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CHRFAM7A Expression in Mice Increases Resiliency after Injury

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SUMMARY:

The CHRNA7 gene encodes the α-7 nicotinic acetylcholine receptor (α7nAchR) that regulates anti-inflammatory responses to injury; however, only humans express a variant gene called CHRFAM7A that alters the function of α7nAChR. CHRFAM7A expression predominates in bone marrow and monocytes/macrophages where the CHRFAM7A/CHRNA7 ratio is highly variable between individuals. We have previously shown in transgenic mice that CHRFAM7A increased emergency myelopoiesis from the bone marrow and monocyte/macrophage expression in lung. Here, we demonstrate that CHRFAM7A expression results in an anti-inflammatory phenotype with improved survival to LPS and decreased acute lung injury in a severe cutaneous burn model compared to WT. These data suggest that the relative expression of CHRFAM7A may alter resiliency to injury and contribute to individual variability in the human systemic inflammatory response (SIRS) to injury.

INTRODUCTION

Acute lung injury resulting from the systemic inflammatory response (SIRS) is a leading cause of late deaths after injury. While an initial hyper-inflammatory response is expected after severe injury, anti-inflammatory mechanisms must be timely activated to prevent secondary organ failure, including acute lung injury. The CHRNA7 gene encodes the α-7 nicotinic acetylcholine receptor (α7nAchR) that regulates cholinergic anti-inflammatory responses. Stimulating the cholinergic anti-inflammatory pathway via direct vagal nerve stimulation or pharmacologic agonists has been shown to decrease SIRS, gut barrier failure, and acute lung injury in models of injury and infection [1].

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Only humans express a unique variant called CHRFAM7A that disrupts the normal α7nAchR homopentamer and alters downstream signal transduction. CHRFAM7A expression predominates in monocytes/macrophages. We have found significant individual variability in the relative expression of CHRFAM7A:CHRNA7. We have previously shown that expression of CHRFAM7A in transgenic mice increases the bone marrow reservoir of $Lin^{-}Scal^{+}cKit^{+}$ (LSK) cells, emergency myelopoiesis, and macrophage trafficking to the lung after severe burn injury. [2]

Based on our previous results, we predicted that CHRFAM7A transgenic mice would exhibit a pro-inflammatory phenotype after injury. Unexpectedly, we demonstrate here that CHRFAM7A transgenic mice have increased resiliency to infection and injury.

MATERIAL AND METHODS

Animals:

CHRFAM7A transgenic mice were prepared in a C57BL/6 background and bred as heterozygotes as described elsewhere [2]. CHRFAM7A mice have been deposited in the Mutant Mouse Resource and Research Center supported by the NIH (https://www.mmrrc.org) and are available by request (Catalog Number MMRRC:065547-UCD). CHRFAM7A mice are compared to gender and age-matched siblings. All animal experiments were approved by the University of California San Diego Institutional Animal Care and Use Committee (IACUC).

Adoptive Transfer of CHRFAM7A.

A bone marrow pellet from donor CHRFAM7A mouse was re-suspended in 1 ml of MacsQuant Running Buffer with 2% BSA and passed through a 70-micron cell strainer, spun at 2700 RPM for 5 minutes, and concentrated in 220 μL of 0.9% normal saline. Each recipient WT mouse was pretreated with 30 mg/kg busulfan for four consecutive days (Sigma #2635) prior to retro-orbital injection of 100 μL of 15e6 donor BM cell suspension. Mice were allowed to recover for 8 weeks before the burn injury.

Survival after LPS.

CHRFAM7A and WT mice were given an intraperitoneal injection of LPS (E. coli O111:B4, Sigma #L4130) 12.5 mg/kg. Mice were returned to their cages and monitored for signs of distress. Moribund mice underwent euthanasia and were considered non-survivors. Survival was followed until 72 hours after injection of LPS.

Severe Burn Model.

Mice weighing 18 to 22g were placed under anesthesia using inhaled isoflurane. Dorsal fur was removed prior to placing the mouse into a template estimating 30% total body surface area for exposure to a 7-second full-thickness steam burn [2]. Following burn, animals received a subcutaneous injection of 1.5ml of normal saline for resuscitation and buprenorphine for pain control. Sham mice underwent the same procedures except for the burn injury.

Lung Vascular Permeability.

Thirty minutes after a retro-orbital injection of 100 μL 70 kDa FITC-Dextran at 50mg/mL (Sigma, FD70-1G), the heart was perfused with 5mL of heparinized saline and lung vascular permeability was evaluated. The right lung was minced in 2 mL of 1X PBS, dissociated, and spun for 5 minutes at 10000 x g. The supernatant was transferred to a fresh tube, and 100 μL in duplicate was used to measure the fluorescence intensity in a 96 well plate using an Omega Flur Star Reader. FITC-Dextran standard curve was constructed by making twofold serial dilutions.

RESULTS

We found that CHRFAM7A mice had improved survival compared to WT after a lethal dose of LPS (Figure 1A). Based on our previous findings that monocyte trafficking to the lungs of CHRFAM7A mice increased after burn injury [2], we next assessed CHRFAM7A effects on burn-induced lung injury. Compared to WT, lung vascular permeability was decreased in CHRFAM7A mice 4 hours after severe burn injury (Figure 1B). These data suggest that CHRFAM7A attenuates the inflammatory response to systemic insults. To understand the effects of BM CHRFAM7A expression on the previously demonstrated lung injury phenotype, we measured lung vascular permeability in WT mice transplanted with CHRFAM7A-expressing BM. Mice transplanted with CHRFAM7A BM had decreased lung permeability at 4 hours after burn injury compared to WT BM (Figure 1C). This demonstrates expression of CHRFAM7A in the BM mitigates the inflammatory response in burn-induced lung injury, adding to our previous work showing that there is a role for CHRFAM7A in mediating the recruitment of myeloid cells to the lung following burn injury [2].

DISCUSSION

Here, we demonstrated that CHRFAM7A expression results in an anti-inflammatory phenotype, with improved survival to LPS and attenuated burn-induced lung permeability compared to WT. Further, adoptive transfer of CHRFAM7A BM suggests that CHRFAM7A expression in BM-derived immune cells is responsible for attenuating the inflammatory response and decreasing secondary lung injury.

Prior studies have demonstrated that CHRFAM7A decreases ligand binding to the α7nAChR and prevents downstream intracellular signal transduction [3,4]. In this model, CHRFAM7A expression is analogous to CHRNA7 knockout (KO) associated with loss of the α7nAChR in animal models. We have shown that CHRFAM7A is highly expressed in human bone marrow (BM) and Mo/MΦ where relative expression of CHRFAM7A and CHRNA7 is highly variable between individuals [5]. This suggests that CHRFAM7A may contribute to individual variability in the SIRS response to injury, a well-recognized phenomenon in the clinical setting.

The inflammatory response generated by monocytes/macrophages depends, in part, on which transcriptional factors have been activated during differentiation [6]. We propose that increased CHRFAM7A expression results in the differentiation of monocytes/macrophages

that are polarized to an anti-inflammatory phenotype and therefore do not require an intact α7nAchR to restrain a pro-inflammatory response (Figure 1D). Therefore, elevated relative CHRNA7 expression in monocytes/macrophages is reserved for pro-inflammatory cells and allows cholinergic agonists to tune the inflammatory response via the α7nAchR when high inflammatory states are sensed by the host. With α7nAChR activation being essential to the regulation of the cholinergic anti-inflammatory response, it is critical to understand the role of both CHRFAM7A and CHRNA7 in modulating human inflammatory responsiveness.

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REFERENCES

1. Costantini TW, Krzyzaniak M, Cheadle GA, et al. Targeting alpha-7 nicotinic acetylcholine receptor in the enteric nervous system: a cholinergic agonist prevents gut barrier failure after severe burn injury. Am J Pathol. 2012;181(2):478–486. [PubMed: 22688057]
2. Costantini TW, Chan TW, Cohen O, et al. Uniquely human CHRFAM7A gene increases the hematopoietic stem cell reservoir in mice and amplifies their inflammatory response. Proc Natl Acad Sci U S A. 2019;116(16):7932–7940. [PubMed: 30944217]
3. Martin-Sanchez C, Ales E, Balseiro-Gomez S, et al. The human-specific duplicated alpha7 gene inhibits the ancestral alpha7, negatively regulating nicotinic acetylcholine receptor-mediated transmitter release. J Biol Chem. 2021;296:100341. [PubMed: 33515545]
4. Chan TW, Langness S, Cohen O, Eliceiri BP, Baird A, Costantini TW. CHRFAM7A reduces monocyte/macrophage migration and colony formation in vitro. Inflamm Res. 2020;69(7):631–633. [PubMed: 32303780]
5. Costantini TW, Dang X, Yurchyshyna MV, Coimbra R, Eliceiri BP, Baird A. A Human-Specific alpha7-Nicotinic Acetylcholine Receptor Gene in Human Leukocytes: Identification, Regulation and the Consequences of CHRFAM7A Expression. Mol Med. 2015;21:323–336. [PubMed: 25860877]
6. Glass CK, Natoli G. Molecular control of activation and priming in macrophages. Nat Immunol. 2016;17(1):26–33. [PubMed: 26681459]

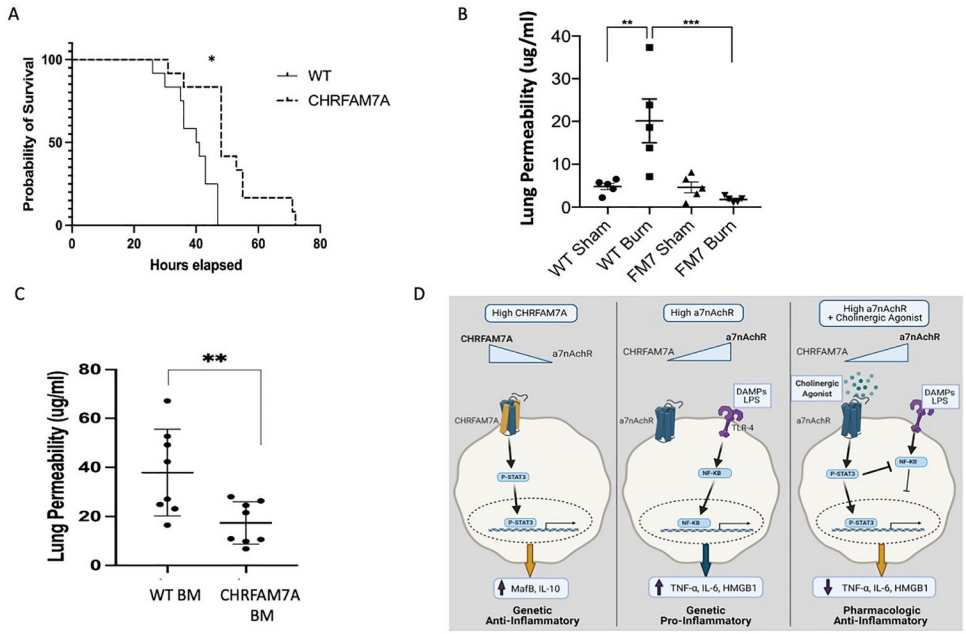

Figure 1: CHRFAM7A expression is associated with an anti-inflammatory phenotype.

(**A**) CHRFAM7A transgenic (n=12) and WT sibling mice (n=12) were injected with LPS (12.5mg/kg) to evaluate the effects of CHRFAM7A expression on survival, and (**B**) lung vascular permeability was measured 4 hours after 30% total body surface area burn (n=5/group). (**C**) Adoptive transfer of CHRFAM7A BM into WT mice prevents burn-induced acute lung injury (n=8/group). (**D**) Proposed model of inflammatory status of monocytes/macrophages based on relative CHRFAM7A expression. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.