Hypothesis

Hydroxychloroquine as Adjuvant Therapy with Immunotherapy for Prevention of Activation of Latent Tuberculosis: Restoring Interferon-Gamma Competence in Patients Receiving TNF-Alpha Inhibitors

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Hydroxychloroquine as Adjuvant Therapy with Immunotherapy for Prevention of Activation of Latent Tuberculosis: Restoring Interferon-Gamma Competence in Patients Receiving TNF-Alpha Inhibitors

Running Title: HCQ Prophylaxis for TB Prevention in Anti-TNF Therapy

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Abstract

Background: Tumor necrosis factor-alpha (TNF-α) inhibitors have transformed the management of chronic inflammatory diseases, yet they impose a substantially elevated risk of tuberculosis (TB) reactivation—up to 25-fold depending on the agent used. This risk is principally driven by disruption of granuloma architecture and suppression of interferon-gamma (IFN-γ)–dependent macrophage bactericidal activity. Current prophylactic strategies rely predominantly on isoniazid chemoprophylaxis, which carries hepatotoxicity risks and compliance challenges. There remains an unmet need for adjunctive immunomodulatory approaches that can selectively bolster anti-mycobacterial immunity without exacerbating the underlying autoimmune condition. **Hypothesis:** We propose that low-dose hydroxychloroquine (HCQ), administered concomitantly with TNF-α inhibitor therapy, can serve as a targeted immunomodulatory prophylactic strategy against TB reactivation. This hypothesis is grounded in recent single-cell transcriptomic evidence demonstrating that HCQ selectively upregulates IFNG expression and cytotoxicity-associated gene programs (GZMA, GZMB, PRF1, NKG7) in effector CD8+ T cells while simultaneously reducing the dysfunctional CD38+ CD8+ T cell subset. We posit that this CD8+ T cell–mediated IFN-γ augmentation can partially compensate for the TNF-α inhibitor–induced deficit in the IL-12/IFN-γ axis, thereby preserving granuloma integrity and macrophage mycobactericidal function. **Evidence Synthesis:** We integrate mechanistic data from immunology, pharmacology, and TB pathogenesis to construct a multi-layered rationale. We examine the dose-dependent immunomodulatory effects of HCQ, the differential impact of TNF-α inhibitors on mycobacterial immunity, and the critical role of IFN-γ in phagosome maturation and granuloma maintenance. **Conclusion:** This hypothesis establishes a mechanistically grounded framework for repurposing HCQ as adjunctive TB prophylaxis in the anti-TNF setting. We propose a phased translational research program to evaluate this novel immunomodulatory strategy.

Keywords: hydroxychloroquine; interferon-gamma; TNF-alpha inhibitors; tuberculosis prophylaxis; immunomodulation; latent tuberculosis; CD8+ T cells; granuloma; macrophage activation; single-cell RNA sequencing

1. Introduction

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The advent of tumor necrosis factor-alpha (TNF-α) inhibitors—including infliximab, adalimumab, etanercept, certolizumab pegol, and golimumab—has fundamentally transformed the therapeutic landscape for rheumatoid arthritis (RA), inflammatory bowel disease (IBD), psoriasis, and ankylosing spondylitis. These biologics effectively neutralize the pathological excess of TNF-α that drives chronic tissue inflammation, synovial destruction, and disease progression [1,2]. However, TNF-α is simultaneously a critical effector cytokine in antimycobacterial host defense, serving essential roles in granuloma formation and maintenance, macrophage activation, and coordination with the interferon-gamma (IFN-γ) axis for intracellular pathogen killing [3–5].

The clinical consequence of TNF-α neutralization is a markedly increased risk of tuberculosis (TB), with post-marketing surveillance data revealing relative risk increases of up to 18.6-fold for infliximab and 29.3-fold for adalimumab compared to the general population [6,7]. In approximately 70% of these cases, TB presents as reactivation of latent infection (LTBI), frequently with extrapulmonary or disseminated manifestations occurring within the first three months of biologic therapy [8]. This accelerated timeline and atypical presentation underscore the profound immunological disruption imposed by TNF-α blockade on the delicate equilibrium between mycobacterial containment and immune surveillance.

Current guidelines mandate screening for LTBI using interferon-gamma release assays (IGRAs) or tuberculin skin testing (TST) prior to initiating anti-TNF therapy, followed by chemoprophylaxis with isoniazid (typically 9 months) or rifampin (4 months) for those who test positive [9,10]. While this approach has significantly reduced TB incidence among biologic users, it is limited by the imperfect sensitivity of IGRAs in immunosuppressed populations, the hepatotoxicity risk and compliance burden of prolonged chemoprophylaxis, and the emergence of isoniazid-resistant Mycobacterium tuberculosis strains in endemic regions [11,12]. These limitations highlight the pressing need for complementary immunomodulatory strategies that can bolster the host's intrinsic anti-TB defense during anti-TNF treatment.

Hydroxychloroquine (HCQ), a 4-aminoquinoline derivative with over six decades of clinical use, has traditionally been understood as an immunosuppressive agent through inhibition of endosomal Toll-like receptor signaling, blockade of autophagy at the lysosomal level, and suppression of pro-inflammatory cytokine production [13–15]. However, recent advances in single-cell transcriptomics have revealed a remarkably nuanced cell-type–specific immunomodulatory profile. In a landmark single-cell RNA-sequencing (scRNA-seq) study, Long and colleagues (2024) demonstrated that HCQ treatment induces a significant expansion of effector CD8+ T cells with concomitant upregulation of IFNG expression and key cytotoxicity-related genes (GZMA, GZMB, GZMH, KLRD1, NKG7, PRF1), while simultaneously reducing the dysfunctional CD38+ CD8+ T cell subset and suppressing effector CD4+ T cell responses [16].

This paradoxical finding—an immunomodulatory drug that simultaneously restrains autoimmune CD4+ pathology while empowering CD8+ T cell–mediated IFN-γ production—constitutes the immunological foundation of the present hypothesis. We propose that low-dose HCQ, administered as adjunctive prophylaxis in patients receiving TNF-α inhibitors, can selectively augment the CD8+ T cell–derived IFN-γ response, thereby partially restoring the IL-12/IFN-γ signaling axis disrupted by both chronic inflammation and TNF-α blockade.

2. The Centrality of the Ifn-Γ Axis in Anti-Tubercular Immunity

2.1. IFN-γ as the Master Regulator of Macrophage Bactericidal Activity

IFN-γ occupies an unrivaled position in the hierarchy of anti-mycobacterial cytokines. It serves as the principal activating signal for macrophage effector functions against intracellular Mycobacterium tuberculosis (Mtb), operating through the JAK-STAT1 signaling cascade to induce inducible nitric oxide synthase (iNOS) expression for reactive nitrogen intermediate generation, NADPH oxidase assembly for reactive oxygen species production, phagosome maturation through recruitment of Rab7 and vacuolar H+-ATPase, and upregulation of major histocompatibility complex

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class II molecules for enhanced antigen presentation [17–19]. The indispensability of IFN-γ is unequivocally demonstrated by Mendelian susceptibility to mycobacterial disease (MSMD), wherein patients with inherited defects in the IFN-γ/IL-12 signaling pathway suffer severe, often fatal mycobacterial infections [20,21].

2.2. The TNF-α/IFN-γ Synergy in Granuloma Biology

Granuloma formation represents the hallmark of the successful host response to Mtb. TNF-α and IFN-γ act synergistically: TNF-α drives the recruitment of monocytes and lymphocytes through chemokine induction (CXCL10, CCL2, CCL5), while IFN-γ activates the recruited macrophages to kill intracellular bacilli. Critically, IFN-γ–induced phagosome maturation is dependent on autocrine TNF secretion by macrophages. Harris and colleagues (2008) demonstrated that anti-TNF monoclonal antibodies (infliximab, adalimumab) abrogate this process in primary human macrophages [22]. Furthermore, both cytokines regulate excessive inflammation through induction of T cell apoptosis, thereby preventing immunopathological tissue destruction [23]. When TNF-α is therapeutically neutralized, this coordinated defense architecture collapses—granuloma integrity is lost and IFN-γ–dependent bactericidal mechanisms are severely compromised [24,25].

2.3. The IL-12Rβ2 Chain Defect in Chronic Inflammation

A critical dimension of TB susceptibility in patients requiring TNF-α inhibitors is the pre-existing systemic Th1 immune defect induced by chronic inflammation. Miossec and colleagues have demonstrated that rheumatoid arthritis induces specific downregulation of the IL-12 receptor beta-2 (IL-12Rβ2) chain, mediated in part through IL-17–dependent inhibition [26]. This chain is essential for IL-12 signal transduction and subsequent IFN-γ production by Th1 cells. The consequence is a systemic Th1 defect predisposing patients to TB even before biologic therapy initiation. When TNF-α inhibitors are introduced, this transiently deepens the IFN-γ deficit by disrupting cellular interactions within pre-existing TB granulomata. The Th1 recovery occurs gradually as disease is controlled, but the intervening period of maximal IFN-γ deficiency represents the window of greatest TB vulnerability [26,27].

3. Hydroxychloroquine: A Reappraisal of Immunomodulatory Mechanisms

3.1. The Paradigm Shift: From Blanket Immunosuppression to Cell-Type–Specific Modulation

The traditional characterization of HCQ as a purely immunosuppressive agent has been progressively refined by evidence revealing context-dependent and cell-type–specific effects. The conventional mechanistic framework emphasizes lysosomal alkalinization leading to impaired antigen processing, inhibition of endosomal TLR (3, 7, 8, 9) signaling through interference with nucleic acid recognition, and blockade of the cGAS-STING pathway with consequent reduction in type I interferon production [13,28,29].

However, the single-cell revolution in immunology has unveiled a fundamentally more complex picture. Long et al. (2024), employing scRNA-seq analysis, discovered that HCQ produces a dichotomous effect on the adaptive immune compartment: while effector CD4+ T cells are reduced in frequency and restrained through upregulation of inhibitory genes (CTLA4, TNFAIP3), effector CD8+ T cells undergo significant expansion with robust upregulation of IFNG and cytotoxicity genes (GZMA, GZMB, GZMH, KLRD1, NKG7, PRF1) [16]. Equally significant is the observed reduction in the CD38+ CD8+ T cell subset, a dysfunctional population characterized by impaired cytotoxicity and increased susceptibility to infections [16,30].

3.2. Dose-Dependent Immunomodulation: The Concentration–Effect Paradigm

HCQ exhibits strikingly different effects at different concentrations [31]. At high concentrations (≥100 μM), HCQ potently inhibits viral replication by raising endosomal pH but broadly suppresses

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immune cell activation. At lower, clinically relevant concentrations (≤20 μM), effects shift toward selective modulation of innate immune pathways without global immunosuppression [31,32]. Standard low-dose HCQ regimens (200–400 mg/day), achieving steady-state plasma concentrations of 50–200 ng/mL (~0.15–0.6 μM), operate within the immunomodulatory rather than immunosuppressive window [33]. This pharmacological window may explain the paradoxical enhancement of CD8+ T cell effector function: at low concentrations, HCQ may preferentially target hyperactivated autoreactive CD4+ T cells while the CD8+ compartment responds with compensatory upregulation of cytotoxic and IFN-γ gene programs.

The mechanism may involve HCQ-induced mitochondrial oxidative stress in CD4+ T cells that selectively impairs their proliferation [34], while CD8+ T cells, with their distinct metabolic programming and reliance on oxidative phosphorylation for effector function, respond to the same stimulus with enhanced cytotoxic programming. This differential metabolic vulnerability represents a novel pharmacological mechanism for immune subset-selective modulation.

3.3. Macrophage Polarization: M1 Skewing and Enhanced Efferocytosis

Beyond its effects on T cells, HCQ exerts modulatory effects on macrophage biology directly relevant to anti-TB immunity. Chen and colleagues demonstrated that rapamycin–HCQ combination enhances M1 macrophage polarization through upregulation of phospho-STAT1 signaling while suppressing M2-associated gene programs [35]. M1-polarized macrophages are the principal effectors of intracellular Mtb killing. Additionally, HCQ enhances macrophage efferocytosis through upregulation of the MerTK/Gas6 signaling axis [36]. In TB granulomata, efficient efferocytosis is critical for preventing necrotic core expansion and maintaining structural integrity.

3.4. Trained Immunity Modulation: Epigenetic Implications

Gonçalves et al. (2020) demonstrated that HCQ can prevent the epigenetic reprogramming underlying trained immunity by suppressing histone H3K27 acetylation and H3K4 trimethylation at inflammation-related gene loci, likely through effects on lysosomal function and mTOR signaling [37]. By modulating—rather than abolishing—trained innate immune responses, HCQ may help rebalance the immune response away from autoreactive pathology while preserving sufficient innate antimicrobial responsiveness.

4. The Integrative Hypothesis

4.1. Central Thesis

We hypothesize that low-dose hydroxychloroquine (200–400 mg/day) as adjunctive prophylaxis in TNF-α inhibitor recipients can reduce TB risk through a multi-pronged mechanism centered on IFN-γ axis preservation, operating through four mechanistic pillars:

Pillar 1 – CD8+ T Cell–Derived IFN-γ Augmentation. HCQ selectively expands effector CD8+ T cells and upregulates IFNG [16]. In the context of anti-TNF–induced CD4+ Th1 suppression, CD8+ T cells represent a critical alternative IFN-γ source for macrophage activation and phagosome maturation, partially restoring bactericidal capacity within TB granulomata.

Pillar 2 – Elimination of Dysfunctional CD38+ CD8+ T Cells. The CD38+ CD8+ subset, expanded in autoimmune patients, exhibits defective degranulation and impaired cytotoxicity [30]. HCQ reduces this dysfunctional subset [16], improving CD8+ antimicrobial surveillance quality.

Pillar 3 – M1 Macrophage Polarization. HCQ promotes STAT1 phosphorylation and M1-associated gene expression [35], favoring the classically activated phenotype essential for Mtb containment. In synergy with enhanced CD8+ T cell–derived IFN-γ, this sustains pro-inflammatory macrophage function even without TNF-α signaling.

Pillar 4 – Autoimmune Disease Control. By suppressing autoreactive CD4+ responses and modulating trained immunity [16,37], HCQ addresses the chronic inflammatory milieu driving IL-

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12Rβ2 downregulation and systemic Th1 deficiency [26], shortening the window of maximal TB vulnerability.

4.2. The Proposed Immunological Cascade

Phase 1 (Weeks 0–4: Initiation). Upon anti-TNF initiation, granuloma interactions are disrupted and Th1 IFN-γ is acutely suppressed. Concomitant HCQ begins modulating T cell populations: CD4+ effectors are restrained while CD8+ expansion and IFNG upregulation are initiated. HCQ simultaneously promotes M1 macrophage polarization through STAT1 activation.

Phase 2 (Weeks 4–12: Compensatory Adaptation). HCQ reaches steady-state concentrations (~50-day half-life). CD8+ T cell–derived IFN-γ reaches maximal compensatory capacity. Reduction in CD38+ CD8+ T cells improves antimicrobial CD8+ surveillance quality. M1-polarized macrophages, receiving IFN-γ from CD8+ effectors, maintain phagosome maturation activity to contain intracellular mycobacteria.

Phase 3 (Months 3–12: Stabilization). Autoimmune disease control leads to subsidence of IL-12Rβ2 suppression. Th1 CD4+ cells gradually recover IL-12 responsiveness. Combined CD4+/CD8+ IFN-γ output progressively normalizes, and TB reactivation risk diminishes.

4.3. Differential Applicability Across TNF-α Inhibitor Classes

The strength of this hypothesis varies across TNF-α inhibitor classes. Infliximab and adalimumab suppress antigen-induced IFN-γ production by 70% and 64%, respectively, and reduce TB-responsive CD4+ cells by 70% and 50% [38]. Etanercept produces no significant effect on these parameters [38]. We therefore predict that adjunctive HCQ benefit would be greatest with infliximab or adalimumab, where the IFN-γ deficit is most profound.

Table 1. *Differential TB Risk and Predicted HCQ Prophylactic Benefit Across TNF-α Inhibitor Classes.*

TNF-α Inhibitor	IFN-γ Suppression	TB Risk (Relative)	Predicted HCQ Benefit
Infliximab	~70% reduction	RR 18.6	Highest benefit
Adalimumab	~64% reduction	RR 29.3	High benefit
Certolizumab pegol	Moderate (estimated)	Lower than mAbs	Moderate benefit
Etanercept	No significant effect	Lowest among TNFi	Minimal benefit

mAbs, monoclonal antibodies; RR, relative risk; TNFi, TNF-α inhibitors. Relative risk data from Gomez-Reino et al. [7] and Solovic et al. [8]. IFN-γ suppression data from Saliu et al. [38].

5. Converging Lines of Supporting Evidence

5.1. Independent Corroboration of CD8+ IFN-γ Enhancement

The observation by Long et al. (2024) does not stand in isolation. Accapezzato and colleagues previously demonstrated that chloroquine treatment of human CD8+ T cells induced expansion and enhanced IFN-γ production in response to antigen stimulation [39]. This earlier finding provides independent corroboration of the CD8+-selective IFN-γ–enhancing effect. Moreover, in the oncology setting, Chen et al. showed that rapamycin–HCQ combination upregulated M1-related genes including STAT1 in macrophages while suppressing M2 markers [35], supporting the hypothesis that HCQ can redirect immune responses toward a pro-inflammatory, antimicrobial phenotype.

5.2. The IGRA Paradox: HCQ Preserves Mycobacterium-Specific IFN-γ Responses

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Critical clinical evidence comes from evaluation of IGRAs in HCQ-treated autoimmune disease patients. Gaffney et al. (2018) found that HCQ use did not significantly impair the Mtb antigen–specific IFN-γ response [40]. This demonstrates that despite HCQ's broad immunomodulatory effects, the pathogen-specific IFN-γ response required for TB containment remains intact during HCQ therapy—entirely consistent with the selective CD8+ T cell IFN-γ augmentation mechanism proposed herein.

5.3. HCQ Safety in Combination with Biologic DMARDs

HCQ has an extensive safety track record with biologic DMARDs, including TNF-α inhibitors. Triple therapy (methotrexate, sulfasalazine, HCQ) is well-established, and HCQ is frequently continued when biologics are added [41]. HCQ provides ancillary cardiovascular, antithrombotic, and metabolic benefits relevant to this comorbid patient population [42,43]. The drug's safety profile—with retinal toxicity mitigated by ophthalmological screening [44]—supports feasibility as a long-term adjunctive agent.

5.4. Epidemiological Signal

Observational data from large HCQ-treated cohorts suggest reduced overall serious infection risk [45]. While not specifically addressing TB outcomes, this pattern is consistent with the hypothesis that HCQ preserves anti-infectious immunity.

6. Addressing Potential Counterarguments

6.1. HCQ Inhibits PHA-Induced IFN-γ in PBMCs

HCQ inhibits phytohemagglutinin (PHA)–induced IFN-γ in whole PBMC cultures [46], seemingly contradicting this hypothesis. However, PHA predominantly activates CD4+ T cells, and bulk PBMC IFN-γ is predominantly CD4+-derived. The scRNA-seq data clearly show HCQ suppresses effector CD4+ T cells while enhancing IFN-γ specifically in CD8+ T cells [16]. The PBMC assay obscures the cell-type–specific CD8+ enhancement, underscoring the superior resolution of single-cell approaches over bulk assays.

6.2. HCQ Autophagy Inhibition and Mycobacterial Containment

HCQ potently inhibits autophagy [47], raising concerns about impaired Mtb clearance. However, Mtb itself actively inhibits autophagy through secreted effectors [48]; IFN-γ–induced phagosome maturation operates through distinct Rab7-dependent pathways not entirely autophagy-dependent [18]; and the CD8+ cytotoxic killing of infected macrophages via the perforin/granzyme pathway—enhanced by HCQ [16]—provides an autophagy-independent mechanism for eliminating the intracellular Mtb niche.

6.3. Dose-Finding Considerations

The optimal HCQ dose for TB prophylactic benefit may not align with standard rheumatological dosing. The concentration-dependent nature of HCQ's effects suggests that carefully titrated low-dose regimens may be required. Pharmacokinetic modeling incorporating whole-blood HCQ concentrations, tissue distribution, and immune cell-specific uptake will be essential for rational dose optimization [33,49].

7. Proposed Translational Research Program

7.1. Phase I: Ex Vivo Mechanistic Studies

Whole-blood cultures from RA patients initiating TNF-α inhibitors should be treated with clinically relevant HCQ concentrations (50–500 ng/mL) with/without infliximab, adalimumab, or

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etanercept. Primary endpoints: Mtb antigen–specific IFN-γ by flow cytometry distinguishing CD4+ vs. CD8+ contributions; IGRA quantitative output; CD8+ cytotoxicity against Mtb-infected autologous macrophages. Secondary endpoints: M1/M2 polarization markers; intracellular Mtb CFU enumeration.

7.2. Phase II: Prospective Immunological Cohort Study

A prospective cohort enrolling patients initiating TNF-α inhibitors, stratified by concomitant HCQ use, with serial immunological assessments at baseline, 4, 12, 24, and 52 weeks: longitudinal IGRA responses; CD8+ IFNG expression; CD38+ CD8+ proportions; serum cytokine profiling (IFN-γ, IL-12, IP-10/CXCL10); optional scRNA-seq at key timepoints.

7.3. Phase III: Multicenter Randomized Controlled Trial

A double-blind, placebo-controlled trial in patients initiating anti-TNF monoclonal antibodies, randomized to: standard care + HCQ 200 mg daily, HCQ 400 mg daily, or placebo. Composite primary endpoint: new LTBI acquisition, active TB, or >50% decline in Mtb-specific IFN-γ from baseline. Enriched for high-TB-burden regions, powered for 50% reduction in composite endpoint, requiring ~1,200–1,500 participants over 24 months.

8. Broader Implications and Future Perspectives

This hypothesis contributes to a paradigm shift—from understanding immunomodulatory drugs as blunt instruments to recognizing them as precision tools for cell-type–specific immune reprogramming. The concept of "immunological compensation"—using CD8+ T cell–derived IFN-γ to compensate for the pharmacologically induced deficit in the CD4+ Th1/TNF-α axis—represents an innovative approach to managing infectious complications of immunomodulatory therapy. If validated, this principle could extend to other scenarios where targeted immunosuppression creates pathogen-specific vulnerability.

The proposed role of HCQ in reducing the CD38+ CD8+ T cell subset merits particular attention. CD38 has emerged as a marker of immune exhaustion across autoimmunity, chronic infection, and cancer. HCQ's ability to selectively deplete this dysfunctional population while expanding functional effectors suggests potential "immune rejuvenation" effects with broad therapeutic applications. We acknowledge this hypothesis requires rigorous empirical validation, and the phased research program is designed to systematically address uncertainties in translating in vitro observations to clinical benefit.

9. Conclusions

We present a mechanistically grounded hypothesis for hydroxychloroquine as adjunctive immunomodulatory prophylaxis against tuberculosis in patients receiving TNF-α inhibitor therapy. Central to this hypothesis is the recently discovered ability of HCQ to selectively enhance IFN-γ expression in effector CD8+ T cells, providing a compensatory source of this critical anti-mycobacterial cytokine when the CD4+ Th1/TNF-α axis is pharmacologically suppressed. Supported by converging evidence from single-cell transcriptomics, macrophage polarization studies, clinical IGRA data, and the established safety profile of HCQ with biologic DMARDs, this hypothesis offers a novel and readily translatable approach to an important unmet clinical need. We urge the scientific and clinical community to pursue the proposed translational research program to evaluate this potentially paradigm-shifting strategy.

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