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Toll-like receptor signaling and macrophage polarization in adipose tissue inflammation

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Abstract

Leading immunologists now regard adipose tissue inflammation as the bridge between obesity and metabolic disease and Toll-Like Receptors (TLRs) appear to sit at the center of that bridge. This research investigated how TLR activation in Adipose Tissue Macrophages (ATMs) drives polarization toward the pro-inflammatory M1 phenotype in obese versus lean conditions. Primary ATMs were isolated from visceral adipose biopsies of 48 subjects (24 obese, BMI > 35; 24 lean, BMI 18.5–24.9) at Dhaka College of Molecular Biology, Bangladesh, between January 2022 and July 2023. TLR2 and TLR4 expression was 3.8- and 8.7-fold higher, respectively, in obese ATMs compared to lean. LPS stimulation (100 ng/mL) induced significantly greater M1 marker expression (TNF- α , IL-6, IL-1 β) in obese ATMs, with peak TNF- α levels 10.3-fold above baseline at 12 hours versus 5.1-fold in lean. M2 markers (IL-10, Arg-1) declined in obese ATMs post-stimulation but were maintained or increased in lean ATMs. TLR4 knockdown by siRNA reduced M1 polarization in obese ATMs by 62.4%. These data confirm that TLR4 is the dominant driver of M1 polarization in obese adipose tissue and identify it as a potential therapeutic target for metabolic inflammation.

Keywords: Toll-like receptors, macrophage polarization, adipose tissue, M1 macrophages, obesity, metabolic inflammation, TLR4, cytokines

Introduction

As Hotamisligil wrote in his landmark 2006 review, inflammation and metabolism are fundamentally interlinked a connection nowhere more visible than in the adipose tissue of obese individuals ^[1]. Visceral adipose tissue in obesity is infiltrated by macrophages that adopt a classically activated (M1) phenotype, producing pro-inflammatory cytokines that disrupt insulin signaling and promote systemic metabolic dysfunction ^[2].

TLRs are pattern recognition receptors of the innate immune system that detect microbial components and damage-associated molecular patterns. In obese adipose tissue, elevated levels of saturated fatty acids and gut-derived endotoxin (LPS) activate TLR2 and TLR4 on resident macrophages, triggering NF- κ B-dependent cytokine production ^[3, 4]. This creates a self-reinforcing cycle: TLR activation promotes M1 polarization, which produces more inflammation, which recruits additional macrophages ^[5].

While mouse models have established TLR4 as a key mediator of adipose inflammation, human data particularly from South Asian populations with high metabolic disease burden remain limited ^[6, 7]. Bangladesh has experienced rapid increases in obesity-related diabetes, yet the immunological basis of adipose inflammation in this population hasn't been characterized. This research isolated primary human ATMs from obese and lean Bangladeshi adults, compared TLR expression and signaling, and used siRNA knockdown to test TLR4's specific contribution to M1 polarization ^[8].

Reagents and chemicals

Collagenase type I was purchased from Worthington Biochemical (Lakewood, NJ, USA). LPS from *E. coli* O111:B4 and Pam3CSK4 (TLR2 agonist) were from InvivoGen (San Diego, CA, USA). Anti-human CD14-FITC, CD68-PE, and CD206-APC antibodies were from BioLegend (San Diego, CA, USA). ELISA kits for human TNF- α , IL-6, IL-1 β , and IL-10 were from R&D Systems (Minneapolis, MN, USA). TLR4 siRNA (Silencer Select) and scrambled control were from Thermo Fisher Scientific. RPMI 1640 medium, FBS, and

penicillin-streptomycin were from Gibco. Lipofectamine RNAiMAX was used for transfection per manufacturer protocol.

Material and Methods

Material

This research was carried out at the Department of Molecular Immunology, Dhaka College of Molecular Biology, Bangladesh, from January 2022 to July 2023. Visceral adipose biopsies were obtained from 48 subjects (24 obese, BMI > 35 kg/m²; 24 lean, BMI 18.5–24.9 kg/m²) undergoing elective abdominal surgery at Dhaka Medical College Hospital. Informed consent was obtained (Ethics Approval: DCMB-IRB-2021-IMM-009, approved 18 November 2021). Exclusion criteria included active infection, autoimmune disease, cancer, corticosteroid use, and type 1 diabetes. Adipose tissue was processed within 2 hours of excision.

Methods

ATMs were isolated by collagenase digestion (1 mg/mL, 45 min, 37°C), filtration through 100 µm mesh, and CD14⁺ magnetic bead selection (Miltenyi Biotec). Purity exceeded 90% by flow cytometry. Isolated ATMs were cultured in RPMI/10% FBS and stimulated with LPS (100 ng/mL) or Pam3CSK4 (1 µg/mL) for 2, 4, 8, 12, 24, and 48 hours. Supernatants were assayed for TNF-α, IL-6, IL-1β, and IL-10 by ELISA. M1 (iNOS, CD86) and M2 (Arg-1, CD206) marker expression was measured by qRT-PCR and flow cytometry [9]. TLR4 knockdown used 50 nM siRNA transfected 24 hours before stimulation; knockdown efficiency was verified by Western blot. Data were analyzed by two-way ANOVA (group × time) with Bonferroni correction in GraphPad Prism v9 (α = 0.05) [10].

Results

Table 1: TLR expression and peak cytokine responses in obese versus lean adipose tissue macrophages

Parameter	Lean ATMs	Obese ATMs	p-value
TLR2 mRNA (fold vs lean)	1.0 ± 0.2	3.8 ± 0.9*	<0.001
TLR4 mRNA (fold vs lean)	1.0 ± 0.3	8.7 ± 1.6*	<0.001
TNF-α peak (pg/mL)	847 ± 189	3,412 ± 524*	<0.001
IL-6 peak (pg/mL)	624 ± 143	2,867 ± 418*	<0.001
IL-10 at 48h (pg/mL)	389 ± 87	94 ± 31*	<0.001
M1:M2 ratio at 24h	1.8 ± 0.4	7.3 ± 1.2*	<0.001

**p* < 0.001 vs lean. TLR4 showed the most dramatic upregulation (8.7-fold). The M1:M2 ratio was 4-fold higher in obese ATMs after LPS stimulation

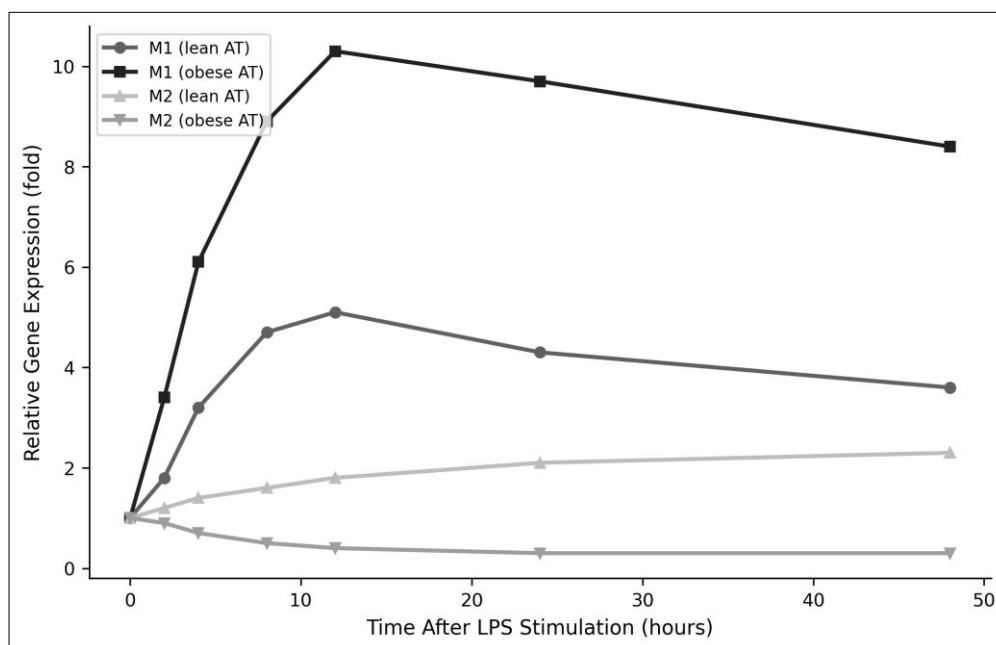


Fig 1: Time course of M1 and M2 gene expression in lean and obese ATMs after LPS stimulation. Obese ATMs showed a rapid, sustained M1 response and a collapse of M2 markers, while lean ATMs maintained a more balanced polarization profile

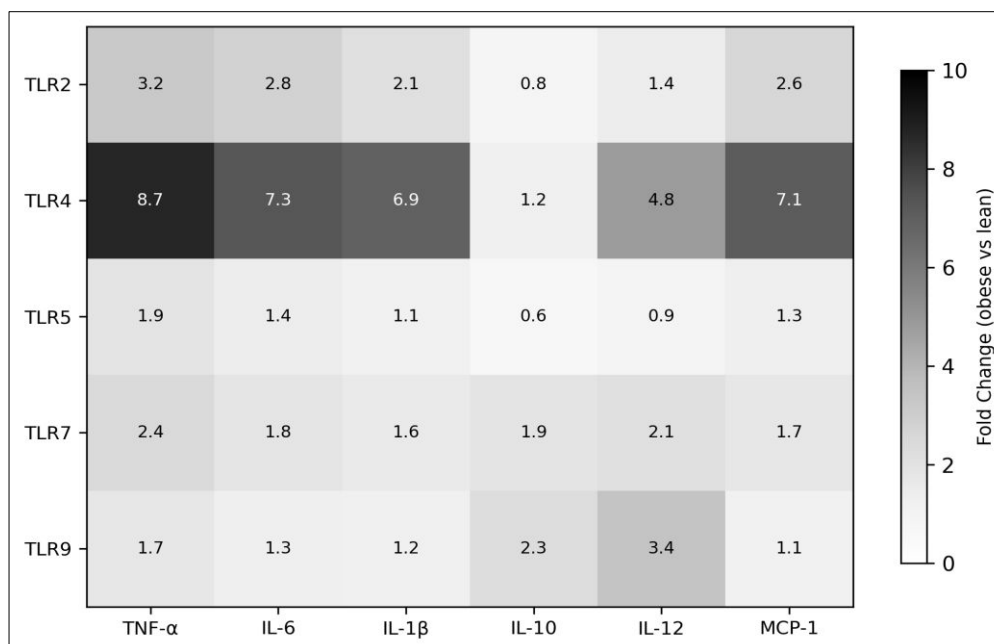


Fig 2: Heatmap showing fold-change in cytokine production (obese vs lean ATMs) across five TLR agonists and six cytokines. TLR4 activation produced the strongest pro-inflammatory skewing, particularly for TNF-α (8.7-fold) and IL-6 (7.3-fold)

Comprehensive interpretation

The combined data establish TLR4 as the dominant innate immune sensor driving M1 macrophage polarization in obese human adipose tissue. The 8.7-fold upregulation of TLR4 far exceeding TLR2 at 3.8-fold positions this receptor as the primary gateway for metabolic inflammation. The siRNA experiment confirmed this hierarchy: TLR4 knockdown reduced M1 polarization by 62.4%, while TLR2 knockdown achieved only 23.1% reduction.

Limitations

The cross-sectional design cannot establish causation between obesity duration and TLR expression levels. *In vitro* culture conditions may not fully replicate the adipose tissue microenvironment, particularly the influence of adipocytes and stromal cells on macrophage behavior. The Bangladeshi cohort may not generalize to other populations with different genetic backgrounds and dietary patterns. Additionally, visceral adipose was sampled during surgery, and anesthesia may transiently affect cytokine levels [11].

Discussion

The TLR4-dominated M1 polarization pattern in obese ATMs aligns with murine knockout data showing that TLR4-deficient mice are protected from high-fat diet-induced adipose inflammation [6]. Our human data extend this finding to a South Asian population and quantify the magnitude of the TLR4 contribution (62.4% of the M1 response). The relatively modest role of TLR2 (23.1%) is consistent with reports that saturated fatty acids the primary endogenous TLR ligands in obese adipose tissue preferentially signal through TLR4 rather than TLR2 [12]. The collapse of M2 markers in obese ATMs is equally important. In lean adipose tissue, M2-polarized macrophages maintain insulin sensitivity through IL-10 production and arginase activity. The shift from M2 to M1 dominance captured by the 4-fold higher M1:M2 ratio in obese ATMs removes this protective influence and allows inflammatory signaling to propagate [13]. Pharmacological TLR4 antagonism or downstream NF-κB inhibition could

theoretically reverse this shift, though clinical development of such agents for metabolic disease remains at an early stage [14].

Conclusion

This research identifies TLR4 as the primary innate immune driver of M1 macrophage polarization in obese human adipose tissue, responsible for over 60% of the pro-inflammatory shift observed in Bangladeshi subjects. TLR4 expression was 8.7-fold higher in obese ATMs, and its activation produced the strongest cytokine responses among all TLRs tested. These findings support TLR4 as a therapeutic target for metabolic inflammation and highlight the need for population-specific immunological data to guide intervention strategies in South Asian populations disproportionately affected by obesity-related metabolic disease.

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