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Impact of CCL2 and CCR2 Chemokine / Receptor Deficiencies on Macrophage Recruitment and Continuous Glucose Monitoring in vivo

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Abstract

The accumulation of macrophages (MΦ) at the sensor-tissue interface is thought to be a major player in controlling tissue reactions and sensor performance in vivo. Nevertheless until recently no direct demonstration of the causal relationship between MΦ aggregation and loss of sensor function existed. Using a Continuous Glucose Monitoring (CGM) murine model we previously demonstrated that genetic deficiencies of MΦ or depletion of MΦ decreased MΦ accumulation at sensor implantation sites, which led to significantly enhanced CGM performance, when compared to normal mice. Additional studies in our laboratories have also demonstrated that MΦ can act as “metabolic sinks” by depleting glucose levels at the implanted sensors in vitro and in vivo. In the present study we extended these observations by demonstrating that MΦ chemokine (CCL2) and receptor (CCR2) knockout mice displayed a decrease in inflammation and MΦ recruitment at sensor implantation sites, when compared to normal mice. This decreased MΦ recruitment significantly enhanced CGM performance when compared to control mice. These studies demonstrated the importance of the CCL2 family of chemokines and related receptors in MΦ recruitment and sensor performance and suggest chemokine targets for enhancing CGM in vivo.

Keywords

Diabetes; Continuous Glucose Monitoring; Macrophage; Chemotaxis; Inflammation

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INTRODUCTION

Implantable glucose sensor technology has come a long way since its first bench top implications more than three decades ago (Updike et al. 1982). Consensus is now that CGM is beneficial in insulin dependent therapy irrespective of the type of diabetes and private and governmental payers are urged to expand and foster CGM implications (Fonseca et al. 2016). Despite the benefits currently an insignificant diabetes patient population uses CGM, which is estimated at 15% of the T1D population and less so for T2D (Foster et al. 2016; Miller et al. 2016). Concerns about sensor accuracy, wearing of a bulky device, the concept of a transdermal sensor with its associated issues of a continuous open wound and concerns of sensor entry infections, all contributes to a less than ideal adoption of the CGM technology. Although technological progress has addressed CGM accuracy for a transdermal sensor usage of up to seven days it is generally accepted that the tissue reaction at site of sensor location is the cause for unpredicted sensor accuracy. Even within this short usage time frame frequent re-calibration of the sensor device, on average every 12 hours, is a necessity to compensate for a less than ideal in vivo sensor performance. Currently the major CGM commercially available systems are the Guardian REAL-Time with a sensor life of 3 days, the DexCom G4 Platinum and G5 Mobile with a sensor life of 7 days, and MiniMed 530G with Enlite with a sensor life of 6 days. Abbott Diabetes Care with its improved Libre sensor lifespan of up to 14 days is not yet available on the US market. The long-term goal is to develop implantable glucose sensors that will function with high accuracy for weeks to months. With that goal in mind it is critical to develop better insights into the causes of sensor failure in vivo, including the mediators and mechanisms that are responsible for sensor biofouling in vivo.

The development of implantable glucose sensors with high accuracy and extended lifespan is critical to the long-term clinical management of diabetes, including the development of closed loop/artificial pancreas technology. Currently, failure of glucose sensors in vivo is frequently associated with “sensor biofouling” attributed to the tissue reaction triad (TRT) of inflammation, fibrosis, and vessel regression (Klueh et al. 2013a, b; Klueh et al. 2014b; Norton et al. 2007; Novak et al. 2013). The importance of inflammation in sensor “biofouling” has been supported by studies in our laboratories (Klueh et al. 2013a, b; Klueh et al. 2007; Klueh et al. 2014b) as with others (Norton et al. 2007), with the belief that MΦ and MΦ related foreign body reactions (FBR) are major players in sensor biofouling. Until recently the importance of macrophages in sensor “biofouling” was primarily based on the histologic existence of macrophages at the tissue sensor interface, but without any direct causal relationships between presence of macrophages, sensor function and CGM (Kvist et al. 2006). Using our murine model of CGM (Klueh et al. 2006), we have recently demonstrated that MΦ deficiency or depletion prevented the accumulation of MΦ at the tissue sensor interface, as well as enhanced continuous glucose monitoring (CGM) in vivo (Klueh et al. 2014b). We have also demonstrated that MΦ can act as metabolic sinks for glucose by depleting local glucose levels in vitro and in vivo and causing anomalous glucose sensor reading (Klueh et al. 2014a). These and other studies support our general hypotheses that MΦ recruitment and activation can directly decrease glucose sensor function and CGM performance in vivo (Klueh et al. 2010a; Klueh et al. 2015; Novak and Reichert 2015;

Novak et al. 2014). Since the recruitment and accumulation of inflammatory leukocytes to injured tissue is dependent on the local generation of leukocyte chemotactic factors (LCF), we chose to investigate the role of monocyte/macrophage LCF in controlling M Φ recruitment into sensor implantation sites and sensor function (Figure 1).

In the present study, we investigated the role of a specific family of cell-derived LCFs that control the recruitment of M Φ in vivo. This family of small molecular weight LCF is known as the CC family of chemokines and receptors. CCL2 is a member of this family and is known as monocyte chemotactic protein-1 (MCP-1) (Semple et al. 2010a; Semple et al. 2010b; Semple et al. 2010c). Inflammatory chemokines, such as CCL2/MCP-1, are a class of small molecular weight chemotactic factors, usually < 10,000 daltons, which are expressed from injured cells and leukocytes. These chemokines induce concentration dependent migration of leukocytes from the vasculature into injured tissue sites. Currently there are four main subfamilies of chemokines: CXC, CC, CX3C and XC, based on cysteine residues that confer 3D configuration to the chemokine. CC chemokines or Beta-chemokines have two (2) adjacent cysteines near the amino terminus of the CC chemokine. CCL2 or monocyte chemotactic protein-1 (MCP-1) is an extremely powerful monocyte chemotactic factor that recruits monocytes from the vasculature into injured tissue and upon activation transforms them into macrophages (Semple et al. 2010b; Semple et al. 2010c). Chemokine receptors, such as CCR2, are G protein-coupled receptors that are expressed on the surface of leukocytes. Expressed CCR2 is responsible for signal transduction for the recruitment of monocytes to sites of tissue injury and inflammation. CCL2 and CCR2 knockout mice have been used to dissect the role of the CC family of chemokines and receptors in a wide variety of diseases and pathology, which are characterized by monocyte infiltrates, such as diabetes, obesity, psoriasis, arthritis, and atherosclerosis (White et al. 2013). When LCFs, such as CCL2, are released from sites of tissue injury, they diffuse outward and ultimately activate vascular endothelial cells, which line blood vessels. This in turn induces adhesion of leukocytes to the vessel wall (Figure 1). CCL chemokines, such as CCL2, bind to specific receptors on the surface of monocytes (e.g. CCR2) and trigger directional migration of monocytes into the site of injury such as sensor implantation sites (Figure 1). In the case of implanted glucose sensors, these recruited/activated monocytes accumulate at the tissue-sensor interface (Figure 1). Since CCL2 can activate monocytes to become macrophages, and have also been implicated in foreign body reactions by inducing foreign body giant cell (FBGC) formation (Kyriakides et al. 2004), the CCL2/CCR2 chemokine family is a prime candidate/target for our investigation into the role of macrophage recruitment as well as FBR (e.g. FBGC formation) in sensor biofouling and CGM in vivo.

To investigate the role(s) of the CCL2/CCR2 chemokine family in tissue reactions at sensor implantation sites as well as CGM, we utilized transgenic mice that are deficient in CCL2 (CCL2^{-/-}) or CCR2 (CCR2^{-/-}). Matched control mice have normal expression of both CCL2 (CCL2^{+/+}) and CCR2 (CCR2^{+/+}) and are designated as normal or wild type mice in our studies. These transgenic knockout mice and their matched control (normal) mice have been used in numerous studies to evaluate the role of CCL2 ligands and receptors in various murine disease models (Semple et al. 2010b; Semple et al. 2010c). Using these two knockout mouse models, and our murine model of CGM, we determined the role of CCL2/CCR2 in controlling Monocyte/Macrophage (M/M Φ) recruitment and sensor function in our

murine model of CGM. The results of these studies demonstrated that both CCL2 and CCR2 knockout mice decreased M Φ recruitment at sensor implantation sites, including the sensor-tissue interface. This decreased M Φ recruitment dramatically improved CGM performance when compared to normal mice (CCL2 and CCR2 sufficient mice).

We believe that the CCL2 induced recruitment and accumulation of CCR2 positive macrophages at the tissue-sensor interface results in direct biofouling of the glucose sensor by creating M Φ based metabolic barriers to glucose diffusion (Klueh et al. 2014a) and possible oxygen diffusion (Figure 1). The inhibition of M Φ recruitment/accumulation would decrease metabolic barriers surrounding the sensor by decreasing the total number of M Φ around the implanted sensor as well as by decreasing FBGC formation. Thus, we hypothesize that the deletion of CCL2 or CCR2 in the KO mice would result in decreased M Φ recruitment and FBGC formation at the sensor implantation site and directly decrease sensor biofouling by preventing the creation of metabolic barriers at the tissue sensor interface. Additionally, CCL2 is also known to indirectly encourage fibrosis via inducing expression of transforming growth factor beta (TGF β), which would likely decrease sensor encapsulation (fibrosis) at the sensor implantation site (Skokos et al. 2011). In conclusion, the present studies suggest that the therapeutic targeting of monocyte / macrophage recruitment, activation, and foreign body giant cell formation, by blocking leukocyte chemotactic factors and receptors such as the chemokine family of CCL2/CCR2, may provide a new approach to decreasing sensor induced tissue reactions. Hence, controlling tissue reactions at the device implantation site will lead to extended glucose sensor performance and CGM in vivo.

MATERIALS AND METHODS

Mouse Models

All mice used in these studies were obtained from Jackson Laboratories, Bar Harbor Maine, and included B6.129S4-Ccl2tm1Rol/J, a transgenic knockout of the chemokine CCL2 (CCL2 $-/-$), also known as MCP-1 (Jax Stock # 004434), and a transgenic knockout of the chemokine CCL2 receptor (CCR2 $-/-$), designated B6.129S4-Ccr2tm1Ifc/J (Jax Stock # 004999). C57BL/6J was utilized as the background strain and control for these transgenic mice (Jax Stock # 00664).

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. Glucose sensors were implanted into mice and continuous glucose monitoring (CGM) was undertaken as described previously (Klueh et al. 2006; Klueh et al. 2011). It should be noted that all sensors used for these studies are non-recalibrated throughout the 4-week test period. This is quite different compared to other animal or human studies, where sensors are recalibrated at least once a day. Since no recalibration occurs throughout the 28-day study period, sensor performance results are reflected in raw nA per mg/dL of blood. 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 the studies involving mice.

Data Analysis

Glucose sensors were implanted into mice of the three genetic backgrounds (CCL2 $-/-$, CCR2 $-/-$, and C57BL/6 (CCL2 $+/+$ and CCR2 $+/+$) and continuous glucose monitoring (CGM) was undertaken for a period up to 28 days as described previously (Klueh et al. 2014b). Blood glucose reference measurements were obtained daily over the 28-day implantation period, using blood obtained from the tail vein and a FreeStyle Blood Glucose monitor. These reference blood measurements were used to calculate the mean absolute relative difference (MARD) over a four-week experiment for the three treatment groups of mice (Obermaier et al. 2013; Zschornack et al. 2013). MARD is calculated by taking the mean of a series of absolute relative differences between the continuous glucose measurement (CGM in mg/dL), calculated from the measurement of electrical current (I in nAmp) within the implanted sensor, and the blood glucose reference measurement (BG in mg/dL) from a standard clinical glucometer (FreeStyle Blood Glucose monitor) (Obermaier et al. 2013). See equations 1.1 through 1.3 below.

$$MARD(mean) = \left(\left(\sum_i^n \frac{|CGM_i - BG_i|}{BG_i} \right) / n \right) \times 100\% \quad \text{Equation 1.1}$$

$$Sensitivity(S) = \left(\left(\sum_i^k (BG_i / I_i) \right) / k \right) \quad \text{Equation 1.2}$$

$$CGM_i = S \times I_i \quad \text{Equation 1.3}$$

Sensitivity (S in mg/dL/nAmp) is calculated for each mouse experiment, based on the BG and I measurements in an initial reference stage of the experiment, i.e. k in Equation 1.2 is approximately 5, for the first initial 5 measurements across 2 days. Mann-Whitney U tests were conducted for statistical comparisons of pairs of average MARD values, as a non-parametric equivalent to student t-tests, because the C57BL/6 control MARD values (any and all weeks) and the week 4 CCR2 $-/-$ MARD values were not normally distributed. Otherwise all other mean MARD values were normally distributed. Consequently, to test for statistical differences among all three-treatment groups at once, a Kruskal-Wallis (K-W) test was conducted on the MARD values, as a non-parametric equivalent to analysis of variance (ANOVA). Microsoft Excel for Mac 2011 (version 14.1.4) and IBM SPSS Statistics 20 (release 20.0.0) were the software packages used for the MARD calculations and statistical analyses respectively. In all cases, $p < 0.05$ was considered significant.

Histopathological Analysis of Tissue Reactions at Glucose Sensor Implantation Sites

In order to evaluate the tissue responses to glucose sensor implantation at various time points, individual mice were euthanized and the tissue containing the implanted sensors was removed. Tissue was then fixed in formalin buffer (Fisher Scientific) for 24-48 hours, followed by 70% ETOH immersion for 24 hours. The complete polymeric composition of the sensor allows histologic sectioning without prior sensor removal from the tissue. Tissue is processed according to standard histological procedure, embedded in paraffin, and sectioned in 5 μ m sections (Klueh et al. 2010a; Klueh et al. 2010b). Generally, histopathological evaluation of tissue reactions at sites of sensor implantation were performed on mouse specimens obtained between 1-28 days post sensor implantation. The tissue samples were examined for signs of inflammation, including necrosis, fibrosis, and vessel regression. Resulting tissue sections were evaluated directly and documented by digitized imaging using the Aperio ScanScope slide scanner.

To provide an initial evaluation of the inflammatory reactions at the sensor tissue interface we utilized semi-quantitative evaluation scoring system from 0-4. For this system the tissue reactions were scored as follows:

- 0 = no inflammation (no leukocyte infiltration present near the implanted sensor)
- 1 = limited inflammation (scattered leukocyte infiltration present near the implanted sensor)
- 2 = mild inflammation (consistent leukocyte infiltration present near the implanted sensor and associated tissues),
- 3 = moderate inflammation (significant leukocyte infiltration near the implanted sensor and associated tissues),
- 4 = severe inflammation (dense leukocyte infiltration near the implanted sensor and associated tissues).

The individual histologic sections were evaluated in a double blind fashion by 2 independent investigators and the mean inflammation index was determined. Since some of the mean inflammation index values were non-normal in distribution, Kruskal-Wallis tests were used to conduct statistical comparisons among the three groups of inflammation index values of different genetic background. Mann-Whitney U tests then were conducted to determine the statistical differences between pairs of mean inflammation index values, where appropriate, otherwise student t-tests were used. The variables which were normal in distribution included inflammation index values for: CCR2^{-/-} 14, 21, and 28 days, CCL2^{-/-} 28 days, and all weeks combined for all three groups, C57BL/6, CCR2^{-/-}, and CCL2^{-/-}. Microsoft Excel for Mac 2011 (version 14.1.4) and IBM SPSS Statistics 20 (release 20.0.0) were the software packages used for the calculations and statistical analyses respectively for both the MARD and inflammation indices.

Immunohistochemical Analysis of Tissue Reactions at Glucose Sensor Implantation Sites

To confirm the observations of the presence of macrophages in tissue sections, we utilized a mouse macrophage specific antibody designated anti-mouse F4/80. Anti-mouse F4/80 (@F4/80, Invitrogen Catalog #A14800) was validated using mouse spleen tissue and standard immunohistochemical (IHC) techniques (Klueh et al. 2014b). For quantification of macrophage influx we determined the Net Macrophage count for each glucose sensor implantation site. To determine the Net Macrophage index, macrophage number, via high power field (HPF), at sensor implantation site was subtracted from the average number of resident MΦ located in the same tissue sample but distant from sensor implantation site.

RESULTS

Role of macrophage chemokine and chemokine receptor CCL2 and CCR2 in CGM in vivo

Since CCL2/MCP-1 is known to be a key macrophage chemotactic factor at sites of injury and inflammation, we hypothesized that deficiencies in CCL2 or CCR2 would reduce MΦ recruitment to sites of glucose sensor implantation, and thereby enhance CGM performance in vivo (Figure 1). To test this hypothesis, CGM was evaluated in transgenic mice that are deficient in CCL2 or the CCR2 receptor. The average MARD over a four-week experiment is utilized to characterize CGM performance, and compared to mice sufficient in CCL2 and CCR2 (C57BL/6) using statistical analyses. As previously demonstrated, control C57BL/6 displayed poor sensor function performance over the 4-week test period as reflected by increasing MARD values (Klueh et al. 2013a, b; Klueh et al. 2014b). Alternatively, both CCL2 deficient and CCR2 deficient mice failed to display any significant loss of sensor function, i.e. no increases in MARD values over the 4-week test period (Figure 2). Specifically, Kruskal-Wallis analysis indicated that although there was no statistically significant difference in MARD among these three (3) groups during weeks 1 and 2, CCL2 $-/-$ and CCR2 $-/-$ deficient mice trend toward significantly better MARD values in week 3, and become significantly better in week 4 (Figure 2). At four weeks, a Kruskal-Wallis test reveals a significant difference among the three groups in the week 4 mean MARD ($p=0.003$). Statistical mean MARD analysis at week 4 of the study indicate that both CCL2 and CCR2 mice had significantly better CGM performance when directly compared to normal control C57BL/6 mice in paired comparisons (Table 1). In addition, a Kruskal-Wallis test revealed a significant difference among the three groups in total mean MARD, i.e. the average MARD among the three groups over the entire four-week experiment ($p=0.048$). These studies indicate that MΦ chemokine CCL2 and the CCL2 receptor (CCR2) play a critical role in controlling sensor function and CGM in vivo. These studies suggest that therapeutic targeting of CCL2 and CCR2 may provide a new approach to extending glucose sensor function and CGM in patients with diabetes.

Histological Evaluation of Tissue Reactions Induced at Glucose Sensor Implantation Sites

In addition to CGM analysis, we also evaluated the effect of CCL2 and CCR2 genetics on inflammation over a four-week experiment for CCL2 $-/-$, CCR2 $-/-$, and C57BL/6 mice. Initial histological analysis of sensor implantation sites demonstrated that although MΦ were present in all mouse strains, there was significantly less inflammation and fewer MΦ at the sensor implantation sites of the CCL2 and CCR2 deficient mice (see Figure 3 and 4)

compared to the wild-type C57BL/6. Further analysis of the tissue reactions at sensor implantation sites demonstrated that the trends in mean inflammation indices over the 4-week test period, varied by genetic background (Figures 3 and 4). The CCL2 ^{-/-} and CCR2 ^{-/-} mice have significantly less inflammation in weeks 1, 2, and 3 than their controls (C57BL/6), but have statistically approximately the same index of inflammation in week 4 (Figure 3). These results confirm the observation that the majority of the inflammatory process is an early, short-term phenomenon following the trauma of sensor implantation in this case, peaking around 2 weeks, and that other wound healing processes, such as fibrosis and vessel regression, dominate the later stages following tissue damage. Also, we consistently found that CCL2^{-/-} and CCR2^{-/-} had less inflammation and better MARD values (lower) than the control C57BL/6 mice (Figures 2, 3 and 4).

Macrophage presence and distribution in tissue at glucose sensor implantation sites

Noticeably CCL2 and CCR2 gene knockouts resulted in a significantly enhanced CGM in the KO mice when compared normal/WT mice (Figure 2), as well as significantly decrease inflammatory reactions, when compared to the normal/WT mice (Figure 3). Although a decrease in the inflammatory reactions in the CCL2/CCR2 KO mice was observed (Figure 3), it was important to determine whether there was a significant reduction in the number or distribution of macrophages at the sensor implantation sites in the KO mice compared to the normal/WT mice. The macrophage specific antibody F4/80 was used in a standard immunohistochemistry protocol to detect macrophages present in the sensor implantation sites from the various KO and WT mice used in Figures 2 and 3. Macrophage levels were dramatically decreased at all time points in both CCL2 and CCR2 mice when compared to normal/WT mice (data not shown). Since day 14 demonstrated the most intense tissue reactions, photomicrographs of H&E and F4/80 staining for all 3 mouse strains were used in these studies and are provided in Figure 4. Representative H&E tissue sections for day 14 post sensor implantation in normal, CCL2 (^{-/-}) and CCR (^{-/-}) mice are presented in Figures 4a, 4c and 4e. The presence and distribution of macrophages, i.e. F4/80 positive cells, is indicated with red arrowheads in these same tissue samples as presented in Figures 4b, 4d and 4f. These studies demonstrated that deletion of CCL2 and CCR2 gene in C57B/6 mice results in a significant decrease in inflammation and macrophage recruitment/accumulation at site of glucose sensor in CCL2 and CCR2 KO mice.

DISCUSSION

The central role of Monocyte Related Cells [(MRC), i.e. macrophages (MΦ), foreign body Giant cells (FBGC) and Dendritic cells (DC)] and their products in tissue reactions associated with a variety of inflammatory diseases, including foreign body reactions, is universally accepted. Although foreign body reactions and MRC, such as MΦ and FBGC, are associated with implantable biosensors in vivo, and MΦ are frequently cited as contributing to the loss of sensor function in vivo, there is very limited data on the specific causal roles, mediators, and pathways that MRC play in the loss of glucose sensor function and CGM in vivo. Usually only the association of MΦ and FBGC at the sensor-tissue interface is the major rationale for invoking a central role of MΦ and FBGC in the loss of

glucose sensor function in vivo. There is a clear need to systematically dissect the roles of MRC such as MΦ and FBGC, in limiting glucose sensor function and CGM in vivo.

Hypothetical model of the role of MΦ in continuous glucose monitoring in vivo

Based on the literature and our current data we developed a theoretical model of the role of CCL2 chemokines and receptors in controlling monocyte/macrophage recruitment and accumulation including the formation of foreign body giant cell formation at sites of glucose sensor implantation in vivo. We hypothesized that in response to sensor implantation trauma and subsequent inflammatory reactions at the implantation site, MCP-1/CCL2 is elaborated to injured tissue and inflammatory cells. This initial local production of CCL2 triggers chemotaxis based recruitment and activation of CCR2 expressing monocytes from the vasculature into the sensor implantation sites. As these CCR2 expressing monocytes accumulate at the sensor-tissue interface, they are activated by CCL2 into CCR2 macrophages. This in turn will amplify the tissue reactions (inflammation, fibrosis, and vessel regression) at the implantation site by further expression of pro-inflammatory and pro-fibrotic mediators including CCL2. Additionally, macrophages can also be fused by CCL2 to form foreign body giant cells. These foreign body giant cells in turn recruit TGFβ, a powerful pro-fibrosis cytokine. This CCL2 induced fibrosis causes blood vessel regression and slows glucose diffusion within the tissue. The combination of CCL2 recruited macrophages and foreign body giant cells ultimately create direct and indirect barriers surrounding the sensor device, resulting in loss of sensor accuracy and function as well as diminished CGM performance in vivo. Using spontaneous and transgenic mouse models and our murine model of CGM, we recently demonstrated that M/MΦ deficiency or depletion significantly decreases tissue reactions at sites of glucose sensor implantation and enhances long term CGM (Klueh et al. 2014b). Implantable glucose sensors used in CGM are known to induce foreign body reactions characterized by the accumulation of macrophages (MΦ) and formation of FBGC at the sensor-tissue interface. Recently, the sensor literature underscored that the presence of MΦ at sites of sensor implantation reduces sensor performance including CGM (Klueh et al. 2014a; Klueh et al. 2014b; Novak and Reichert 2015; Novak et al. 2014). Moreover, it is known that mice deficient in CCL2 (MCP-1) experience minimal fibrotic reactions in response to an implant (Castro et al. 2014; Moore et al. 2015; Skokos et al. 2011). Recent studies suggested that this diminished fibrotic response is at least in part due to a compromised TNF and NFκB induction in these CCL2 deficient mice. It should be noted, that this study was based on intraperitoneal inflammation models, which may be different from an analysis done in other tissues, such as skin (Moore et al. 2015). Alternatively, subcutaneous implantation studies showed that the recruitment and migration of macrophages in CCL2 KO mice is similar to WT mice (Kyriakides et al. 2004), suggesting it is not a player in MΦ recruitment. Nevertheless it is known that CCL2 is a requirement in the formation of FBGC (Castro et al. 2014). It must be stated that the phenotype and number of macrophages induced in response to an inflammatory signal are dependent on the type of injury, tissue site, implant and/or biomaterial, as well as the time point of the evaluation of the inflammatory response (e.g. 3 days versus 28 days post tissue injury) (Avula et al. 2016; Helton et al. ; Helton et al.). Also, it has recently been suggested that biomaterial geometry, size and shape are players to dictate macrophage behavior (Luu et al. 2015). An even more complicating factor is that a variety of CCL2 or CCR2 mutant/KO

mice have been used in various implant/biomaterial studies. These mutant/KO mice are available in varying strain backgrounds and as combinations with other mutations/KO. This level of validation complicates comparison with other CCL2/CCR2 studies. Nevertheless it is not unreasonable to expect a mixture of macrophage phenotypes at the site of injury. In our study, we utilized a transdermal implanted glucose sensor and evaluated the sensor functionality and tissue response over a 28-day period. Over this 1-month period we showed the presence of a significantly lower macrophage number at sites of sensor location in KO mice when compared to control mice. Subsequently, sensors implanted in KO mice demonstrated considerable better overall performance when compared to control mice. As such, we concluded that substantial lower number of macrophages at sensor implantation sites is the key factor in enhanced device performance. Furthermore, lower inflammatory cell numbers at sensor sites minimize the formation of a “metabolic sink” effect (Klueh et al. 2014a). Since macrophages are very metabolic active cells, they consume glucose and oxygen at the periphery of the device location prior to glucose diffusion to sensor sites. As such macrophage glucose consumption leads to an artificial lower glucose level at sensor sites, which is not physiological relevant (Klueh et al. 2014a; Novak et al. 2014).

Since it is known that FBGC are responsible for an impaired glucose sensor function, we investigated the role of CCL2 and CCR2 as it relates to sensor performance over a 28-day period. Our glucose sensor performance studies demonstrated significantly better sensor function over the tested time frame. As expected, histological evaluation at 4-week post sensor implantation demonstrated that WT mice experienced substantial tissue reactions and M Φ accumulation at sites of sensor location. CCR2 KO mice showed a lower number of macrophage accumulation at sites of sensor location when compared to WT but significant more when compared to CCL2 KO mice (Figure 4). Although the inflammation index was elevated 14-weeks post implantation in only CCR2 KO mice but not in CCL2 KO mice (Figure 3), interestingly, sensor performance showed no difference between these two knockout mice (Figure 2). This underscores the important role of M Φ /FBGC in glucose sensor function in vivo including the usefulness of therapeutically targeting leukocyte chemotactic factors to reduce tissue reactions at sensor implantation sites as well as enhancing CGM in vivo.

Impact of macrophage chemokines and chemokine receptors CCL2 and CCR2 on continuous glucose monitoring in vivo

Chemokines, such as CCL2, are pivotal chemical mediators that are produced by both tissue and inflammatory cells in response to tissue injury. Chemokines control inflammation and wound healing of injured tissue by the recruitment and activation of both inflammatory cells as well as tissue cells (fibroblast and vascular endothelial cells) in vivo. Although importance of leukocyte chemotactic factors is recognized in a wide variety of disease and tissue injuries, there have been no studies to our knowledge, of the specific nature and pathways that LCF play in tissue responses and sensor biofouling seen at the site of glucose sensor implantation. Since sensors are known to induce FBR at sites of implantation with the appearance of hallmark macrophages and related FBGC, we investigated the role of monocyte/macrophage chemotactic factor CCL2 in controlling M Φ recruitment and accumulation at the sensor-tissue interface at sensor implantation sites using transgenic

knockout mice that lack CCL2 or CCR2. The results of these studies demonstrate that the CCL2/CCR2 family of chemokines is critical to the recruitment and accumulation of M Φ to the sensor-tissue interface, which subsequently influences sensor performance as reflected in the MARD analysis. There is still no uniform system to evaluate continuous glucose monitor (CGM) performance in vivo, whereas the performance of blood glucose meters is evaluated according to standards comparable with the International Organization for Standardization (ISO). Typically CGM is evaluated by comparing the sensing output with a measured blood glucose value. The deviation between these two signals is then used to determine accuracy as an indication for sensor performance. This deviation is taken into account when assessing sensor accuracy. A frequent used method to assess continuous glucose sensor accuracy and precision is the mean absolute relative difference (MARD). MARD is the mean value of all calculated deviations from a sensor output value compared to a measured blood glucose value. A lower MARD value is indicative of a better CGM performance. In our studies we utilized MARD to compare CGM performance between the different mice strains. MARD analysis was conducted to assess sensor performance in CCL2 and CCR2 knockout mice.

CCL2 is known to induce M Φ fusion forming FBGC where as Khan et al have recently demonstrated that CCL2 and CCR2 are both required for the formation of FBGC (Khan et al. 2015). FBGC can act as both direct biofouling agents by creating barriers to glucose and oxygen diffusion to the implanted sensor, as well as indirect biofouling agent by expression of TGF β . TGF β is a powerful fibrosis inducing cytokine that would lower tissue glucose levels by causing vessel regression and slowing glucose diffusion to the sensor. These initial data are extremely exciting and could open the door to future investigations into the role of CCL2 and other leukocyte chemotactic factors responsible for controlling tissue reactions and sensor function in vivo. Our present study demonstrates the CCL2/CCR2 chemokine system as a major player in glucose sensors induced tissue reactions and subsequent sensor performance in vivo. In the future, we anticipate that incorporation of CCL and CCR inhibitors into the coating of the implanted sensor device will provide local inhibition of CCL2 expression and obstruct recruitment of CCR2 expressing leukocytes via receptor antagonists. We anticipate that these inhibitors would be released in a steady state during the lifetime of the implant (Figure 5). Possible local drug delivery treatment options for inhibitors of CCL2 monocyte chemoattractant protein (MCP-1) expression are bindarit (Carfi et al. 1999; Ge et al. 2012) and or CCR competitive antagonists such as BMS CCR2 22 (Carter et al. 2012; Cherney et al. 2008; You et al. 2010). Notwithstanding the choice of drug delivery or sensor coating, there is a large array of CCL2 inhibitor and CCR2 antagonists for consideration for incorporation in sensor coatings as drug delivery (Deshmane et al. 2009).

CONCLUSION

M/M Φ are key cells in both innate and acquired immunity (2000; 2002). When controlled, M Φ play positive key roles in host defense and wound healing. Uncontrolled, M Φ can be extremely destructive, as is the case for chronic inflammations, such as autoimmune diseases and granulomas, including foreign body granulomas. In fact, the accumulation of macrophages and formation of related foreign body giant cells (FBGC) is a hallmark of

chronic inflammation including FBR. Cytokine networks, including chemokines, control recruitment, activation, and suppression of M/M Φ in vitro and in vivo. Our present studies demonstrate the importance of an impaired FBR at the glucose sensor-tissue interface in relation to sensor function and CGM in vivo. Evaluation of CGM in transgenic mice that have deficiencies in the M Φ chemokine CCL2 or the corresponding CCL2 receptor CCR2 displayed a decrease in M Φ recruitment/accumulation at the sensor implantation sites four weeks post implantation. The diminished FBR at sites of sensor implantation demonstrated a dramatic improvement in CGM performance as reflected by the MARD analysis. This study could serve as a foundation for future studies to investigate the pathways and chemotactic factors that control the recruitment of leukocytes and tissue cells, such as fibroblasts and endothelial cells, to the sensor implantation site. This information could identify target molecules, which can act as specific agonists and antagonists to control tissue reactions at sensor implantation sites as well as enhance sensor function and CGM in vivo.

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References

- Macrophages. Oxford University Press; Oxford New York: 2000.
- The Macrophage. Second edition. Oxford University Press; New York: 2002.
- Avula M, Jones D, Rao AN, McClain D, McGill LD, Grainger DW, Solzbacher F. Local release of masitinib alters in vivo implantable continuous glucose sensor performance. *Biosens Bioelectron*. 2016; 77:149–156. [PubMed: 26402593]
- Carfi A, Smith CA, Smolak PJ, McGrew J, Wiley DC. Structure of a soluble secreted chemokine inhibitor vCCI (p35) from cowpox virus. *Proc Natl Acad Sci U S A*. 1999; 96(22):12379–12383. [PubMed: 10535930]
- Carter PH, Brown GD, King SR, Voss ME, Tebben AJ, Cherney RJ, Mandlekar S, Lo YC, Yang G, Miller PB, Scherle PA, Zhao Q, Decicco CP. Discovery of an orally-bioavailable CC Chemokine Receptor 2 antagonist derived from an acyclic diaminoalcohol backbone. *Bioorganic & medicinal chemistry letters*. 2012; 22(9):3311–3316. [PubMed: 22475558]
- Castro PR, Marques SM, Viana CT, Campos PP, Ferreira MA, Barcelos LS, Andrade SP. Deletion of the chemokine receptor CCR2 attenuates foreign body reaction to implants in mice. *Microvasc Res*. 2014; 95:37–45. [PubMed: 25020267]
- Cherney RJ, Mo R, Meyer DT, Nelson DJ, Lo YC, Yang G, Scherle PA, Mandlekar S, Wasserman ZR, Jezak H, Solomon KA, Tebben AJ, Carter PH, Decicco CP. Discovery of disubstituted cyclohexanes as a new class of CC chemokine receptor 2 antagonists. *Journal of medicinal chemistry*. 2008; 51(4):721–724. [PubMed: 18232650]
- Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. *J Interferon Cytokine Res*. 2009; 29(6):313–326. [PubMed: 19441883]
- Fonseca VA, Grunberger G, Anhalt H, Bailey TS, Blevins T, Garg SK, Handelsman Y, Hirsch IB, Orzech EA, Roberts VL, Tamborlane W. Consensus Conference Writing, C. Continuous Glucose Monitoring: A Consensus Conference of the American Association of Clinical Endocrinologists and American College of Endocrinology. *Endocr Pract*. 2016
- Foster NC, Miller KM, Tamborlane WV, Bergenstal RM, Beck RW, Network TDEC. Continuous Glucose Monitoring in Patients With Type 1 Diabetes Using Insulin Injections. *Diabetes Care*. 2016; 39(6):e81–82. [PubMed: 27208319]

- Ge S, Shrestha B, Paul D, Keating C, Cone R, Guglielmotti A, Pachter JS. The CCL2 synthesis inhibitor bindarit targets cells of the neurovascular unit, and suppresses experimental autoimmune encephalomyelitis. *Journal of neuroinflammation*. 2012; 9:171. [PubMed: 22788993]
- Helton KL, Ratner BD, Wisniewski NA. Biomechanics of the sensor-tissue interface-effects of motion, pressure, and design on sensor performance and foreign body response-part II: examples and application. *J Diabetes Sci Technol*. 5(3):647–656. [PubMed: 21722579]
- Helton KL, Ratner BD, Wisniewski NA. Biomechanics of the sensor-tissue interface-effects of motion, pressure, and design on sensor performance and the foreign body response-part I: theoretical framework. *J Diabetes Sci Technol*. 5(3):632–646. [PubMed: 21722578]
- Khan UA, Hashimi SM, Bakr MM, Forwood MR, Morrison NA. CCL2 and CCR2 are Essential for the Formation of Osteoclasts and Foreign Body Giant Cells. *Journal of cellular biochemistry*. 2015
- Klueh U, Antar O, Qiao Y, Kreutzer DL. Role of interleukin-1/interleukin-1 receptor antagonist family of cytokines in long-term continuous glucose monitoring in vivo. *J Diabetes Sci Technol*. 2013a; 7(6):1538–1546. [PubMed: 24351180]
- Klueh U, Antar O, Qiao Y, Kreutzer DL. Role of vascular networks in extending glucose sensor function: Impact of angiogenesis and lymphangiogenesis on continuous glucose monitoring in vivo. *Journal of biomedical materials research Part A*. 2013b
- Klueh U, Frailey JT, Qiao Y, Antar O, Kreutzer DL. Cell based metabolic barriers to glucose diffusion: macrophages and continuous glucose monitoring. *Biomaterials*. 2014a; 35(10):3145–3153. [PubMed: 24461328]
- Klueh U, Kaur M, Montrose DC, Kreutzer DL. Inflammation and glucose sensors: use of dexamethasone to extend glucose sensor function and life span in vivo. *J Diabetes Sci Technol*. 2007; 1(4):496–504. [PubMed: 19885112]
- Klueh U, Kaur M, Qiao Y, Kreutzer DL. Critical role of tissue mast cells in controlling long-term glucose sensor function in vivo. *Biomaterials*. 2010a; 31(16):4540–4551. [PubMed: 20226521]
- Klueh U, Liu Z, Cho B, Ouyang T, Feldman B, Henning TP, Kaur M, Kreutzer D. Continuous glucose monitoring in normal mice and mice with prediabetes and diabetes. *Diabetes Technol Ther*. 2006; 8(3):402–412. [PubMed: 16800762]
- Klueh U, Liu Z, Feldman B, Henning TP, Cho B, Ouyang T, Kreutzer D. Metabolic biofouling of glucose sensors in vivo: role of tissue microhemorrhages. *J Diabetes Sci Technol*. 2011; 5(3):583–595. [PubMed: 21722574]
- Klueh U, Liu Z, Feldman B, Kreutzer DL. Importance of Interleukin 1 / Interleukin 1 Receptor Antagonist in Short Term Glucose Sensor Function in Vivo. *Diabetes Science and Technology*. 2010b; 4(5):1073–1086.
- Klueh U, Qiao Y, Czajkowski C, Ludzinska I, Antar O, Kreutzer DL. Basement Membrane-Based Glucose Sensor Coatings Enhance Continuous Glucose Monitoring in Vivo. *J Diabetes Sci Technol*. 2015
- Klueh U, Qiao Y, Frailey JT, Kreutzer DL. Impact of macrophage deficiency and depletion on continuous glucose monitoring in vivo. *Biomaterials*. 2014b; 35(6):1789–1796. [PubMed: 24331705]
- Kvist PH, Iburg T, Bielecki M, Gerstenberg M, Buch-Rasmussen T, Hasselager E, Jensen HE. Biocompatibility of electrochemical glucose sensors implanted in the subcutis of pigs. *Diabetes Technol Ther*. 2006; 8(4):463–475. [PubMed: 16939371]
- Kyriakides TR, Foster MJ, Keeney GE, Tsai A, Giachelli CM, Clark-Lewis I, Rollins BJ, Bornstein P. The CC chemokine ligand, CCL2/MCP1, participates in macrophage fusion and foreign body giant cell formation. *The American journal of pathology*. 2004; 165(6):2157–2166. [PubMed: 15579457]
- Luu TU, Gott SC, Woo BW, Rao MP, Liu WF. Micro- and Nanopatterned Topographical Cues for Regulating Macrophage Cell Shape and Phenotype. *ACS Appl Mater Interfaces*. 2015; 7(51):28665–28672. [PubMed: 26605491]
- Miller K, Foster N, Tamborlane W, Bergenstal R, Beck R. Continuous glucose monitoring in T1D patients using injections of insulin: a report from the T1D Exchange clinic registry. *Diabetes Technol Ther*. 2016; 18:A-27. Abstr. 069.

- Moore LB, Sawyer AJ, Charokopos A, Skokos EA, Kyriakides TR. Loss of monocyte chemoattractant protein-1 alters macrophage polarization and reduces NFkappaB activation in the foreign body response. *Acta Biomater.* 2015; 11:37–47. [PubMed: 25242651]
- Norton LW, Koschwanetz HE, Wisniewski NA, Klitzman B, Reichert WM. Vascular endothelial growth factor and dexamethasone release from nonfouling sensor coatings affect the foreign body response. *Journal of biomedical materials research Part A.* 2007; 81(4):858–869. [PubMed: 17236219]
- Novak MT, Reichert WM. Modeling the Physiological Factors Affecting Glucose Sensor Function in Vivo. *J Diabetes Sci Technol.* 2015; 9(5):993–998. [PubMed: 26134832]
- Novak MT, Yuan F, Reichert WM. Predicting glucose sensor behavior in blood using transport modeling: relative impacts of protein biofouling and cellular metabolic effects. *J Diabetes Sci Technol.* 2013; 7(6):1547–1560. [PubMed: 24351181]
- Novak MT, Yuan F, Reichert WM. Macrophage embedded fibrin gels: an in vitro platform for assessing inflammation effects on implantable glucose sensors. *Biomaterials.* 2014; 35(36):9563–9572. [PubMed: 25175597]
- Obermaier K, Schmelzeisen-Redeker G, Schoemaker M, Klotzer HM, Kirchsteiger H, Eikmeier H, del Re L. Performance evaluations of continuous glucose monitoring systems: precision absolute relative deviation is part of the assessment. *J Diabetes Sci Technol.* 2013; 7(4):824–832. [PubMed: 23911163]
- Simple BD, Bye N, Rancan M, Ziebell JM, Morganti-Kossmann MC. Role of CCL2 (MCP-1) in traumatic brain injury (TBI): evidence from severe TBI patients and CCL2-/- mice. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism.* 2010a; 30(4):769–782.
- Simple BD, Frugier T, Morganti-Kossmann MC. CCL2 modulates cytokine production in cultured mouse astrocytes. *Journal of neuroinflammation.* 2010b; 7:67. [PubMed: 20942978]
- Simple BD, Kossmann T, Morganti-Kossmann MC. Role of chemokines in CNS health and pathology: a focus on the CCL2/CCR2 and CXCL8/CXCR2 networks. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism.* 2010c; 30(3):459–473.
- Skokos EA, Charokopos A, Khan K, Wanjala J, Kyriakides TR. Lack of TNF-alpha-induced MMP-9 production and abnormal E-cadherin redistribution associated with compromised fusion in MCP-1-null macrophages. *The American journal of pathology.* 2011; 178(5):2311–2321. [PubMed: 21514443]
- Updike SJ, Shults M, Ekman B. Implanting the glucose enzyme electrode: problems, progress, and alternative solutions. *Diabetes Care.* 1982; 5(3):207–212. [PubMed: 7172985]
- White GE, Iqbal AJ, Greaves DR. CC chemokine receptors and chronic inflammation--therapeutic opportunities and pharmacological challenges. *Pharmacological reviews.* 2013; 65(1):47–89. [PubMed: 23300131]
- You H, Altier C, Zamponi GW. CCR2 receptor ligands inhibit Cav3.2 T-type calcium channels. *Molecular pharmacology.* 2010; 77(2):211–217. [PubMed: 19864434]
- Zschornack E, Schmid C, Pleus S, Link M, Klotzer HM, Obermaier K, Schoemaker M, Strasser M, Frisch G, Schmelzeisen-Redeker G, Haug C, Freckmann G. Evaluation of the performance of a novel system for continuous glucose monitoring. *J Diabetes Sci Technol.* 2013; 7(4):815–823. [PubMed: 23911162]

How are MQ recruited to sensor implantation sites?

Figure 1. Role of CC chemokines in leukocyte recruitment and accumulation at the glucose sensor-tissue interface in vivo

Figure 1A is a photomicrograph of a histological section (H&E) from tissue obtained at an Abbott navigator glucose sensor implant site in a C57B/6 mouse. The figure demonstrates leukocyte (L) recruitment and accumulation at the glucose sensor-tissue interface in vivo. In response to sensor implantation, inflammatory leukocytes are recruited from the vasculature (V) to the implanted sensor via leukocyte chemotactic factors, e.g. CCL2, produced by injured cells. With time, these recruited leukocytes (L) amass at the tissue-sensor interface (white arrow) and eventually deter sensor function and CGM in vivo. Figure 1B is a theoretical model demonstrating a chemokine gradient in response to tissue injury (e.g. sensor implantation). Leukocytes (e.g. MΦ) follow the chemokine gradient and migrate to the source of insult.

Figure 2. Summary of cumulative MARD trend analysis of four week CGM data from Abbott Navigator sensors implanted in normal (C57BL/6), CCL2 (CCL2^{-/-}) and CCR2 (CCR2^{-/-}) knockout mice

Figure 2 represents the trends in cumulative average mean MARD from weeks 1 to 4, by genetic background. The p-values represent the significance in CGM variability among the three treatment groups in average mean MARD value for each individual week, by the Kruskal-Wallis test (as non-parametric equivalent to ANOVA). The error bars around each data point represent a standard error of the mean MARD for the particular time point and genetic group. All sensors used for these studies were non-recalibrated throughout the 4-week test period as opposed to other animal or human studies where the sensors are typically recalibrated at least once a day. Therefore, our sensor performance results are reflected as raw nA per mg/dL of blood.

Figure 3. Inflammation index trend analysis at sensor implantation sites for CCL2 -/-, CCR2 -/-, and C57BL/6 mice

Figure 3 represents the trends in mean inflammation indices from weeks 1 to 4, by genetic background for CCL2 -/-, CCR2 -/-, and C57BL/6 mice. Inflammation indices range from a minimum of 0 (no inflammation) to a maximum of 4 (severe inflammation). p-values obtained by Kruskal-Wallis (K-W) tests as a non-parametric equivalent to analysis of variance (ANOVA) represent the significance of difference among the three groups for mean inflammation index values for each individual week. The error bars around each data point represent a standard error of the mean inflammation index value for the particular time point and genetic background.

Figure 4. Evaluation of glucose sensor induced histopathology at glucose sensor implantation sites in CCL2 $-/-$, CCR2 $-/-$, and C57BL/6 mice: H&E and anti-F4/80 staining

Figure 4 is a photomicrograph of representative histopathologic evaluations of glucose sensor implantation sites in CCL2 $-/-$, CCR2 $-/-$, and C57BL/6 mice at the day of peak tissue reaction i.e. day 14 post sensor implantation. Representative tissue reactions as evaluated by H&E staining in normal, CCL2 $-/-$ and CCR $-/-$ mice are presented Figures 4a, 4c and 4e. The presence and distribution of macrophages (representative examples designated with red arrow heads <) in these same tissue samples are presented in Figures 4b, 4d and 4f. Sensor implantation site within the tissue sample is designated using the * sign. These studies demonstrated that deletion of CCL2 and CCR2 gene in C57B/6 mice, results in dramatic decrease in inflammation and macrophage recruitment at site of glucose sensor in normal and CCL2 and CCR2 KO mice.

Figure 5. Model of suppression of tissue inflammation, and enhanced glucose sensor function in vivo using glucose sensor coatings for local delivery of CCL2 expression inhibitors and CCR2 competitive antagonists

Figure 5 is a graphic representation of proposed local delivery of CCL2 expression inhibitors and CCR2 competitive antagonists incorporated into sensor coatings. It outlines the effects of these inhibitors/antagonists on CCL2 expression in tissue cells and recruited monocyte/macrophages as well as the impact of CCR2 competitive antagonist on MΦ recruitment at sensor implantation sites. In response to sensor implantation trauma and subsequent inflammatory reactions at the implantation site, MCP-1/CCL2 is elaborated to injured tissue and inflammatory cells (Figure 5A). This initial local production of CCL2 triggers chemotaxis based recruitment and activation of CCR2 expressing monocytes from the vasculature into the sensor implantation sites (Figure 5A). As these CCR2 expressing monocytes accumulate at the sensor-tissue interface, they become activated by CCL2 into CCR2 macrophages, which will amplify the tissue reactions (inflammation, fibrosis, and vessel regression) at the implantation site by further expression of pro-inflammatory and pro-fibrotic mediators including CCL2. Additionally, CCR2 macrophages are fused by CCL2 to form foreign body giant cells. Monocyte/macrophages are the centerpiece of chronic inflammation and foreign body reactions. Preventing the recruitment of these leukocytes by inhibiting monocyte chemokine expression, as well as directly antagonizing

chemokine receptors on monocytes/macrophages would decrease chronic inflammation and enhance glucose sensor performance (Figure 5B). Abbreviations/symbols used in this figure include: Glucose sensor (black tube); sensor coating (yellow mass surrounding sensor) macrophages (cells with kidney bean nucleus); tissue cells (example fat cell, yellow cell); blood vessel (large red oval); tissue damage (red lightning bolt); CCL2 chemokine/monocyte chemotactic peptide (1CCL2/MCP-1), CC Receptor 2 (CCR2), CCR2 receptor antagonists (CCR2-I), Bindarit (B), Blockade of gene expression (Red X).

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Table 1

Cumulative mean absolute relative difference (MARD) values at four weeks for normal (C57BL/6), as well as CCL2 (CCL2^{-/-}) and CCR2 (CCR2^{-/-}) knockout mice.

	C57BL/6 median MARD = 24.59% +/- 9.39% n=15	CCL2 ^{-/-} median MARD = 16.02% +/- 5.14% n=10	CCR2 ^{-/-} median MARD = 19.19% +/- 5.58% n=11
C57BL/6 median MARD = 24.59% +/- 9.39% n=15	-----	0.0023	0.0045
CCL2 ^{-/-} median MARD = 16.02% +/- 5.14% n=10			0.7045
CCR2 ^{-/-} median MARD = 19.19% +/- 5.58% n=11			-----

Table 1 lists the medians of the cumulative Mean Absolute Relative Difference (MARD) values at four weeks for the three groups of mice with CCL2^{-/-}, CCR2^{-/-}, and C57BL/6 genetic backgrounds. Error values following the +/- following the median percent value are the interquartile ranges (difference between the third and first quartile range, 75% -25% percentile) of the individual treatment group's MARD values. p-values within the boxes represent the statistical significance of the comparisons of the two treatment groups indicated in the axes, calculated by Mann-Whitney U tests, as non-parametric equivalents to student t-tests.