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SYMPOSIUM

Importance of Interleukin-1 and Interleukin-1 Receptor Antagonist in Short-Term Glucose Sensor Function *in Vivo*

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Abstract

Background:

The importance of the interleukin (IL)-1 cytokine family in inflammation and immunity is well established as a result of extensive *in vitro* and *in vivo* studies. In fact, much of our understanding of the *in vivo* importance of interleukin-1beta (IL-1B) is the result of research utilizing transgenic mice, such as overexpression or deficiencies of the naturally occurring inhibitor of IL-1 known as interleukin-1 receptor antagonist (IL-1RA). For the present studies, we utilized these transgenic mice to determine the role of IL-1B in glucose sensor function *in vivo*.

Methods:

To investigate the role of IL-1B in glucose sensor function *in vivo*, we compared glucose sensor function in transgenic mice that (1) overexpressed IL-1RA [B6.Cg-Tg(Il1rn)1Dih/J] and (2) are deficient in IL-1RA (B6.129S-*Il1rn*^{tm1Dih}/J), with mice that have normal levels of IL-1RA (C57BL/6).

Results:

Our studies demonstrated that, during the first 7 days post-sensor implantation (PSI), mice deficient in IL-1RA had extensive inflammation and decreased sensor function when compared to normal or IL-1RA-overexpressing mice.

Conclusion:

These data directly support our hypothesis that the IL-1 family of cytokines and antagonists play a critical role in controlling tissue reactions and thereby sensor function *in vivo* during the first 7 days PSI.

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Abbreviation: (CGM) continuous glucose monitoring, (DPI) days post-implantation, (HES) hematoxylin eosin stain, (icIL1RA) intracellular interleukin 1 receptor antagonist, (IL) interleukin, (IL-1B) interleukin-1beta, (IL-1RA) interleukin-1 receptor antagonist, (IL-1RA-KO) interleukin-1 receptor antagonist knockout mice (B6.129S-*Il1rn*^{tm1Dih}/J), (IL-1RA-OE) interleukin-1 receptor antagonist overexpressing mice (B6.Cg-Tg(Il1rn)1Dih/J), (IL-1R) interleukin-1 receptor, (IL-1RN) interleukin-1 receptor antagonist gene designation, (LCF) leukocyte chemotactic factor, (MQ) macrophage, (PMN) polymorphonuclear leukocyte, (PSI) post-sensor implantation, (sIL-1RA) soluble interleukin-1 receptor antagonist, (TNF) tumor necrosis factor, (VEGF) vascular endothelial growth factor

Keywords: angiogenesis, biosensor, diabetes, fibrosis, inflammation, interleukin-1, interleukin-1 receptor antagonist, tissue responses

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Introduction

Tissue reactions at sites of glucose sensor implantation, e.g., inflammation, fibrosis, and loss of vasculature, are generally thought to be major contributors to the loss of glucose sensor function *in vivo*. This loss of sensor function *in vivo* is a result of tissue reactions that include biofouling of the sensor, sensor encapsulation (fibrosis), formation of metabolic barriers by inflammatory cells,[1,2] and loss of vasculature (vessel regression). Unfortunately, the specific mediators and mechanisms involved in the loss of sensor function *in vivo* remains unclear. However, the importance of inflammation in this loss of sensor function is well established. For example, our laboratory demonstrated that anti-inflammatory drugs such as corticosteroids (dexamethasone) not only suppresses inflammation at sites of sensor implantation, but also extends sensor lifespan *in vivo*.[3] Unfortunately, steroids have many drawbacks. For example, although corticosteroids are effective anti-inflammatory agents for short-term suppression of inflammation, steroids can have significant negative side effects when used long-term.[4,5] Additionally, corticosteroids are broad-spectrum anti-inflammatory agents that affect a wide range of inflammatory and wound-healing pathways, thus the specific mechanism(s) and mediators that are affected by corticosteroids are not completely catalogued or understood. Interestingly, part of the mechanisms of the anti-inflammatory effects of steroids appears to be related to suppression of cytokine expression by various cells.[6–8] For example, steroids are known to suppress interleukin-1beta (IL-1B) expression in various cell types.[9–11] Since there is a significant body of literature that clearly indicates that cytokine and growth factor networks are critical to controlling inflammation and wound healing, it is likely that cytokine networks play a significant role in controlling inflammation and wound healing that impact sensor function *in vivo*.

Cytokines are small molecular weight glycoproteins, which range from 6000 to 70,000 Da (generally <20,000 Da), that play a critical role in controlling innate and acquired immunity, inflammation, and wound healing (angiogenesis, regeneration, and fibrosis) in a wide variety of diseases and infections. Among the primary cytokine families involved in inflammation and wound healing is the IL-1 and tumor necrosis factor (TNF) families. For example, IL-1B and TNF-alpha (TNFa) are considered to be key initiators of a wide range of proinflammatory cell and tissue reactions (i.e., prime cytokines) because of their ability to amplify tissue inflammation by inducing additional expression of other proinflammatory cytokines. Thus IL-1 and TNFa are central to immunity and host defense, as well as acute and chronic inflammatory diseases such as rheumatoid arthritis,[12,13] inflammatory bowel disease,[14,15] and interstitial lung disease[16] to name a few. We hypothesize that proinflammatory cytokines, such as IL-1B and TNFa, likely play critical roles in tissue reactions at sites of sensor implantation via regulation of leukocyte and tissue cell activation and mediator expression at these sites. Interleukin-1B is a powerful proinflammatory cytokine, and its regulation/blockade is critical to preventing uncontrolled inflammation and tissue destruction, including foreign body reactions. Crucial to controlling IL-1B-mediated inflammation is the naturally occurring IL-1B antagonist IL-1 receptor antagonist (IL-1RA).[17] Interleukin-1RA competes with IL-1 for binding to the IL-1 receptors and thereby prevents IL-1 activation of both recruited leukocytes and resident tissue cells.[18] For the purpose of this study, the term "resident tissue cells" refers to the population of cells normally present at the site of sensor implantation, i.e., in the skin. The importance of IL-1RA in controlling inflammation has also been supported by studies using transgenic mice that demonstrate that overexpression of IL-1RA in these mice suppresses inflammation,[19] and IL-1RA knockout (KO) mice have increased inflammation and tissue destruction in response to injury.[17,19] For our present studies, we have focused on the role of the IL-1 family, particularly IL-1B (agonist), and its naturally occurring antagonists IL-1RA in glucose sensor function and related tissue reactions utilizing these transgenic mice in our mouse model of continuous glucose monitoring (CGM).[1,20] We specifically hypothesize that inflammation at sensor implantation sites will be increased and sensor function decreased in IL-1RA-KO mice, when compared to wild-type mice and IL-1RA overexpressing (OE) mice.

Methods and Materials

Interleukin-1 Receptor Antagonist Knockout and Interleukin-1 Receptor Antagonist Overexpressing Mouse Models

For these *in vivo* studies, female IL-1RA-KO and IL-1RA-OE mice were used. IL-1RA-KO (B6.129S-*Il1rn*^{tm1Dih}/J) and IL-1RA-OE mice (B6.Cg-Tg(Il1rn)1Dih/J) were obtained from Jackson Laboratory (Bar Harbor, ME).[21] Additionally, female C57BL/6 mice were used as normal controls

for these studies and were also obtained from Jackson Laboratory. All mice were maintained on antibiotic water for the duration of the experiment. Generally, mice used were between 20 and 30 g. At least 10 mice per mouse strain (e.g., wild type, IL-1RA-KO, IL-1RA-OE) were used in these studies.

Glucose Sensors, Implantation, and Murine Continuous Glucose Sensor System

All modified Navigator glucose sensors used in these *in vivo* studies were obtained from Abbott Diabetes Care. Sensors were modified by removal from the standard transdermal insertion unit and by the attachment of wires to the electrode contact pads. Glucose sensors were implanted into IL-1RA-KO, IL-1RA-OE, or C57BL/6 mice, and CGM was undertaken for a period of 7 days as previously described.[1,20] For the present studies, all sensor data are presented as raw current signals (nA) in order to evaluate the true noncalibrated signal dynamics, i.e., no sensor calibration or recalibration. Current data at 60 s intervals were overlaid on blood glucose reference measurements in dual *y*-axis plots to obtain a best visual fit. Blood glucose reference measurements were obtained at least daily using blood obtained from the tail vein of the mouse and a FreeStyle® blood glucose monitor. The Institutional Animal Care and Use Committee of the University of Connecticut Health Center (Farmington, CT) approved all mice studies. Representative examples of CGM data for each strain of mouse are provided in figures included in this article.

Histopathologic Analysis of Tissue Reactions at Glucose Sensor Implantation Sites

In order to evaluate tissue responses to glucose sensor implantation at various time points, individual mice were euthanized and the full thickness of the skin and sensors were removed *en bloc* in approximately 3 × 3 cm sections and immediately placed in tissue fixative. Tissue was fixed in zinc buffer for 24 h, followed by standard processing, embedded in paraffin, and sectioned. The resulting 4–6 μm sections were then stained using standard protocols for hematoxylin eosin stain (HES) and Masson trichrome (fibrosis). Histopathologic evaluation of tissue reactions at sites of sensor implantation was performed on mouse specimens obtained at 1, 3, and 7 days post-implantation (DPI) of the glucose sensor. The tissue samples were examined for signs of inflammation, including necrosis, fibrosis, angiogenesis, and vessel regression. Resulting tissue sections were evaluated directly and documented by digitized imaging using an Olympus digital microscope. Representative examples are shown in figures included in this article.

Results

Glucose Sensor Function in Normal Mice (C57BL/6)

To begin our studies, we first determined the CGM profile of normal C57BL/6 mice over a 7-day post-sensor implantation (PSI) time period (**Figure 1**). As expected, the analog-to-digital converter-modified Navigator sensors displayed excellent CGM during the first 7 DPI, with glucose sensing closely following highs and lows of mouse blood glucose levels (**Figure 1**). Data presented in **Figures 1A** and **1B** show a sudden increase in the blood glucose level around days 2 and 3, respectively, PSI. The reason for this sudden increase in blood glucose levels is not always obvious. However, we believe that, since the mouse had a low blood sugar level (around 50 mg/dl), which might have been the result of the stress associated with the initial sensor implantation period, the mouse started eating and developed a more physiological blood glucose level at 2 and 3 DPI. On the other hand, we have also observed that any acute stress (e.g., cage changes, isoflurane administration, or loud noise) can also lead to a temporary increase in blood sugar level. Regardless of cause, the modified Navigator sensor tracked both hyperglycemic and hypoglycemic events in the normal mice (**Figure 1**). These data clearly demonstrate that the modified Navigator sensor has a very good response profile throughout the first week post-implantation and is consistent with our previously published data.[1,20]

Glucose Sensor Function in Interleukin-1 Receptor Antagonist Knockout Mice

Since we hypothesized that IL-1RA is important in controlling tissue reactions at sites of sensor implantation, we next determined the impact of IL-1RA deficiency on sensor function using the IL-1RA-KO mice. Over the 7-day period of CGM, sensor output failed to reliably track with blood glucose levels in the IL-1RA-KO mice (**Figures 2A–D**). Specifically, sensor output in the IL-RA-KO mice during 1–3 days PSI consistently failed to correlate with blood glucose levels in the IL-1RA-KO mice (**Figure 2**). Additionally, sensor output beyond 3 days PSI was occasionally erratic in the IL-1RA-KO mice (**Figure 2A**) but, in most cases, regained function and correlated well with the sporadic blood glucose reference measurements (**Figures 2B–D**). In summary, unlike normal C57BL/6 mice (**Figures 1A–D**), sensor output in IL-1RA-KO mice during 7 days of CGM failed to track hyperglycemic and hypoglycemic events consistently in these mice (**Figure 2**), particularly within the first 72 h PSI. These data directly support our hypothesis that IL-1B and IL-1RA play an important role in short-term CGM *in vivo*.

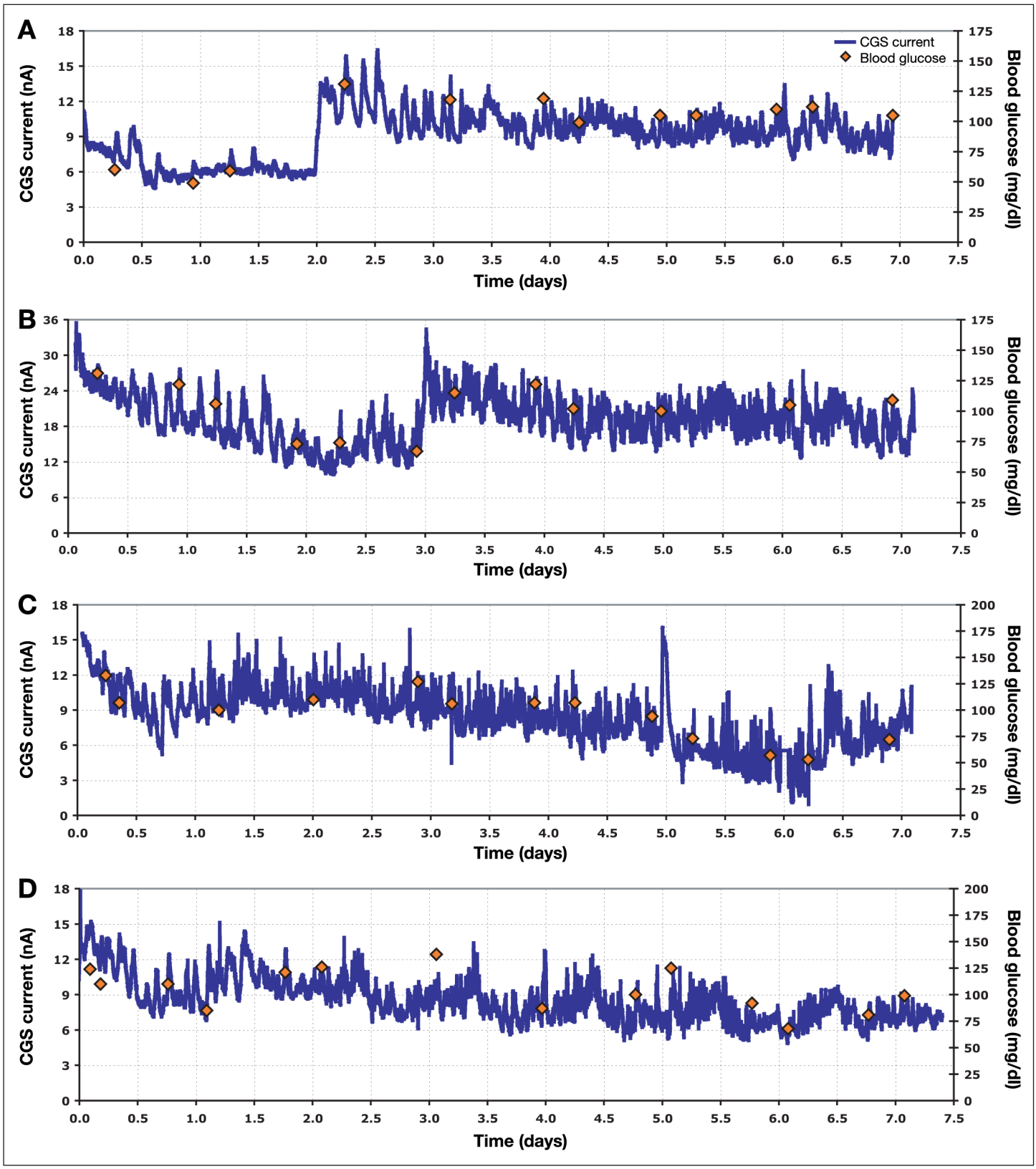

Figure 1. Continuous glucose monitoring in normal C57BL/6 mice over a seven-day time period. Sensor output is expressed as CGS output (nA) and is represented by the blue curves. Blood glucose levels are represented by red diamonds.

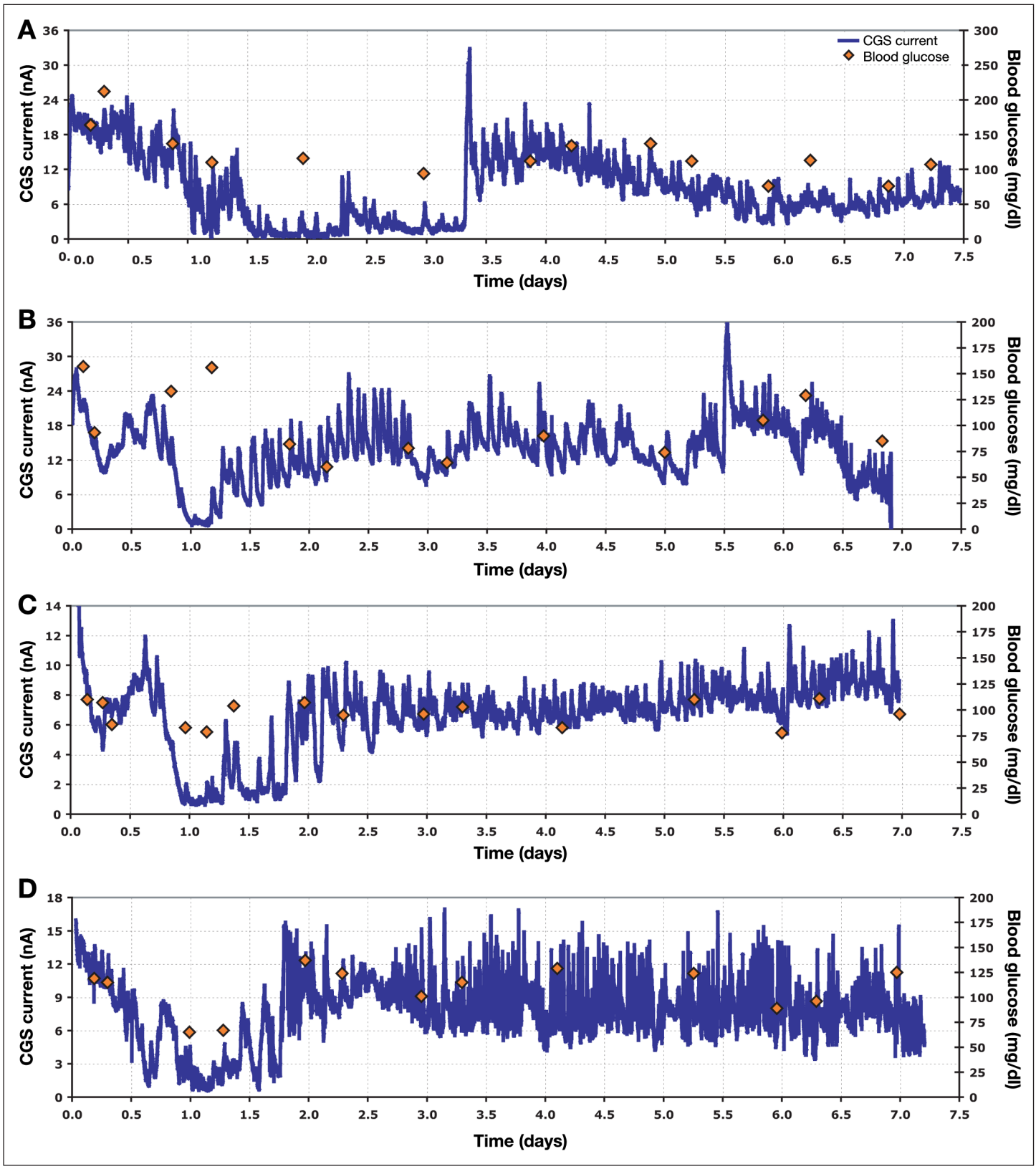

Figure 2. Continuous glucose monitoring in IL-1RA-KO mice over a seven-day time period. Sensor output is expressed as CGS output (nA) and is represented by the blue curves. Blood glucose levels are represented by red diamonds.

Glucose Sensor Function in Interleukin-1 Receptor Antagonist Overexpressing Mice

The IL-1RA-KO studies described here indicated that the absence of IL-1RA negatively impacts glucose sensor function *in vivo*. We hypothesize that sensor function in the IL-1RA-OE mice would be as good or possibly better than sensor function in wild-type mice. To test this hypothesis, we investigated the impact of overexpression of IL-1RA on sensor function using IL-1RA-OE mice. As was the case with C57BL/6 mice, sensor output in IL-1RA-OE mice correlated well with the reference blood glucose measurement during the entire 7-day testing period (**Figure 3**). As with the wild-type mice, there was no dramatic loss of sensor function in the IL-1RA-OE mice. These data support the concept of the importance of IL-1RA in controlling IL-1 sensor function, likely by controlling tissue reactions during the initial days PSI.

Inflammation and Fibrosis at the Sites of Glucose Sensor Implantation

The sensor function in normal, IL-1RA-KO, and IL-1RA-OE mice described here clearly demonstrates the key role of the IL-1 family of cytokines, i.e., IL-1/IL-1RA, in controlling sensor function *in vivo*. The next obvious question was, "how did alterations in IL-1RA expression influence tissue reactions *in vivo*?" We hypothesized that IL-1 drives inflammation and fibrosis at sites of sensor implantation, which is normally inhibited by the IL-1B antagonist IL-1RA. Therefore, we would predict that, by removing IL-1RA control of the proinflammatory cytokine IL-1B (i.e., IL-1RA deficient/KO mice), there would be an increase in inflammation and fibrosis at sites of sensor implantation, ultimately resulting in a loss of sensor function. To investigate this possibility, we evaluated sensor–tissue sites using HES (tissue histology) as well as trichrome staining (collagen deposition) technology at 1, 3, and 7 days PSI in wild-type IL-1RA-KO and IL-1RA-OE mice. As expected, tissue reactions in wild-type C57BL/6 mice were limited over the first 7 days PSI, i.e., minimum inflammation and tissue destruction (see **Figures 4A–C**).[1] The early stages (1–2 days) of the tissue reactions in wild-type mice were characterized by the presence of polymorphonuclear leukocytes (PMNs) with a predominance of mononuclear leukocyte at the later stages of the tissue reactions (i.e., days 3–7). IL-1RA-OE mice displayed tissue reactions similar to wild-type mice over both the early and later days PSI (see **Figures 4G–I**). Alternatively, IL-1RA deficiency (e.g., IL-1RA-KO mice) resulted in a dramatically increased tissue inflammation (**Figures 4D–F**) when compared to normal (**Figures 4A–C**) or IL-1RA-OE (**Figures 4G–I**) mice. Inflammation was consistently intense in the IL-1RA-KO mice at both early stages PSI (i.e., days 1–3) as well as later stages PSI (day 7) (**Figures 4D–F**). It should be noted that leukocyte accumulation in early stages (days 1–2 PSI) of the tissue reactions in IL-1RA-KO mice was characterized by the presence of PMNs. At later stages of the tissue reactions (days 5–7 PSI) in IL-1RA-KO mice, macrophages (MQs) were the dominant leukocytes at the sensor implantation site. Of particular interest was that there was significantly higher accumulation of PMNs and MQs at the interface of the sensor with tissue in IL-1RA-KO mice (**Figures 4D–F**) when compared to normal (**Figures 4A–C**) or IL-1RA-OE mice (**Figures 4G–I**). This increase in MQs at the interface of the sensor is likely significant because it is known that MQs are key cells in controlling inflammation and fibrosis at sites of tissue injury, including foreign body reactions. Using trichrome staining techniques, we evaluated the impact of IL-1RA deficiency (IL-1RA-KO mice) or overexpression (IL-1RA-OE mice) on fibrosis at the site of sensor implantation. It should be noted that, because of the relatively short time period of 7 days, it was expected that only limited fibrosis could occur at the implantation sites. As expected, in normal mice (C57B6), there was no significant collagen associated with implanted sensors during days 1–3 PSI (**Figures 5A** and **B**), and by day 7, there was only limited collagen association with the implanted sensors (**Figure 5C**). As it relates to IL-1RA-OE mice, we also saw limited collagen association with the implanted sensors at days 1–3 and slightly more by 7 DPI (**Figures 5G–I**), effectively the same as the wild-type mice. In the case of IL-1-KO mice, there appeared to be slightly higher association between loosely packed collagen and the implanted sensor by day 7 (**Figure 5F**). This slight increase in collagen–sensor association in IL-1-KO mice was likely the result of the high level of inflammation seen at the site of sensor implantation in IL-1-KO mice (**Figures 4D–F**). The impact of IL-1RA deficiency on inflammation and fibrosis at the tissue-sensor interface would likely contribute negatively to extending sensor function *in vivo* seen in IL-1RA-KO mice. It is likely that any increased occurrence in collagen deposition in the IL-1RA-KO mice would be more pronounced at a later time point PSI (e.g., 28 days). Since IL-1B is known to have a major role in controlling fibroblast function *in vivo*, it is possible that the lack of IL-1RAs at the sensor–tissue interface in the IL-1RA-deficient mice may contribute to the increase in fibrosis at the sensor implantation sites. Alternatively, since it is known that IL-1 controls fibroblast function, it is possible that overexpression of IL-1RA may directly decrease both the recruitment and activation of fibroblasts at the site of sensor implantation. Of course, it is likely that both factors could contribute

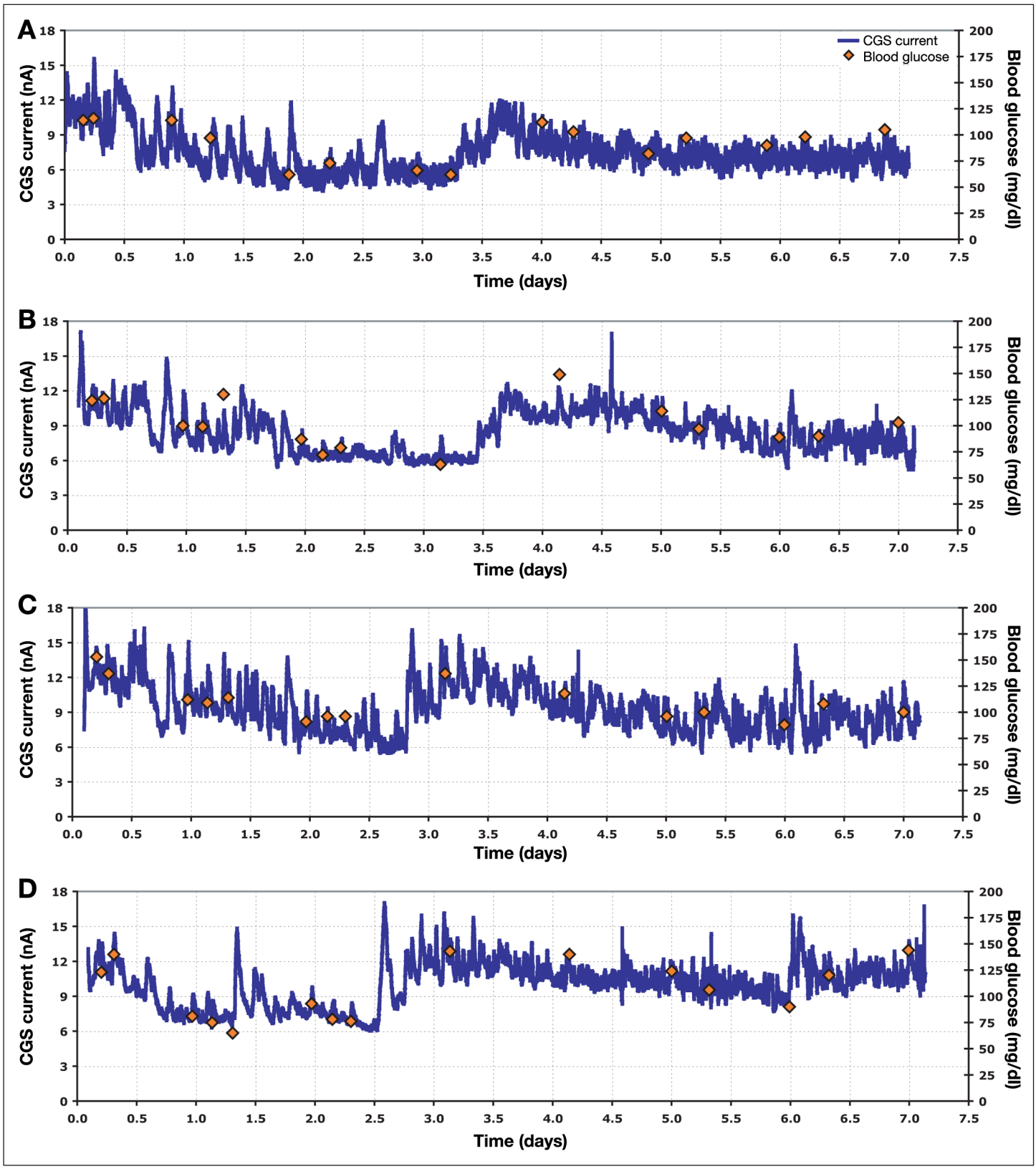

Figure 3. Continuous glucose monitoring in IL-1RA-EO mice over a seven-day time period. Sensor output is expressed as CGS output (nA) and is represented by the blue curves. Blood glucose levels are represented by red diamonds.

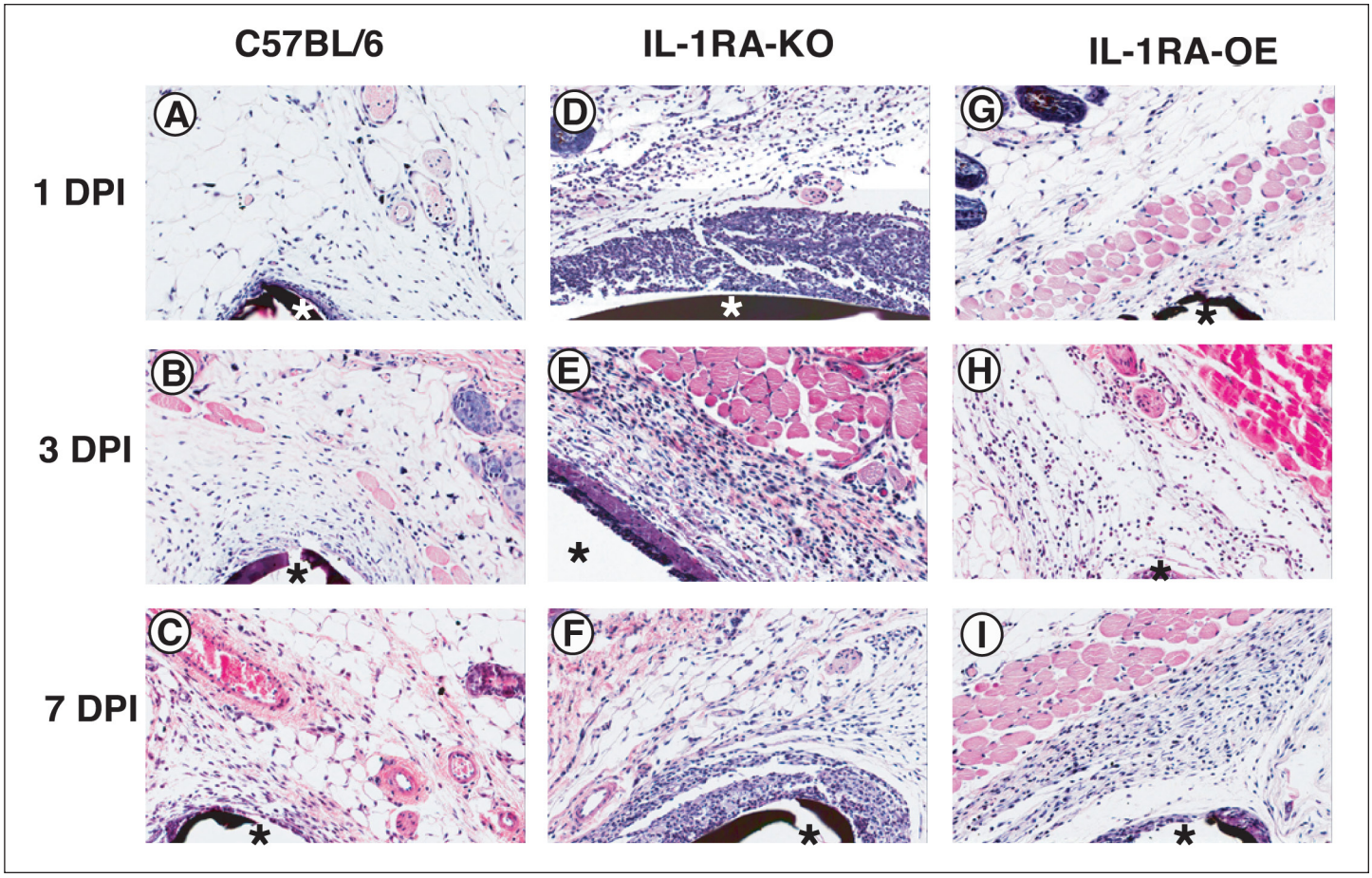

Figure 4. Tissue reactions induced at sites of glucose sensor implantation in C57B/6, IL-1RA-KO, and IL-1RA-OE mice over a seven-day period. Histopathologic analysis of tissue from sensor implantation sites in C57BL/6 (A–C), IL-1RA-KO (D–F), and IL-1RA-OE (G–I) mice was evaluated using standard HES techniques. All histological images were taken at a 20× magnification. Location of the sensor in the tissue is designated by the asterisk. In HES sections, the residual sensor coating appears as a black layer associated with the asterisk.

equally to a decrease in fibrosis in IL-1RA-OE mice during long-term implantation (i.e., 28 days). Future studies addressing testing sensor function post-7-days implantation will most likely reveal more about the importance of IL-1 in sensor function long-term, e.g., 28 days PSI.

Discussion

Currently, all commercially available glucose sensors for human use are approved for an implantation period of 7 days or less. These sensors generally function well over the first 7 DPI. However, even within this time-frame unexplainable sensor output excursions can occur, and a number of data analysis methods and algorithms have been proposed and developed in order to predict false alarms for hypoglycemic and hyperglycemic events.[22,23]

It is generally believed that tissue reactions induced during the initial seven days of sensor implantation can impact both positively and negatively on long-term sensor function because of tissue destruction associated with the implanted sensor. Developing a better understanding of the role of the cells, factors, and tissue reactions that occur at sites of sensor implantation and their relationship to sensor function will likely provide better rationales and approaches to extending glucose sensor function *in vivo*. Because of the importance of IL-1 family inflammation and wound healing, we initially focused on the role of the proinflammatory cytokine IL-1B and its naturally occurring antagonist IL-1RA on sensor function and tissue reactions *in vivo*.

To investigate the role of IL-1B and IL-1RA in glucose sensor function and tissue reactions *in vivo* using our murine model of CGM,[1,20] we compared sensor function in transgenic mice that (1) overexpress IL-1RA [B6.Cg-Tg(IL1rn)1Dih/J] and (2) are deficient in IL-1RA (B6.129S-*Il1rn*^{tm1Dih}/J) with mice that have normal levels of IL-1RA (C57BL/6). These studies clearly indicate that (1) the IL-1 family of cytokines, likely IL-1B, play a critical role in controlling tissue reactions and sensor function *in vivo*, and (2) the IL-1 antagonist IL-1RA is critical in

controlling tissue reactions and sensor function *in vivo*. These studies suggest that targeting the IL-1 family of cytokines, e.g., local delivery of IL-1 antagonists at sites of sensor implantation will likely enhance short-term sensor function *in vivo* and possible long-term sensor function *in vivo*.[24]

Interleukin-1 Cytokine Family and Inflammation

Cytokines are low molecular weight glycoproteins secreted by tissue, inflammatory, and tumor cells that can regulate cell functions in an autocrine or paracrine fashion. The cytokine IL-1 is best known as a key regulator of inflammation and immune response. Interleukin-1 has been appreciated as a multifunctional cytokine able to affect virtually all cell types.[16,25] The IL-1 family consists of two agonists (IL-1a and IL-1B), a competitive antagonist [IL-1 receptor antagonist (IL-1RA/IL-1ra)], and two receptors (IL-1RI and IL-1RII). Interleukin-1a and IL-1B show approximately 25% amino acid homology; IL-1a is the acidic form while IL-1B is the neutral form. Both IL-1a and IL-1B are synthesized as 31 kDa precursors, which are cleaved into 17 kDa proteins. Interestingly, these cytokines lack classical signal peptides (for secretion), yet IL-1a and IL-1B exert their physiological effects by binding to specific receptors. While IL-1a remains intracellular and is released upon cell death, IL-1B is secreted out of the cell. Interleukin-1 is a potent inducer of inflammation, and unlike other cytokines, IL-1-mediated cellular activation is regulated at multiple levels. Crucial to controlling an inflammatory event is the concentration of the interleukin-1 antagonist (IL-1RA) and the ratio of IL-1RA/IL-1 within the tissue microenvironment. The IL-1RA competes for binding to the IL-1Rs and thereby prevents IL-1 from activating the receptor. Isoforms of IL-1RA have been identified and include one secreted form (sIL-1RA) and three intracellular forms (icIL1RA 1, 2, and 3).[26,27] While sIL-1RA competitively inhibits IL-1 receptor binding, icIL1ra may not only inhibit IL-1 binding, but also regulate IL-1 responses beyond the receptor level. Interleukin-1RI is an 80 kDa

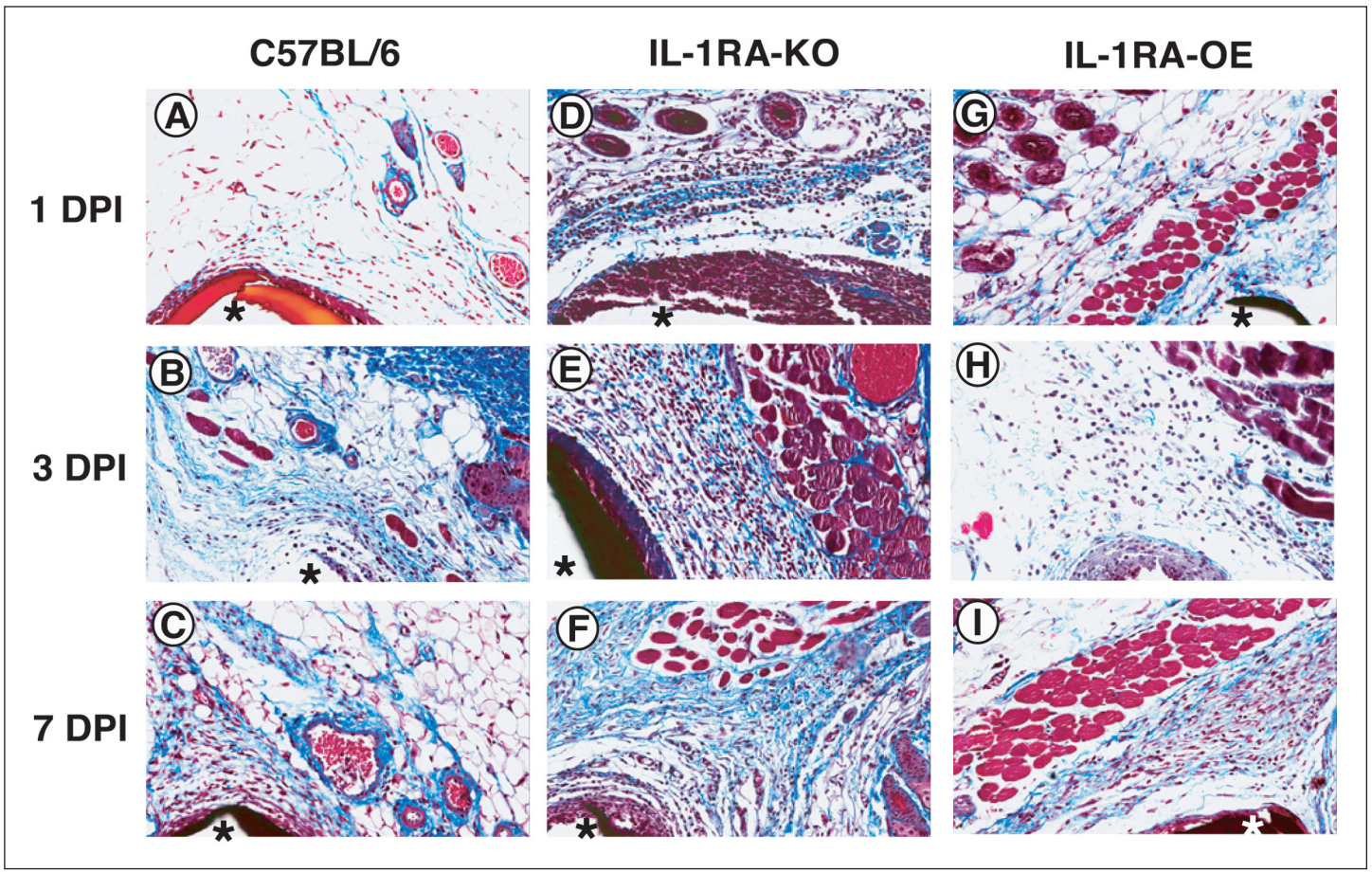

Figure 5. Evaluation of fibrotic tissue response to implanted glucose sensors over a seven-day period. To evaluate the collagen distribution in tissue response associated with various segments of the glucose sensor implanted in the mice for up to seven days, mouse tissue from the sensor sites was obtained and processed for trichrome staining (collagen stains blue in the sections). **A–C** show the histopathologic analysis of tissue from sensor implantation sites in C57BL/6 mice, **D–F** are from IL-1RA-KO mice, and **G–I** are from IL-1RA-OE mice. All histological images were taken at a 20× magnification. In the Masson trichrome sections, the residual sensor coating appears as an orange layer associated with the asterisk.

membrane-bound receptor, while IL-1RII is a 68 kDa protein, but both are members of the immunoglobulin superfamily. The two receptors share 28% homology in their extracellular domains but differ in their cytoplasmic regions. Where IL-1RI has a 213 amino acid cytoplasmic domain, IL-1RII contains only 29 amino acids in this region. Interleukin-1RI is the signal-transducing receptor, and IL-1RII does not transduce a signal when IL-1 is bound to it and is considered an IL-1 "sink." Additionally, IL-1RII not only exists as a membrane-bound form, but can also be found as a soluble form in the circulation of healthy adults. Therefore, IL-1RI mediates IL-1 signal transduction, and IL-1RII is involved in down-regulation or inhibition of IL-1 activation. Lastly, IL-1 activation requires that IL-1/IL-1RI complex associate with IL-1 receptor accessory protein (IL-1RacP) to mediate signal transduction.[28] The mechanism by which IL-1 mediates its activity is via activation of the inhibitor of |B/nuclear factor-|B (I|B/NF|B) and AP-1 transcription factor pathways.[29] Nuclear factor|B has been shown or implicated in the regulation of a number of protumorigenic activities, including (A) regulation of invasiveness/metastasis factors such as metalloproteinase,[30] urokinase plasminogen activator,[31] and endothelial cell adhesion molecules (selectins) critical for angiogenesis[32] and (B) a number angiogenic/mitogenic cytokines such as growth-regulated oncogene protein,[33] IL-8, vascular endothelial growth factor (VEGF), basic fibroblast growth factor, and TNF as well as the motility factor, IL-6.[34]

Continuous Glucose Monitoring in Normal Mice

Currently, all commercial continuous glucose sensors are approved for 3–7 days in humans. It is generally accepted that the tissue responses to the implanted sensor will become increasingly more important as the implantation period is increased. In order to work toward long-term glucose sensing, it is important to appreciate that the severity of the tissue reaction occurring in the initial phase of sensor implantation (e.g., tissue injury) will have an impact on the tissue repair at the site of sensor implantation. Therefore, in an effort to begin to unravel potential mediators and mechanisms that control sensor-related tissue reactions within the first seven DPI, we utilized our murine model of CGM.[1,20] Because the IL-1 family of cytokines is known to be key mediators of inflammation and repair, we chose to focus on determining the role of IL-1/IL-1RA in glucose sensing using genetically engineered mice, which lack IL-1RA or overexpress IL-1RA. Initially, we evaluated CGM in normal C57BL/6 mice. As expected, CGM during the first seven days was excellent, and sensor output closely paralleled blood glucose levels monitored externally (**Figure 1**). Our modified Abbott Navigator glucose sensor consistently detected both hyperglycemic and hypoglycemic events during the seven days of CGM (**Figure 1**). These results were used for comparison of CGM in IL-1RA-KO and IL-1RA-OE mice described next.

Continuous Glucose Monitoring in Interleukin-1 Receptor Antagonist Knockout Mice

The importance of the IL-1 family of cytokines in controlling inflammation and wound healing is well established, but the role of the IL-1 family in controlling glucose sensor function *in vivo* is not. Because of the powerful proinflammatory and profibrotic activity of IL-1B, we hypothesized that, by removing IL-1 antagonist IL-1RA expression *in vivo*, it would allow overexpression of proinflammatory activity of locally produced IL-1B, resulting in enhanced inflammation and fibrosis and decreased glucose sensor function. Our present studies clearly demonstrate that deficiency of IL-1RA in IL-1RA-KO mice results in a dramatic increase in inflammation at the site of sensor implantation (**Figures 4D–F**), which correlated with loss of sensor function within the first few days PSI (**Figure 2**). The CGM results in the IL-1RA-KO mice support our core hypothesis of the importance of IL-1 family in CGM. What was particularly interesting was that sensor functionality was briefly lost, typically within the first 24 h post-implantation, and in most cases, this temporary loss of sensor functionality lasted for the first 1–3 days. We hypothesize that initial implantation of the sensors triggered the release of local inflammatory mediators from tissue cells and plasma proteins, resulting from an increased vasopermeability, including leukocyte chemotactic factors (LCFs). These locally expressed LCFs in turn recruit both PMNs and monocyte/MQs. Both PMNs and MQs are known to express IL-1, and MQs are a key source of locally produced IL-1RA. We further speculate that the initial increase in vasopermeability associated with sensor implantation trauma would act to inhibit acute IL-1B activity as well as supplement local MQ expression IL-1RA. Since IL-1RA-KO mice are deficient in the antagonist IL-1RA, IL-1B expression is not regulated during this critical phase of sensor implantation and tissue injury. Therefore, IL-1B expression levels are severely increased, which has a dramatic effect on sensor functionality PSI, typically within the first 24 h. Within the first 24 h, sensor output can decline rapidly and sharply (**Figures 2B** and **C**) or decline continuously over a few hours (**Figures 2A** and **D**). This dramatic loss of sensor function typically lasts for a day but can also span over several days before the sensor output increases again and starts correlating with the reference blood glucose measurements. It should

be noted that this acute lose of sensor function was associated with the accumulation of PMNs at the sensor–tissue interface. We believe that the PMN accumulation at sensor site results in the release of highly tissue-toxic products (e.g., oxygen radicals and proteases) from activated PMNs that can destroy tissue function as well as biofoul the sensor. Additionally, because of the short lifespan of PMNs, the dying PMNs will passively release tissue- and sensor-toxic substances from both cytoplasmic and granule-derived toxic products. Additionally, PMNs are extremely metabolically active and likely create a "metabolic barrier" for glucose that results in apparent loss of sensor function *in vivo*. The return of sensor function seen at the later stages of the tissue reactions (days 3–5 PSI) was associated with the early accumulation of MQs at the sensor–tissue interface. These MQs contribute not only to the removal of PMN and tissue debris, but also to the initiation of wound-healing processes, including inducing new blood vessels (angiogenesis). It is possible that the return of sensor functionality seen during days 3–5 likely attributed to both (1) removal of the PMN/tissue debris that would otherwise contribute to further tissue destruction and sensor biofouling and (2) initiation of wound healing. During wound healing, new blood vessels are formed to allow the passage of proteins and cells to the site of tissue injury. Interestingly, IL-1B is known to induce angiogenesis in wound healing and cancer, i.e., blocking IL-1B inhibits angiogenesis in animal models.[35] It may be that the absence of IL-1RA in the IL-1RA-KO mice would result in increased angiogenesis, neovascularization, and blood flow into the sensor implantation. Carmi and colleagues[36] have demonstrated that IL-1B can induce VEGF expression in cells. This information supports our hypothesis that the failure to block IL-1B activity in the IL-1RA-KO mice may be responsible for the return of sensor function seen in the IL-1-KO mice, because without the IL-1RA present, IL-1B can induce VEGF expression and angiogenesis at the tissue sites at days 3–7 PSI (36). It is therefore possible that this IL-1B-induced formation of new vessels leads to a better diffusion of the analyte glucose to the sensing element, allowing the sensor output to increase to its initial value.

Continuous Glucose Monitoring in Interleukin-1 Receptor Antagonist Overexpressing Mice

The CGM studies utilizing IL-1RA-KO mice clearly support the role of IL-1/IL-1RA in controlling both tissue reactions and glucose sensor function at sites of sensor implantation. We hypothesized that an overexpression of IL-1RA would allow blocking of proinflammatory activity of locally produced IL-1B, resulting in decreased inflammation and fibrosis and increased glucose sensor function. Our present studies demonstrate that, unlike the sensor function and tissue reactions seen in IL-1KO mice, overexpression of IL-1RA in IL-1RA-OE mice results in sensor function and tissue reactions (**Figures 4G–I**) comparable to wild-type mice (**Figures 4A–C**). These studies suggest that, if there is any decrease in systemic and/or local IL-1RA expression, inflammatory reaction will be increased and sensor function will be compromised.

Hypothetical Model of the Roles of Interleukin-1B and Interleukin-1 Receptor Antagonist in Glucose Sensor Function and Tissue Reactions in Vivo

The results developed in the present studies support our hypothesis that the IL-1 family of cytokines (agonists and antagonists) are important in controlling tissue reactions and thereby sensor function at sites of glucose sensor implantations. Specifically, we hypothesize that, in the cases of normal mice (C57B/6), the initial sensor-associated tissue trauma induces both leukocyte accumulation, via local expression of LCFs (**Figure 6A, step 1**), as well as increased vasopermeability (**Figure 6A, step A**), which causes an influx of plasma-derived IL-1RA. We believe that this initial influx of plasma IL-1RA is adequate to control the initial levels of IL-1B produced at the site of sensor implantation but not the increased local production of IL-1B by both activated leukocytes (recruited) and tissue cells (**Figure 6A, step 2**). Of particular importance to our hypothesis is the concept that, if IL-1B is not controlled by IL-1RA, IL-1B will induce expression of the M1 class of proinflammatory cytokines (e.g., IL-6, IL-8, monocyte chemotactic protein, and interferon gamma) from M1 MQs (**Figure 6A, step 2**). This expression of pro-inflammatory cytokines ultimately increases inflammation and tissue destruction and decreases sensor function.[37,38] We believe that, in due course, this IL-1B expression is likely neutralized by upregulation of IL-1RA expression in M2 MQs and activated tissue cells (**Figure 6A, step B**). This IL-1RA-based inhibition of IL-1B reduces inflammation and tissue injury, both of which enhance glucose sensor function and life span (**Figure 6A, step 2**). In the case of the IL-1RA-KO mice, we hypothesize that the lack of plasma- or cell-derived IL-1RA allows the dominance of IL-1B-induced proinflammatory cytokines both at early stages (**Figure 6B, steps 1** and **A**) and later stages (**Figure 6B, steps 2** and **B**) PSI. Alternatively, in the case of the IL-1RA-OE mice we hypothesize that the proinflammatory actions of IL-1B is limited in both the early (**Figure 6C, steps 1** and **A**) and late stages (**Figure 6C, steps 2** and **B**) PSI, similar to what is seen in the normal mice, i.e., plasma levels of IL-1RA plus the

expression of IL-1RA by both recruited leukocytes and tissue cells effectively suppress any initial IL-1B expression associated with sensor implantation (**Figure 6C, steps 1** and **A**). Additionally, we hypothesize that the continued overexpression of IL-1RA by both MQs and tissue cells continues to suppress IL-1B activation of MQs and tissue cells (**Figure 6C, steps 2** and **B**). We believe that the overexpression of IL-1RA results in (1) a decrease in the expression of IL-1B, (2) IL-1B-induced proinflammatory cytokines, as well as (3) an increase in functional M2 class of anti-inflammatory MQs. The end result of all these IL-1RA-driven events is to decrease inflammation and fibrosis, as well as increase neovascularization at the site of sensor implantation. The overall anti-inflammatory effect of IL-1RA overexpression results in maintenance of accurate glucose sensor function *in vivo* over the first seven days PSI similar to the normal mice. The question remains as to the impact of IL-1RA on long-term glucose sensor function and related tissue reactions. It is possible that the overexpression of IL-1RA and or the IL-1RA deficiency may have even greater effects on long-term glucose sensor than it has on near-term (7-day) glucose sensor function.

Conclusion

Our present studies not only demonstrate the importance of the IL-1 family of cytokines (agonists and antagonists) in controlling tissue reactions and glucose sensor function at sites of sensor implantation, but they also support the potential of local delivery of IL-1B inhibitors and antagonists (e.g., local delivery of recombinant IL-1RA, IL-1RA gene therapy, antibodies to IL-1B, local delivery of recombinant soluble IL-1 receptors, and IL-1 receptor gene therapy) to limit inflammation and fibrosis and promote glucose sensor function *in vivo*. The studies clearly demonstrate the importance of the IL-1 family of cytokines in tissue reactions and sensor function over the initial seven days PSI and provide the rationale to investigate the role of IL-1 and IL-1RA in controlling long-term tissue reactions at sites of sensor implantation as well as long-term continuous glucose sensing *in vivo*. In conclusion, these studies not only implicate the IL-1 family of cytokines in glucose sensor function and associated tissue reaction, but also suggest that local delivery of IL-1 antagonists may be useful in extending glucose sensor function *in vivo*. These studies clearly demonstrate the importance of the IL-1 family of cytokines in glucose sensor function *in vivo*. Future studies to dissect the mechanisms involved in IL-1 and IL-1RA effects on sensor function could lay the foundation for rationale approaches and agents that can control sensor function *in vivo*. Using these same mouse models, future studies to determine the impact of the

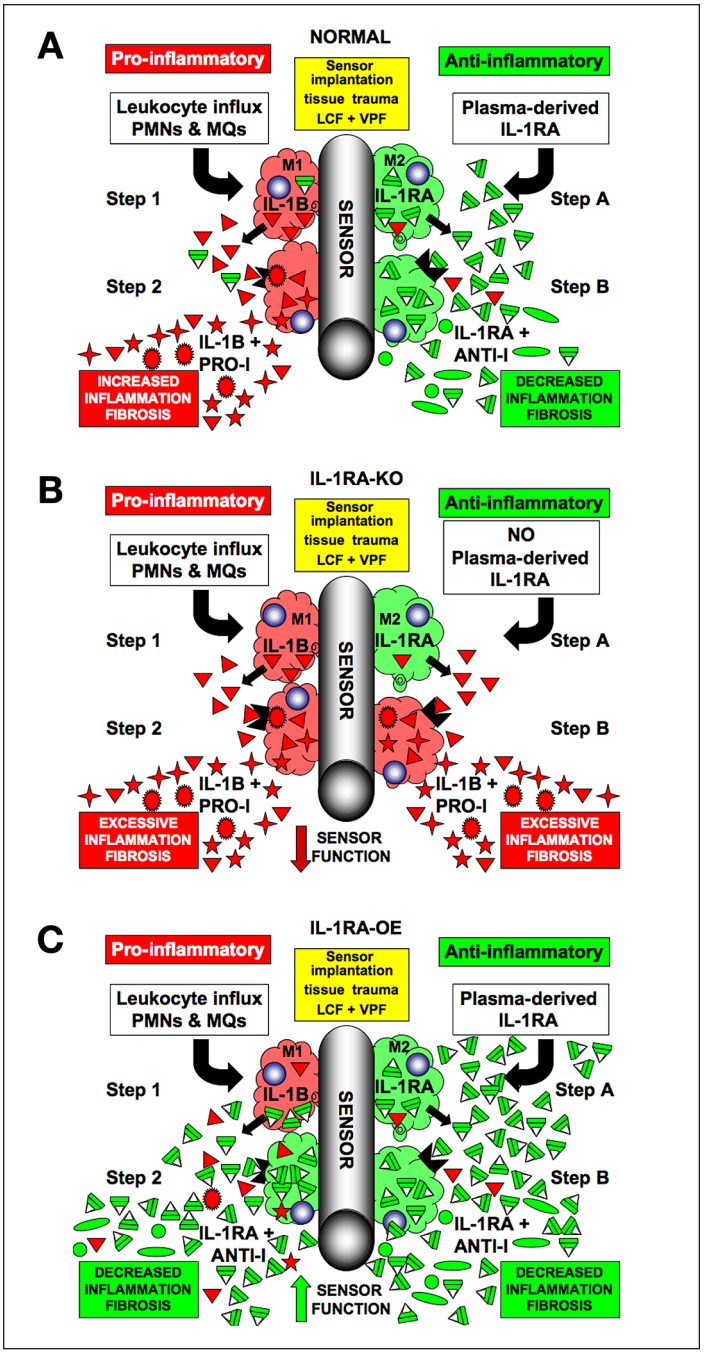

Figure 6. Hypothetical model of IL-1B and IL-1RA tissue and sensor interactions at sites of glucose sensor implantation in normal tissue. This hypothetical model outlines the various possible IL-1-related pathways that are involved in controlling tissue reactions at sites of glucose sensor–tissue reactions as well as glucose sensor function *in vivo* in **(A)** normal mice, **(B)** IL-1RA KO mice, and **(C)** IL-1-OE mice. M1 MQs, red cells; M2 MQs, green cells; IL-1B, red triangles; IL-1RA, green triangles; proinflammatory and pro-fibrotic factors, red stars; anti-inflammatory and anti-fibrosis factors, green circles and ovals; VPF, vasopermeability factors. Red arrows down represent the loss of sensor function, and green arrows up represent the extended sensor function and lifespan.

IL-1 family of cytokines and antagonists on sensor function will provide equally important insights into the role of IL-1 and IL-1RA in long-term glucose sensing. Extending glucose senor function *in vivo* is critical to the development of long-term closed-loop systems that will be of great benefit in the treatment of patients with diabetes.

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