulation of gene expression                              | 91    | 9.87%      | 3.54 X 10^-2 |
| myeloid leukocyte mediated immunity                        | 4     | 0.43%      | 3.65 X 10^-2 |
| I-kappaB kinase/NF-kappaB cascade                          | 11    | 1.19%      | 6.94 X 10^-2 |
| **Day 60 DR\textit{lyp}/\textit{lyp} (n=1979 probe sets)** |       |            |               |
| antigen processing and presentation of exogenous antigen    | 14    | 0.71%      | 6.54 X 10^-9 |
| antigen processing and presentation                        | 22    | 1.11%      | 4.47 X 10^-6 |
| antigen processing and presentation of peptide antigen      | 17    | 0.86%      | 3.55 X 10^-5 |
| immune system process                                      | 95    | 4.80%      | 3.50 X 10^-3 |
| I-kappaB kinase/NF-kappaB cascade                          | 24    | 1.21%      | 6.23 X 10^-3 |
| immune response                                            | 65    | 3.28%      | 1.00 X 10^-2 |
| myeloid leukocyte mediated immunity                        | 6     | 0.30%      | 2.15 X 10^-2 |
| leukocyte migration                                        | 9     | 0.45%      | 8.73 X 10^-2 |
| **Day 60 DR+/+ (n=2904 probe sets)**                       |       |            |               |
| regulation of transcription                                | 260   | 9.33%      | 8.75 X 10^-8 |
| regulation of gene expression                              | 276   | 9.90%      | 2.13 X 10^-7 |
| regulation of transcription, DNA-dependent                 | 237   | 8.50%      | 3.84 X 10^-7 |
| regulation of signal transduction                          | 91    | 3.27%      | 1.83 X 10^-4 |
| regulation of transcription from RNA polymerase II promoter | 81    | 2.91%      | 2.64 X 10^-5 |
| negative regulation of transcription, DNA-dependent        | 38    | 1.36%      | 9.95 X 10^-3 |
| antigen processing and presentation of exogenous antigen    | 8     | 0.29%      | 1.21 X 10^-2 |
| negative regulation of transcription                       | 49    | 1.76%      | 1.34 X 10^-2 |
| antigen processing and presentation                        | 17    | 0.61%      | 1.40 X 10^-2 |
| negative regulation of gene expression, epigenetic         | 5     | 0.18%      | 2.17 X 10^-2 |
| I-kappaB kinase/NF-kappaB cascade                          | 25    | 0.90%      | 3.33 X 10^-2 |
| immune system process                                      | 105   | 3.77%      | 3.75 X 10^-2 |
| **Day 40 PBS-treated DR\textit{lyp}/\textit{lyp} (n=1526 probe sets)** |       |            |               |
| antigen processing and presentation                        | 27    | 1.73%      | 2.63 X 10^-11|
| antigen processing and presentation of peptide antigen      | 21    | 1.35%      | 1.89 X 10^-9 |
| immune system process                                      | 96    | 6.17%      | 1.96 X 10^-6 |
| immune response                                            | 67    | 4.30%      | 2.31 X 10^-5 |
| antigen processing and presentation of exogenous antigen    | 9     | 0.58%      | 1.92 X 10^-4 |
| I-kappaB kinase/NF-kappaB cascade                          | 25    | 1.61%      | 1.98 X 10^-4 |

* Genes identified in Figures 1, 2, and 4 were analyzed for significantly regulated annotations by The Database for Annotation, Visualization and Integrated Discovery (DAVID). If an annotation was identified in more than one of the analyses, it is listed accordingly. Complete lists of annotations are provided in Supplement B. The P-value defines the significance of the association of a particular biological process with the gene list analyzed.
## Table 2. Cytokine/Chemokine levels in DR/lyp/lyp, DR+/+ and BN rats

| Cytokine | day 60 DR/lyp/lyp | day 60 DR+/+ | day 180 BN | Lower Detection Limit pg/ml |
|----------|------------------|--------------|------------|---------------------------|
|          | Mean pg/ml +/− S.E.M. | Mean pg/ml +/− S.E.M. | Mean pg/ml +/− S.E.M. |                           |
| IL-1a    | 0.0 0.0           | 0.0 0.0      | 0.0 0.0    | >27                       |
| IL-1b    | 15.6 4.4          | 11.7 6.0     | 11.8 8.1   | >27                       |
| IL-2     | 20.6 6.9          | 23.8 9.7     | 3.9 3.9    | >75                       |
| IL-4     | 1.9 1.9           | 5.1 3.9      | 6.7 3.3    | >27                       |
| IL-5     | 19.6 10.8         | 0.0 0.0      | 0.0 0.0    | >10                       |
| IL-6     | 1.6 1.3           | 0.0 0.0      | 1.8 1.8    | >250                      |
| IL-9     | 224.1 43.9        | 105.0 28.5   | 102.9 55.8 | >250                      |
| IL-10    | 107.2 30.0        | 58.2 19.4    | 42.7 24.2  | >27                       |
| IL-13    | 90.4 †            | 21.1         | 14.1 2.2   | >27                       |
| IL-17    | 0.0 0.0           | 0.0 0.0      | 0.0 0.0    | >10                       |
| IL-18    | 14.6 3.0          | 12.4 3.3     | 3.2 1.6    | >10                       |
| G-CSF    | 0.0 0.0           | 0.0 0.0      | 0.0 0.0    | >10                       |
| GM-CSF   | 6.9 5.2           | 0.0 0.0      | 2.9 2.9    | >27                       |
| GRO/KC   | 302.5 45.0        | 227.1 29.9   | 199.8 34.7 | >27                       |
| Eotaxin  | 0.9 0.9           | 0.0 0.0      | 8.4 2.8    | >27                       |
| IFNg     | 3.9 2.3           | 1.6 1.0      | 0.0 0.0    | >27                       |
| IP-10    | 0.0 0.0           | 0.0 0.0      | 0.2 0.2    | >10                       |
| Leptin   | 1727.4 607.1      | 2478.8 602.3 | 1438.4 203.9 | >10                     |
| MCP-1    | 80.0 32.9         | 45.8 21.1    | 87.6 7.3   | >75                       |
| MIP-1α   | 0.0 0.0           | 0.0 0.0      | 0.0 0.0    | >10                       |
| Rantes   | 1496.9 416.0      | 6757.0 4493.9| 1530.2 176.7| >27                      |
| TNFa     | 1.6 0.9           | 0.0 0.0      | 16.9 3.1   | >10                       |
| VEGF     | 0.0 0.0           | 0.0 0.0      | 0.0 0.0    | >27                       |
| IL-12p70 | 0.0 0.0           | 0.0 0.0      | 0.0 0.0    | >27                       |
| IL-33    | 8.9 0.4           | 6.8 0.1      | 5.6 0.5    | >7                        |
| LPS **   | 1.30‡             | 0.2          | 1.34‡      | 0.2                       |

Each sample was tested in duplicate using the Millipore BeadLyte cytokine assay kit, with the exception of IL-33 and LPS. Values represent the mean and standard error of 6 rats per group.

* p<0.01 Wilcoxon rank sum test vs BN
† p<0.01 Wilcoxon rank sum test DR/lyp/lyp vs DR+/+
‡ p<0.05 Wilcoxon rank sum test vs BN
** Conversion from endotoxin units (EU) to pg is based upon 1EU=100pg
Table 3. IL-1β levels in conditioned medium after PBMC culture with DR/lyp/lyp, DR+/+ and BN sera.

| Duration | day 60 DR/lyp/lyp Mean +/- S.E.M. | day 60 DR/lyp/lyp+IL1-Ra Mean +/- S.E.M. | day 60 DR+/+ Mean +/- S.E.M. | day 180 BN Allogeneic Mean +/- S.E.M. | day 180 BN Autologous Mean +/- S.E.M. |
|----------|-----------------------------------|------------------------------------------|-------------------------------|---------------------------------------|--------------------------------------|
| 0 hour   | 2.9 +/- 1.7                       | 1.7 +/- 1.2                              | 4.4 +/- 2.0                   | 2.2 +/- 1.3                           | 2.0 +/- 2.0                          |
| 1 hour   | 5.3 +/- 2.3                       | 5.3 +/- 2.0                              | 4.4 +/- 1.8                   | 0.0 +/- 0.0                           | 0.7 +/- 0.8                          |
| 3 hours  | 4.7 +/- 2.2                       | 7.7 +/- 6.7                              | 4.1 +/- 2.3                   | 3.3 +/- 2.9                           | 0.8 +/- 0.5                          |
| 6 hours  | 3.6 +/- 1.4                       | 5.2 +/- 1.9                              | 7.7 +/- 3.5                   | 2.7 +/- 2.5                           | 1.9 +/- 0.9                          |
| 12 hours | 7.0 +/- 2.5                       | 10.3 +/- 3.8                             | 4.6 +/- 1.7                   | 9.0 +/- 4.0                           | 6.6 +/- 3.3                          |
| 24 hours | **12.4** +/- **3.3**              | **10.1** +/- **5.4**                     | **9.5** +/- **4.1**           | **1.2** +/- **0.7**                    | **4.0** +/- **1.8**                   |

Each culture was tested in duplicate using IL-1β Quantikine ELISA kit (R&D Systems). Values represent the mean and standard error of 4 cultures per group.

In cultures possessing autologous BN sera supplemented with 1ng/ml IL-1β, on average 816.3 ± 51.7 pg/ml was detected across the 6 time points.

Assay sensitivity: >5 pg/ml.

*<p<0.05 Student’s t-test vs 0 hour time point.
†<p<0.05 Student’s t-test vs day 180 allogeneic BN sera at 24 hours of culture.
Transcriptional signatures in the diabetic prone BB rat

Figure 1

A

B

C

Expression Ratio
Relative to Autologous

| Gene    | Entrez ID | DR hyp/hyp | DR hyp/hyp + IL-1Ra | DR hyp/hyp |
|---------|-----------|------------|----------------------|------------|
| Ccl2    | 24770     | 4.59       | 5.45                 | 4.04       |
| Cr14    | 35829     | 3.33       | 3.25                 | 3.62       |
| Gcr1    | 57301     | 2.88       | 2.53                 | 2.17       |
| Hmox1   | 24451     | 2.49       | 2.19                 | 2.18       |
| Thbd    | 83580     | 2.26       | 2.22                 | 2.16       |
| CtsH    | 25697     | 2.94       | 1.83                 | 1.56       |
| Nek6    | 360161    | 1.80       | 1.79                 | 1.53       |
| Trf4    | 29269     | 1.43       | 1.40                 | 1.26       |
| Kif4    | 114565    | 1.88       | 3.44                 | 2.03       |
| Bcl3    | 306330    | 1.28       | 2.55                 | 1.93       |
| Klf2    | 50692     | 1.56       | 1.91                 | 1.11       |
| Atf1    | 117540    | 1.67       | 1.35                 | 1.57       |
| Plaur   | 29427     | 1.84       | 1.59                 | 1.31       |
| Fgfr3   | 300662    | 1.47       | 1.64                 | 1.28       |
| Rppk2   | 362491    | 1.46       | 1.59                 | 1.49       |
| Thr1    | 500592    | 1.52       | 1.93                 | 1.69       |
| RT1-Bb  | 288774    | 1.28       | 1.50                 | 1.27       |
| Traf7   | 317445    | 1.26       | 1.33                 | 1.33       |
| Mapk6   | 498165    | 1.28       | 1.33                 | 1.33       |
| Map4k1  | 292763    | 1.22       | 1.68                 | 1.52       |
| Map4k2  | 293694    | 1.31       | 1.94                 | 1.33       |
| Mx1     | 24575     | 1.30       | 1.26                 | 1.25       |
| Birc3   | 78971     | 1.29       | 1.21                 | 1.09       |
| Ikar1   | 31480     | 1.23       | 1.97                 | 1.17       |
| Lta4h   | 299732    | 1.41       | 1.15                 | 1.32       |
| Kcnn1   | 25464     | 1.23       | 1.31                 | 1.54       |
| Eif2a   | 317163    | 1.30       | 1.43                 | 1.03       |
| RhoC    | 309341    | 1.22       | 1.28                 | 1.16       |
| Psme1   | 29639     | 1.27       | 1.11                 | 1.13       |
| Trfas1   | 156767    | 1.20       | 1.05                 | 1.20       |
| Faln    | 140930    | 1.23       | 1.13                 | 1.02       |
| Gs58    | 20648     | 1.19       | 1.07                 | 1.05       |
| Hsp1    | 79245     | 1.29       | 1.29                 | 1.51       |
| Gard1   | 304314    | 1.13       | 1.29                 | 1.43       |
| Cola    | 24339     | 1.13       | 1.13                 | 1.04       |
| Nfh2b   | 309452    | 1.04       | 1.02                 | 1.33       |
| Lat     | 81511     | 1.04       | 1.02                 | 1.33       |
| Gosm3   | 406163    | -1.07      | 1.14                 | 1.11       |

fold of change relative to autologous
Figure 2

A, B, C, D: Heatmaps showing transcriptional signatures in different diabetic prone BB rat strains. Each heatmap represents different conditions and control groups, with fold change indicated by the color scale. The overlap (intersection) of genes in DR\textsuperscript{lyp/lyp} and DR\textsuperscript{+/-} is highlighted in the central Venn diagram.
Figure 3
Transcriptional signatures in the diabetic prone BB rat

Figure 4

A

![Graph showing percent diabetes free vs age in days](image)

B

![Images of hIL-1Ra and saline treated groups at onset and day 40](image)

C

![Venn diagram showing unique and union sets](image)

D

![Heatmap showing expression ratio relative to autologous](image)

E

| Gene | PBS-treated | hIL-1Ra-treated | DR lyp/yp | DR lyp/yp |
|------|-------------|-----------------|-----------|-----------|
| Sphk1* | 179667 | 1.44 | 1.11 |
| Il1ra | 294273 | 1.47 | 1.04 |
| Bc10 | 325924 | 1.32 | 1.23 |
| Tsc22d2 | 381857 | 1.35 | 1.14 |
| Lib | 117106 | 1.35 | 1.14 |
| Arc | 149934 | 1.35 | 1.11 |
| Sig1r | 660111 | 1.33 | 1.38 |
| Hla-dimb | 294274 | 1.40 | 1.13 |
| Prkcb | 25023 | 1.33 | 1.02 |
| Rtt-8b | 208622 | 1.26 | 1.02 |
| Fasn | 549026 | 1.24 | 1.10 |
| Jak3 | 22329 | 1.27 | 1.01 |
| Icam1 | 25646 | 1.24 | 1.64 |
| Gadd45a | 29216 | 1.19 | 1.78 |
| Socs1 | 25124 | 1.17 | 1.98 |
| Cskb1 | 8760 | 1.22 | 1.92 |
| Rtt-8l1 | 294270 | 1.26 | 1.02 |
| Pamb1 | 24368 | 1.24 | 1.03 |
| Card11 | 304214 | 1.16 | 1.97 |
| Cdy1O | 12153 | 1.20 | 1.11 |
| Ctla4 | 25469 | 1.20 | 1.02 |
| Gpama3 | 409163 | 1.14 | 1.06 |
| Icam3 | 361690 | 1.12 | 1.06 |
| Sltk1 | 211523 | 1.13 | 1.11 |
| Tr2 | 288774 | 1.11 | 1.20 |
| Stat2 | 81255 | 1.10 | 1.00 |
| Nild0 | 290644 | 1.10 | 1.00 |
| Nild0a | 309452 | 1.10 | 1.00 |
| Nild0b | 35463 | 1.06 | 1.02 |
| Nild0c | 30033 | 1.11 | 1.03 |
| Nild0d | 61101 | 1.07 | 1.03 |

Fold of change relative to autologous