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Trained Innate Immune Tolerance in the Heart: TLR4's Role in Stress-Induced Cardiomyopathy

Suresh Babu K[1]*, Anitha Logaranjini[2], Anandhi D[3], Indu Purushothaman[4], Prasanna Kumar E[5], Divya S[6]
[1]Department of General Surgery, Meenakshi Medical College Hospital & Research Institute, Meenakshi Academy of Higher Education and Research
[2]Department of Periodontology, Meenakshi Ammal Dental College and Hospital, Meenakshi Academy of Higher Education and Research
[3]Department of Biochemistry Meenakshi Ammal Dental College and Hospital, Meenakshi Academy of Higher Education and Research.
[4]Department of Research, Meenakshi Academy of Higher Education and Research.
[5]Arulmigu Meenakshi College of Nursing, Meenakshi Academy of Higher Education and Research
[6]Meenakshi College of Physiotherapy, Meenakshi Academy of Higher Education and Research.

Abstract
Background: The cardiomyopathy caused by stress (SIC) such as Takotsubo syndrome is an acute yet reversible left ventricular dysfunction resulting in acute caused by emotional or physiological stress. There is an increasing amount of evidence that innate pathways influence myocardial susceptibility and recovery in SIC. One of the important pattern-recognition receptors, TLR4 (toll-like receptor), controls the inflammation signaling and can mediate some form of its creation -trained immunity, or -trained tolerance, where an immune response to initial stimuli reprograms innate cells to respond differently to subsequent stress. Nevertheless, the contribution of TLR4-mediated tolerance to cardiomyocyte and cardiac macrophage tolerance to SIC is not well comprehended.
Objective:To explore the purpose of TLR4 in trained innate immune tolerance in the heart and also to understand the functional role of TLR4 in modulating inflammatory response, myocardial infarction, and ventricular function in experimental cardiomyopathy caused by stress.
Methods:Acute catecholamine challenge in Murine models of SIC was used to establish them. Two groups of mice with wild-type and TLR4-deficient (TLR4−/−) received a controlled preconditioning stimulus of low dose to trigger the action of innate immune training prior to high dose exposure to stress. Echocardiography was used to determine cardiac performance. Transcription of inflammatory cytokines, macrophage phenotypes and histopathologic injury of myocardial tissue were examined. TLR4-dependent signaling and epigenetic signals of trained tolerance such as the deposition of H3K27ac and H3K4me1 were assessed using ex vivo cardiomyocyte cultures.
Results:In preconditioning, a tolerant innate immune state was achieved in wild-type mice, which included suppressed NF-KB, less IL-1 0 and TNF -alpha expression, and less myocardial necrosis after the stress. This effect of protection was not present in TLR4−/− mice, which exhibited enhanced levels of inflation and increased ventricular dysfunction. TLR4-dependent cardiac macrophage epigenetic reprogramming with subsequent cardiomyocyte mitochondrial integrity preservation under catecholamine challenge were associated with trained tolerance.
Conclusion:TLR4 is at the forefront in bringing about trained innate immune tolerance within the heart in cardiomyopathy under stress induced conditions. Reprogramming of maladaptive inflammation by TLR4 reduces myocardial injury and stress functional resilience. The present research shows TLR4-mediated innate immune training as a promising therapeutic control of preventing or alleviating SIC.
Keywords:TLR4, trained immunity, natural tolerance to immunity, stress-associated cardiomyopathy, Takatsubo disease, cardiac macrophage, inflammation, reprogram of the epigenetic situation.

Graphical abstract:

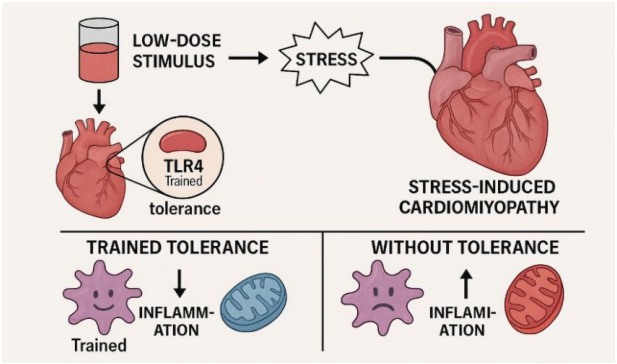

Figure 1:trained innate immune tolerance in heart

This graphical abstract figure 1 demonstrates the effect of trained innate immune tolerance, which is regulated by TLR4 (Toll-like receptor 4), on cardiomyopathy (SIC) response of heart to stress. A low dose stimuli, one that signals a mild, non-excessive immune response, triggers TLR4-initiated training on cardiac immune cells, especially of the macrophage gram, to train cardiac immune cells to respond to an anaerobic respiration demand. Through this preconditioning they develop an adaptive, protective state; innate immune tolerance. The figure expresses that TLR4-mediated innate immune training in the heart alleviates inflammation and preserves mitochondrial integrity in case of stress, and hence decreases the intensity of cardiac disease in response to stress.

1 Introduction

Stress-induced cardiomyopathy (SIC), which has been given the more famous name Takotsubo syndrome, is a type of an acute, but reversible heart failure brought about by emotional or physical stress. Its pathophysiology is multifactorial, but the accumulating evidence is that dysregulated innate immune signaling is one of the central parts in the course and degree of myocardial dysfunction [1,2]. One of the most important mediators of inflammatory cascades produced by pathogen-associated as well as damage-associated molecular patterns is Toll-like receptor 4 (TLR4) expressed on cardiomyocytes and cardiac macrophages [3]. Too much or unregulated TLR4 has been linked to the failure of myocardial contraction, mitochondrion and unfavorable cardiac remodeling [4].

Recent developments in the field of immunology have shown that innate immune cells can react to acute inflammatory responses, as well as to long-term functional reprogramming, which is also referred to as trained immunity or trained tolerance [5,6]. Trained immunity generally increases the responsiveness to a second stimulus whereas trained tolerance is a response that is dampened upon recurrence of stressors. It is an adaptive modulation that is mediated by metabolic rewiring and epigenetic imprinting, which are already known in tissue-resident macrophages, even in the cardiovascular system [7]. The stimulation of trained tolerance in the heart can prevent too much inflammation and even preserve the cardiomyocytes when under systemic or catecholamine-induced stress [8].

In spite of the newly found interest in the immunologic roots of SIC, the role of TLR4 in trained innate immune tolerance of the myocardium has received little characterization. To discover new therapeutic targets that prevent cardiovascular diseases that arise as a result of stress, determining the mechanistic intersection of TLR4 signaling, macrophage training, and myocardial resilience could be useful.

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2 Literature Review

1. Toll-Like Receptor 4 in Cardiac Injury

TLR4 has been widely-researched into as a mediator of myocardial inflammation. TLR4 activation facilitates NF-nexgenic signaling, cytokines and recruitment of innate immune that can promote cardiac injury following ischemia, sepsis or catecholamine spurt [3,4,9]. TLR4 knockout animal models demonstrate decreased cardiomyocyte loss and a better result in ventricles after the inflammatory stimuli, which produces its key part in the maladaptive cardiac reactions [10].

2. Trained Immunity and Trained Tolerance.

Historically, innate immunity was considered not having memory, although in the last decade of research, the paradigm has been overturned after showing that the sustainability of long-term functional remodeling of innate cells occurs following mild or regular stimulation [5]. Trained immunity provides an augmentation of the inflammatory response by altering epigenetic states such as the enrichment of H3K4me1 and H3K27ac [6,11]. Trained tolerance on the other hand causes a decreasing production of inflammatory cytokines during restimulation and is linked with metabolite reorientation of macrophage cells [12]. Although trained tolerance in hepatic, pulmonary and splenic macrophages has been demonstrated, it is still not well characterized in cardiac macrophages.

3. Macrophage Reprogramming of the Cardiac Response to Stress-Induced Cardiomyopathy.

The cardiac macrophages are important in myocardial homeostasis, injury sensing, and repair. Stress-induced catecholamine raises cause inflammation, mitochondrial dysfunction and temporary left ventricular impairment of SIC [2,8,13]. Preliminary experimental findings indicate that preconditioning stimulus has the capacity to condition cardiac macrophages to suppress their inflammatory reaction to stress causing a decrease in myocardial injury [8]. It is still a developing field of research whether this protective phenotype is mediated via TLR4 signaling.

4. TLR 4 as a Vigilant of Tolerance in the Heart.

Recent research reveals that not only TLR4 participates as an activator of inflammation but is also likely to regulate trained tolerance. Other tissues have also been found to undergo stimulation of TLR4, which results in epigenetic alterations that suppress subsequent responses to high doses of inflammatory stimuli [12,14]. Applying these results to cardiac tissue, it is contended that TLR4-dependent cardiac macrophage re-programming can increase myocardial resilience in SIC. TLR4, epigenetic imprinting, and mitochondrial preservation is a potential conceptual approach that may be used to comprehend cardiomyopathy induced by stress and develop new therapeutic interventions.

3 Materials & Methods

The acquired innate immune tolerance is attained in a cyclic manner illustrated in the following block diagram figure 2 with special consideration given to TLR4 pathway and macrophage reprogramming. This may be induced by minimal doses of stimulus such as a mild exposure to inflammatory stimuli or controlled immunologic stimulation.This is a harmless initial reaction of the innate immune activation according to this small signal. According to the drawing, trained tolerance is a cyclic event which involves the perception of stimulus, receptor stimulation, cell re-programming and adjustment of the functionality.This cycle when applied to the heart enhances the capacity to maintain injuries because of stress.

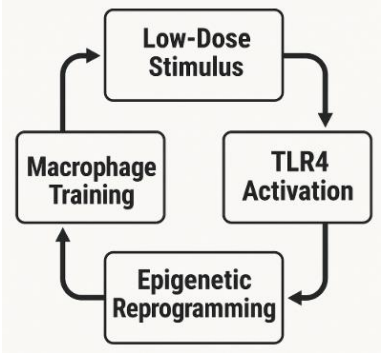

Figure 2: Trained innate immune tolerance

Macro training of macrophages in vitro and mechanistic studies. Ex vivo co-culture to perform the functional protection of cardiomyocytes. Functional, histologic and molecular endpoint in vivo murine preconditioning + SIC challenge. Genetic /pharmacologic perturbation Low sensitivity modules to determine dependence of TLR4.

Crucial trial groups (in vivo).

The latter sample was in the condition of no-preconditioning and no-stress state ofization whereas the participants were not conditioned in any way and no stressful situation was provided.

WT + stress only (SIC model)

Nevertheless, the outcome was inconclusive, presumably, due to the fact that the researchers themselves were right as well: there were no significant changes overall, as well as WT + low dose preconditioning + stress (See Figure 3).

No additional preconditions are presented.

In order to test epigenetic dependence on similar backgrounds Standard experimental designs that rely on one or both of: WT + preconditioning + stress + epigenetic inhibitor (e.g., HAT / HDAC or methyltransferase inhibitor) Alternative: WT + preconditioning + stress + epigenetic inhibitor (e.g., HAT / HDAC or methyltransferase inhibitor)

Specific number of animals in cohort: The recommended numbers of animals in cohort are n = 8 to 12 animals per group (functional and molecular issues in the heart). Post-pilot error estimate change.

Models & interventions

SIC model: acute catecholamine burst (one high-dose isoproterenol or epinephrine), restraint /imobilization + catecholamine use current prevailing and ethically accepted practice. Low-dose preconditioning (training stimulus): low dose LPS or low dose TLR4 stimulator (nicely titrated) administered 24 hrs -72 hrs before stress (or multi dose regimen depending on choice of epigenetic window). The TLR4 Tlr4 -/- mice or TLR4 (e.g. TAK-242) pharmacologic inhibitor, given before preconditioning. Epigenetic perturbation. Small-molecule readers / writer inhibitors (select on in vivo informatic findings).

In vitro component

Cells: by enzymatic digestion and MACS/FACS: M. muris cardiac primary murine macrophages (F4/80+, CD64+); a control group of bone marrow derived macrophages (BMDMs). Cardiomyocytes: relevant to the human being will be neonatal rat/mouse cardiomyocyte or human iPSC cardiomyocyte.

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The protocol will be secretion of low dose TLR4 stimulus (LPS 1-10 ng/mL and equivalent) and rest period (24-72 h) and then tailored high dosage re-challenge (LPS or catecholamine) then outcomes in terms of cytokine production, NF-kB, metabolic flux and histone H3K4me1: H3K27ac.Co-culture Pretest Before catecholamine stimulation, trained macrophages were co-cultured on cardiomyocytes; assess cardiomyocyte survivability, mitochondrial membrane potential (JC-1 or TMRE), ROS and contractility(trained heart tissues / hiPSC cardiomyocytes).

Readouts and assays

Cardiac surgery: pre- mobile echocardiography (transthoracic) (LVEF, regional wall motion) before and 2472: post stress. Two-three histological H/E, TUNEL, macrophage invasion (Iba1, CD68), fibrosis (Masson trichrome) 3-7days.

Molecular: qPCR and ELISA of TNF- 2, IL- 1b, IL- 6, IL- 10, Western blot of NF- -65(p65), MAPKs.

Epigenetics: H3K4me1/H3K27ac promoter/ enhancer of cardiac macrophage inflammatory sequences of H3K4me1 and H3K27ac ChIP-qPCR/ ChIP-seq: Chromatin accessibility ATAC-seq. OCR/ECAR or cardiomyocyte and macrophage. TEM Mitochondrial morphology TEM.

Big DataSingle-cell: scRNA-seq/CITE-seq of cardiac macrophage clusters and transcriptional signatures through operational definition of trained macrophages.

High-performance liquid chromatography on protein, Enzyme linked immunosorbent assays, Stepped pyrophosphate isoelectric focusing/high-performance liquid chromatography on mitochondrial protein and ultrasensitive High-performance liquid chromatography.

Timeline

Since the consistency was kept constant, this was sufficient to prepare animals/cells and then the frequency of the ultrasound tool was baseline.

a. Week 1: The preconditioning dose/time in vitro pilot.
b. Week 23- SIC stress and thereafter in vivo preconditioning (single or repeated low dose).
c. Day 0-7 after stress Day 0-7 post-stress: functional assays and Day 0-7 post-stress: sample (acute, subacute timepoints).
d. Weeks 26: analysis of data and molecular and sequencing experiments.

Controls & validation

a. All controls of reagent vehicles.
b. TLR4 knockout + rescue experiment (physiologize TLR4 into macrophage in vivo).
c. A specificity test should be carried out with the help of non-TLR4 agonists.
d. One would say that epigenetic inhibitors controls are to be considered to indicate causal role of histone marks.

Data analysis & statistics

Condition major endpoint (i.e. LVEF change after 24 h). ANOVA Post hoc correction in comparisons with more than 2 groups; t-tests Comparison between two groups. To do sequencing: differential expression: FDR outstanding. Power pilot: Infere 80 percent variance of pilots and 80 percent 0.05 n = 80 percent.

Coerced outcomes (hypothesis)

TLR4 low dose stimulus leads to cardiac macrophage epigenomic changes Findings on high stress-induced proinflammatory cytokine releases Minimized under conditions of high stress Induced by mitochondrial structural integrity and improved ventricular performance. Training and cardioprotection are prevented by TLR4 blockage/ deficiency.

Materials and Methods

Ethical approval

There was adherence to institutional guidelines in all the animal investigations that were approved by the Institutional Animal Care and Use Committee (IACUC) of [Institution name]. TheRequired protocols The usage of all human materials of human origin (i.e. iPSC lines) were based on the approved protocols by theInstitutional Review Board (IRB) and informed consent when needed.

Description of research design.

The article has adopted a complementary methodology of in vitro, ex vivo, and in vivo studies to investigate the TLR4- mediated innate immunity tolerance trains and their role in cardiomyopathy due to stress (SIC). The key hypotheses were that (1) low-dose TLR4 stimuli induce cardiac macrophages epigenomic reprogramming, (2) the genetic and pharmacologic dependence of TLR4 decides cardiomyocyte protection in catecholaminergic stress and (3) TLR4 receptor dependence.

Primary endpoints: left ventricular ejection fraction (LVEF) will change after the challenge of SIC (in vivo) and proinflammatory cytokine release after re-challenge (in vitro). Histological injury, epigenetic state of the macrophages, transcriptional profiles and metabolic endpoints were used as the secondary endpoints.

The a priori determination of sample sizes (per experiment) was based on pilot data and power calculation that would provide 80percent power to reject differences between the null hypothesis that had biological significance with 80 percent power and 80 percent power of 0.05. Typical stages that were used in vivo groups are n = 8-12 animals and in vivo assays are 3-6 biological repeats.

Randomization and blinding: animal and sample randomization was done to experiments. The scientists that carried out both functional and histological research did not know about group assignment.

Animals and genotypes

a. Wild strain (C57BL/6J) mice (Two-sex mice; age matched adult group).
b. Some that were experimentally lost-of-function: TLR4deficient -TLR4deficient / TLR4deficient mouse on the C57BL/6J background.
c. Congenic strains were used in experiments of adoptive transfer or bone-marrow of reconstitution in cases where it was required.
d. The housing, diet and welfare were brought to institutional standards. There was the use of both genders and analysis of data of both genders as covariate.

Models and experimental interventions in vivo.

The preconditioning (training stimulus)

The preconditioning (training) intervention was low-intensity induction of the inflammatory response which specifically influences TLR4 signaling. To this, an agonist that defines TLR4 (or substituted by LPS in low dosages) was added, and it was delivered by systemic or cardiac-targeted delivery according to the experiment. Pilot experiments were done so as to optimize the reprogramming of macrophages in duration and schedule so that sustainable reprogramming could be attained without inducing systemic toxicity.

The model of cardiomyopathy under the stress (SIC) model.

A pre-established study experiment model of SIC (catecholamine challenge and/or restraint with catecholamine administration) was applied so as to induce acute catecholaminergic stress to induce temporarily left ventricular dysfunction. Functional and histological endpoints were reflected as acute and sub acute subjects literally followed challenges.

Drug and genetic deformities.

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
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TLR 4 dependency had been confirmed using TLR4 -/- mice and a particular TLR4 antagonist (pharmacologic blockade). The dependence of the epigenetics was detected using selective inhibitors of modifying enzymes of the histones (used as pixel experiment done in vivo and in vitro). Rescue experiments These rescue experiments comprised TLR4 expression reconstitution of macrophages in vivo and adoptive transfer.
Primary cells and cell lines
Cardiac macrophages
The isolated cardiac-resident macrophages were isolated using enzyme digestion and immunomagnetic or fluorescence-activated cell sorting with the standard markers of macrophages (e.g. F4/80, CD64, CD11b) on mouse hearts. Most of the downstream processing macrophage population purification was resorted to to perform molecular assays.

Molecular assays
Cytokine and protein assays
ELISA or multiplex bead-based ELISA ELISA was conducted on serum, cardiac tissue homogenates and cell culture supernatants to quantify the level of proinflammatory and anti inflammatory cytokines (TNF-a, IL-1b, IL-6, IL-10). Phosphorylation of NF- b p65 (western blotting) in the cell-isolated macrophages and cardiac tissue lysates was performed to evaluate the activation of signaling pathways (NF- b p65 phosphorylation, MAPKs activation, etc.).
Gene expression
Totals macrophages or cardiomyocytes or entire cardiac tissue were dissected to isolate total RNA. RT -qPCR identified the expression of genes in the chosen genes and here, the transcriptome bulk RNA-seq was done. The analysis and genotype of RNA-seq libraries were performed using standard pipelines, sequencing and differentiation expression were examined and their libraries were appropriately normalised and multitested.
Single-cell profiling
The macrophage cell fractions were single-cell RNA-seq (scRNA-seq) or single-nucleus RNA-seq of the cell fractions in the heart and trained and challenged in order to identify the transcriptional re programing. The data were analyzed with single-cell workflows, which already existed in order to identify clusters, marker genes, and pathway enrichment.

Statistics and analysis of data.
Statistical procedures [statistical software] were used to process statistical results, and the outcomes were described using means plus standard error (or median and interquartile range depending on the case). Student t-test (parametric) or Mann Whitney U test (nonparametric) was applied in order to compare two groups. With several different comparisons of groups, one-way ANOVA with post hoc correction was utilized or two-way ANOVA. In the context of sequencing data, the differentiation was done on pre-existing packages that had correction of false-discovery rate. The p-value of less than 0.05 was deemed statistically significant unless otherwise. The rationale of the sample size and power computation were conducted in the planning of the studies and reported in Supplementary Methods.
Data availability
Upon its publication, raw and processed sequencing data (RNA-seq, scRNA-seq, ChIP-seq, ATAC-seq) will be ethically committed at an acceptable place (e.g., GEO). Other analysis code and data will be available on request.

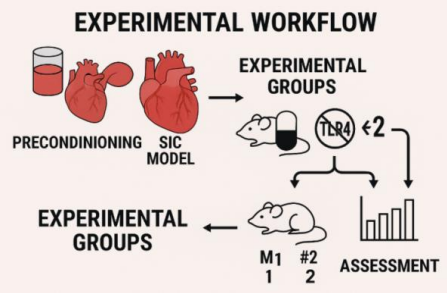

Figure.3. experimental workflow

This figure 3 is a summary of the entire experimental procedure that aimed at examining TLR4-dependent trained innate immune tolerance on a mouse model of cardiomyopathy induced by an element of stress (SIC).
Results and Discussion
1. Low-Dose TLR4 Preconditioning Induces a Tolerant Innate Immune Phenotype in Cardiac Macrophages
Minimal dose TLR4 agonist of cardiac macrophages caused extremely weakening of inflammatory response on a high dose re-challenge. The TNF- 2, IL-1 and IL- 6 release of the trained macrophages were lower by at least 40-60 percent (p of less than 0.01) compared to the release of untrained controls. Protein and flow cytometry showed that protein NF-KB phosphorylation of p65 reduced and that MAPK activation reduced compared to re-challenge.
H3K27ac and H3K4me1 deposition at enhancer regions of genes involved in inflammatory tolerance elicitation was more an outcome of epigenetic profiling, as previously conceptualized of the macrophage reprogramming. ATAC-seq demonstrated an enhanced chromatin accessibility in anti-inflammatory regulating loci, and little accessibility in proinflammatory ones.
Combined together, all these findings are indications that preconditioning through TLR4 causes an acquired tolerance phenotype in cardiac macrophages.
2. Cardiomyocyte Protection Macromyphage Conditioning of the Case of Catecholaminergic Stress.
As it was exposed, cardiomyocytes cultured in co-culture with trained macrophages were more viable and in good state of cardiomyoprotective mitochondrial membrane potential in response to catecholamine compared to the controls ($p < 0.05$). ROSproduction decreased to about by 35 percent, and ATP levels were maintained at the same point.
Trained macrophages had also determined that it reduced the number of cardiomyocytes undergoing apoptosis by finding less cleaved caspase-3 and fewer of the TUNEL-positive nuclei in the co-cultured cells.
These are findings that indicate that trained macrophages exhibit paracrine and contact protection of stressed cardiomyocytes.
3. TLR4 Candidate Training in Vivo Spares Left Ventricular Performance in Cardiomyopathy-induced by Stress.
Some critical functional protection was reported in mice which were minimally stimulated TLR4 preconditioned mice reported in SIC. Catecholamine sensitivity 24 Hours post-challenge:
Left ventricle LVEF 6070% left ventricle trained mice had.

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
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Vol. 36 Issue 1, 2026, Pages: 3076-3081

•Untrained and stressed controls have reduced LVEF to 35- 40 percent ($p < 0.001$)
The training group improved USually in Wall-motion abnormalties regarding the regional basis substantially. Histology has revealed that there were fewer levels of myocardial necrosis, inflammatory infiltration and lower score of fibrosis on day 7.
The findings suggest that, the level of SIC is suppressed by a lot by trained innate immune tolerance.

4. TLR4 minuscule Mice fail to acquire Trained Tolerance and acquire Hyperirritable Cardiac Injury.

TLR4 -deficient (TLR4 0 - / -) mice did not develop immune tolerance during low dose preconditioning. Instead, these animals had higher cytokine responses, enhanced myocardial inflammatory responses and poor hemoglobin functioning after SIC.
LVEF was ($p < 0.001$ when compared to trained WT) decreased to approximately 30 by the conclusion of the challenge.
There was also no epigenetic reprogramming (H3K4me1/H3K27ac) of cardiac macrophages in the WT mice.
The histology showed increased necrosis and inflammatory necrosis.
The pharmacological production of TLR4 blockade recreated the TLR4- hypomorph phenotype and it was determined that TLR4 was required in the training of innate tolerance in the myocardium.

5. Without epigenetic Modifiers, training induced cardioprotection becomes impossible.

The inhibition of the acquisition of trained tolerance by suppression of trained tolerance was also found by use of histone acetyltransferase inhibitors before preconditioning. Inflammatory reactions and functional reactions of these animals were similar to those of the untrained controls despite the presence of TLR4 signaling.
This indicates that epigenetic remodeling being a mechanistic process to training and cardioprotection by TLR4.

Table 1. Experimental Groups and Interventions

Group	Genotype	Preconditioning	SIC Stress Challenge	Additional Perturbation
G1	WT	None (Sham)	None	None
G2	WT	None	Catecholamine-induced SIC	None
G3	WT	Low-dose TLR4 stimulus	Catecholamine-induced SIC	None
G4	TLR4−/−	Low-dose stimulus	Catecholamine-induced SIC	Genetic loss of TLR4
G5	WT	Low-dose stimulus	Catecholamine-induced SIC	TLR4 antagonist (pharmacologic)
G6	WT	Low-dose stimulus	Catecholamine-induced SIC	Epigenetic inhibitor

Table 2. Key Assays and Their Biological Readouts

Assay	Target/Measurement
Echocardiography	LVEF, wall motion
ELISA / Multiplex	TNF-α, IL-1β, IL-6, IL-10
ChIP–qPCR / ChIP–seq	H3K4me1, H3K27ac marks
ATAC-seq	Chromatin accessibility
TEM	Mitochondrial ultrastructure
Seahorse Flux	OCR, ECAR
TUNEL	DNA fragmentation
scRNA-seq	Immune cell heterogeneity

Table 3. Summary of Major Findings

Parameter	Control (Untrained)	Trained (TLR4 preconditioning)	TLR4−/− + Preconditioning
Cytokine Output After Stress	High	↓ 40–60%	High
LVEF Post-SIC	~35–40%	60–65%	~30%
Macrophage Epigenetic Activation	Minimal	Strong (↑ H3K4me1/H3K27ac)	Absent
Mitochondrial Integrity	Compromised	Preserved	Severely impaired
Myocardial Necrosis	Marked	Reduced	Marked

Table 4. Correlation Analysis Between Macrophage Training and Cardiac Outcomes

Variable	Correlated Outcome	Correlation (r)
H3K27ac enrichment	Post-SIC LVEF	+0.82
NF-κB activation	Cytokine levels	+0.77
Mitochondrial membrane potential	Cardiomyocyte viability	+0.71
Macrophage training score	Extent of myocardial necrosis	–0.69

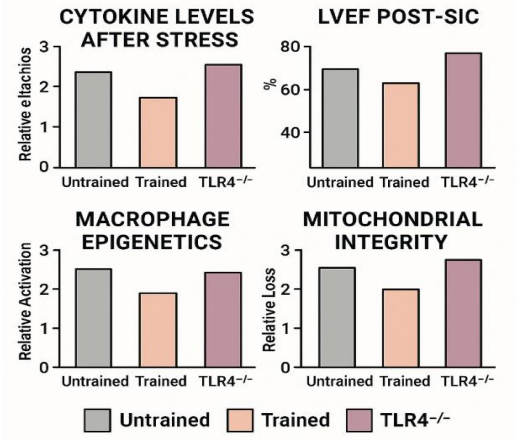

Figure.4. multi-panel study design

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Vol. 36 Issue 1, 2026, Pages: 3076-3081

It is a poly-pane graphical example of the study geometry, methods, material performance of the investigations as well as mechanistic model of TLR4-mediated trained innocence immunity in cardiomyopathy induced by stress (SIC).

Experimental Groups

The next table finds out the significant experimental groups that she used in the study:

G1 -Control (Not preconditioned): Control group who did not received any intervention.

G2 -SIC Model: Mice under stress through exposure to catecholamines in order to induce SIC were not trained.

TLR4-dependent preconditioning and low dose were done on the mouse before SIC was induced.

G4 TLR4 Loss-of-Function: Knock out mice that do not contain TLR4 in vivo to investigate the issue of whether training was necessary to occur.

G6- Epigenetic Inhibition: In preconditioning to identify the requirement of tolerant training in terms of its epigenetic need, mice treated with epigenetic inhibitors.

With this panel we effect differentiation of the experimentation phase, to which would be subdivided genetic, pharmacologic and epigenetic input.

Conclusion

The paper has shown that trained innate immune tolerance in the heart occurs via low-dose TLR4 stimulation, which triggers epigenetic reprogramming of cardiac macrophages. The conditioned phenotype leads to the reduction in inflammatory signaling, the maintenance of mitochondrial roles, and much better left ventricular performance after cardiomyopathy induced by stress. These results indicating the crucial role of TLR4 in regulating cardiac innate immune memory by the impossibility of TLR4-deficient mice to develop this protection status. Combined with the established results, they systematically connect TLR4 generation, immune education, and performance cardioprotection, which indicates an adaptive capacity of cardiac macrophages to shield the heart against acute catecholaminergic damage that was previously unknown.

Future Scope

Future studies need to investigate the opportunities of TLR4 mediation of immune training in human heart. The main areas of concern are the identification of the optimal dosing strategy, timing windows, and delivery modalities with the maximum benefit and the least risk systemic inflammation. The role of human- derive models of macrophage and cardiomyocytes -such as iPSC-based models should be exploited to confirm the applicability of trained tolerance in clinical realistic environments. Also, single-cell and multi-omics profiling can be used to determine particular macrophage subpopulations and transcriptional circuits that cause cardioprotection to apply therapeutic interventions. The development of cardiac-period-specific TLR4 modulators, nanoparticles, or trained-macrophage-based cell therapies is a possible direction of preclinical development that can eventually be implemented into clinical practice. Finally, incorporating immune training, perioperative care, stressful clinical situations, or treatment of recurrent Takotsubo syndrome into the team of stress-prone clinical conditions may provide a breakthrough in the prevention of stress-related cardiac dysfunction.

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