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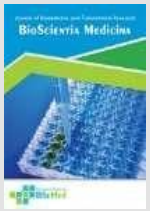

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Single-Dose Intralesional Bacillus Calmette-Guérin (BCG) Immunotherapy Induces Complete and Sustained Remission of Recalcitrant Anogenital Condylomata: A Mechanistic Case Series

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A B S T R A C T

Background: Anogenital condylomata acuminata caused by Human Papillomavirus (HPV) presents a significant therapeutic challenge due to high recurrence rates after conventional cytodestructive therapies. Intralesional immunotherapy aims to induce a host-mediated immune response, offering a promising alternative. This report investigates the efficacy, safety, and immunological rationale of a novel, single-dose Bacillus Calmette-Guérin (BCG) protocol in an immunologically primed population. **Methods:** In this prospective case series, three immunocompetent patients with extensive, therapy-refractory anogenital condylomata were enrolled. Following a standardized protocol, each patient received a single, calculated intralesional injection of BCG vaccine into the largest index lesion. The primary outcome was complete clinical and dermoscopic clearance. Patients were evaluated at regular intervals for efficacy and safety over a 12-month follow-up period. **Result:** All three patients achieved complete clinical and dermoscopic clearance of both the injected and distant, untreated lesions within a rapid timeframe of 6 to 10 weeks. The treatment was well-tolerated, with adverse events limited to anticipated and transient local inflammatory reactions. No recurrences were documented in any patient during the 12-month follow-up period. **Conclusion:** Single-dose intralesional BCG immunotherapy appears to be a highly effective, durable, and safe therapeutic strategy for recalcitrant anogenital condylomata. The observed pan-lesional clearance strongly suggests the induction of a systemic, cell-mediated anti-HPV immune response. These compelling preliminary findings provide a strong rationale for validation through larger, randomized controlled trials.

1. Introduction

Anogenital warts, clinically designated as condyloma acuminata, represent one of the most prevalent sexually transmitted infections (STIs) globally, imposing a substantial burden on public health systems and the psychosocial well-being of affected individuals.[1] The global incidence is estimated in the tens of millions of new cases annually, and the associated direct medical costs for treatment are considerable.[2] Beyond the physical symptoms, the psychological sequelae—encompassing anxiety, depression, feelings of shame, and significant psychosexual dysfunction—can be profound and debilitating. The etiological agents are human papillomaviruses (HPV), with low-risk, non-oncogenic types 6 and 11 accounting for over 90% of clinical presentations.[3] Nevertheless, the potential for co-infection with high-risk, oncogenic HPV types such as

HPV-16, 18, 31, and 33 is a significant concern, as it confers a risk for the development of anogenital dysplasia and carcinoma, particularly in the context of persistent or extensive disease.

The current therapeutic landscape for anogenital warts is extensive but is fundamentally constrained by high rates of post-treatment recurrence, which can range from 20% to over 60% within six months of completing therapy.[4] The therapeutic armamentarium is broadly bifurcated into two categories: patient-applied topical agents (imiquimod 5% cream, podophyllotoxin 0.5% solution) and provider-administered, predominantly destructive procedures. The latter category, which includes cryotherapy with liquid nitrogen, application of trichloroacetic acid, surgical excision, and laser (CO_2) ablation, remains the cornerstone of management for patients with a large burden of disease or lesions refractory to topical treatments.[5]

The fundamental principle underlying these destructive modalities is the physical eradication of keratinocytes that are visibly infected with HPV.[6] However, their primary and critical limitation is an inherent inability to address the latent or subclinical viral reservoir present in the clinically and dermoscopically normal-appearing epithelium surrounding the visible lesions. This untreated viral sanctuary is now understood to be the principal driver of post-treatment recurrence, leading to frustrating and demoralizing cycles of therapy, patient disillusionment, and escalating healthcare expenditures. Furthermore, these destructive methods are frequently associated with significant procedural and post-procedural morbidity. This includes acute pain, ulceration, delayed wound healing, and the potential for permanent sequelae such as scarring, dyschromia (hypo- or hyperpigmentation), and anatomical distortion, which can have devastating functional and cosmetic consequences in the sensitive anogenital region.[7]

These formidable challenges have catalyzed a paradigm shift in dermatologic therapeutics, moving away from simple physical destruction and towards sophisticated immunomodulation. The central tenet of immunotherapy is to re-educate and harness the patient's own immune system to recognize and eliminate HPV-infected cells, thereby targeting the root etiology of the disease rather than merely its superficial manifestation.[8] This immunological approach offers profound theoretical advantages over traditional methods. It has the capacity to target both clinically apparent warts and the subclinical latent viral reservoir simultaneously. Moreover, by inducing specific immunological memory, it holds the potential to provide durable, long-term protection against recurrence. A variety of intralesional antigens, including *Candida albicans* antigen, measles-mumps-rubella (MMR) vaccine, and purified protein derivative (PPD), have been investigated with this goal in mind. These agents have demonstrated variable but promising efficacy, functioning primarily by inducing a localized, non-specific Type IV hypersensitivity reaction that breaks the state of local immune tolerance and facilitates subsequent viral clearance.

Among this class of immunotherapeutic agents, the Bacillus Calmette-Guérin (BCG) vaccine, a live attenuated strain of Mycobacterium bovis, is distinguished as an exceptionally potent immunomodulator. Its formidable capacity to stimulate the immune system is most famously leveraged in the field of oncology, where intravesical BCG instillation remains the undisputed gold standard for adjuvant therapy in non-muscle-invasive bladder cancer, significantly reducing recurrence and progression.[9] The mechanism of action is predicated on its ability to robustly engage both innate and adaptive arms of the immune system. Mycobacterial cell wall components, rich in pathogen-associated molecular patterns (PAMPs) such as lipoteichoic acid and peptidoglycan, are recognized by pattern recognition receptors (PRRs)—most critically Toll-like receptors 2 and 4 (TLR2, TLR4)—on the surface of antigen-presenting cells (APCs) like dendritic cells and macrophages. This molecular engagement triggers an intracellular signaling cascade, culminating in the activation of transcription factors like NF-κB and the

subsequent secretion of a powerful suite of pro-inflammatory cytokines, most critically Interleukin-12 (IL-12), IL-1β, and Tumor Necrosis Factor-alpha (TNF-α). IL-12 is the master cytokine that orchestrates the differentiation of naive T-helper (Th) cells towards the Th1 phenotype. This Th1 polarization is essential for effective antiviral and intracellular bacterial immunity, as Th1 cells are the primary source of Interferon-gamma (IFN-γ), which in turn activates cytotoxic T-lymphocytes (CTLs) and Natural Killer (NK) cells—the primary effector cells that recognize and destroy virus-infected host cells. This process directly counteracts the immunosuppressive, Th2-skewed local microenvironment that HPV cunningly establishes to evade immune surveillance and ensure its persistence.[10]

Despite this compelling immunological rationale, the clinical evidence for intralesional BCG in the treatment of anogenital warts has been limited, consisting mainly of small case series or reports that typically involve multiple, often painful, injection sessions administered over several weeks. The novelty of our study lies in the investigation of a single-dose treatment protocol. We hypothesized that in a population from a tuberculosis-endemic region like Indonesia, where mandatory neonatal BCG vaccination leads to widespread and long-lasting immunological priming, a single intralesional challenge with BCG could elicit a rapid and powerful anamnestic (memory) immune response. We posited that this amplified secondary response would be sufficient to achieve complete and durable viral clearance without the need for repeated administrations. Therefore, the primary objective of this study was to prospectively evaluate the efficacy, safety, and long-term outcomes of a single-session intralesional BCG immunotherapy protocol for recalcitrant anogenital condylomata.

2. Methods

This work was conducted as a prospective, single-center case series at the dermatology and venereology outpatient clinic of Dr. Moewardi Regional General Hospital, a tertiary referral center in Surakarta, Indonesia. The study protocol received full approval from the hospital's Institutional Review Board and Health Research Ethics Committee. The study was performed in strict accordance with the ethical principles for medical research involving human subjects as outlined in the Declaration of Helsinki. Comprehensive written informed consent, which included detailed information about the investigational nature of the treatment, potential risks and benefits, and consent for clinical photography and anonymous data publication, was obtained from all participants prior to their enrollment.

We enrolled the first three consecutive patients who presented to our clinic between January 2024 and March 2024 who met all of the following inclusion criteria: (1) Age 18 years or older; (2) A definitive diagnosis of anogenital condyloma acuminata, confirmed both clinically by visual inspection and dermoscopically; (3) A documented history of treatment failure to at least two different first-line therapeutic modalities (cryotherapy, podophyllin, imiquimod), thereby qualifying as recalcitrant disease; (4) Immunocompetent status, verified by a negative serological test for Human Immunodeficiency Virus (HIV) and no personal or medical history of primary or secondary immunodeficiency, organ transplantation, or current use of systemic immunosuppressive medications (corticosteroids, chemotherapy). Exclusion criteria included pregnancy or lactation, a personal history of active or latent tuberculosis infection, known hypersensitivity or prior adverse reaction to the BCG vaccine, and unwillingness or inability to comply with the follow-up schedule.

All enrolled patients underwent a comprehensive baseline evaluation. This included a detailed medical history, a full-body dermatological examination to document the extent of the disease, and serological screening for syphilis (Venereal Disease Research Laboratory [VDRL] test with Treponema Pallidum Hemagglutination [TPHA] confirmation) and HIV (third-generation ELISA). To confirm prior immunological sensitization to mycobacterial

antigens—a key component of our study's hypothesis—a baseline Tuberculin Skin Test (TST) was performed on all patients. This involved the intradermal injection of 5 Tuberculin Units (TU) of purified protein derivative into the volar forearm. The transverse diameter of palpable induration was measured by a trained clinician at 48-72 hours post-injection. For select cases, a shave biopsy of a representative lesion was performed for histopathological confirmation and/or HPV genotyping was conducted on a cervical or lesional swab using a polymerase chain reaction (PCR)-based assay.

The commercially available lyophilized BCG vaccine (Bio Farma, Indonesia, Pasteur strain 1173 P2) was used. Each vial was reconstituted immediately prior to use with 1 mL of sterile 0.9% sodium chloride solution, as per the manufacturer's instructions. Based on the manufacturer's product specifications, this reconstitution yields a suspension containing a viable bacterial count between 2-8 x 10^5 colony-forming units (CFU) per 0.1 mL dose. The total volume of the BCG suspension to be injected was meticulously calculated for each patient based on the aggregate surface area of the warts. The dosage was standardized at 0.1 mL for every 2 cm^2 of estimated lesion surface area. This ratio was empirically derived based on a review of intralesional immunotherapy literature for other cutaneous conditions and our preliminary clinical experience, aiming to provide a sufficient antigenic load to trigger a robust immune response while minimizing the risk of excessive local reactions. For solitary or well-defined plaque-like lesions, the surface area was estimated using the formula for the area of an ellipse: Area = $\pi \times (d_1/2) \times (d_2/2)$, where d_1 and d_2 represent the maximal and minimal diameters of the lesion, respectively. For cases with multiple, confluent, or irregularly shaped papules, the total affected area was estimated by overlaying a transparent sterile grid (1 cm x 1 cm squares) and counting the number of squares occupied by the lesions.

The procedure was performed under aseptic conditions without the use of local anesthesia. Using a 30-gauge needle attached to a 1 mL tuberculin syringe, the calculated volume of the BCG vaccine suspension was injected intradermally into the base of the largest accessible wart (the index lesion). To ensure even distribution of the antigen within the lesion, the total volume was administered via multiple small depots (0.01-0.02 mL per puncture) using a fanning technique throughout the base of the index lesion. In all cases, only the largest lesion or confluent plaque was injected; smaller satellite or distant lesions were intentionally left untreated to serve as internal controls for assessing a systemic immune response.

The primary efficacy outcome was defined as complete clinical and dermoscopic clearance of all anogenital warts (both injected and distant). This was defined as the total absence of any visible or dermoscopically identifiable papillomatous structures or characteristic vascular patterns (glomerular or hairpin vessels), as confirmed independently by two board-certified dermatologists.

Patients were followed up in person every two weeks for the first three months, then at 6 and 12 months post-injection. At each follow-up visit, the treatment area was examined, and standardized clinical photographs were taken using a high-resolution digital camera with consistent lighting and positioning. Dermoscopy (DermLite DL4, 10x magnification, polarized mode) of the target areas was performed to assess for subclinical residual disease. Safety and tolerability were assessed at each visit. Patients were systematically queried for local adverse events (pain, erythema, edema, ulceration, crusting) and systemic symptoms (fever, malaise, myalgia, arthralgia) using a standardized checklist. Injection sites were examined for signs of scarring, atrophy, or pigmentary changes.

3. Results

A summary of the patient demographics, clinical characteristics, and treatment outcomes is presented in Table 1.

Case 1

A 19-year-old, otherwise healthy female presented with significant psychological distress due to a rapidly enlarging "cauliflower-like" growth on her perineum for one month (Table 1). She reported dyspareunia and considerable anxiety related to the lesion's appearance. Her medical history was notable for having failed two courses of topical podophyllin resin 25% and four sessions of cryotherapy, after which the lesion promptly recurred and enlarged. Baseline TST showed 15 mm of induration. On examination, a large, solitary, exophytic, and highly verrucous plaque measuring approximately 3.1 cm x 2.2 cm (calculated area ≈ 6.0 cm^2) was present on the posterior fourchette of the vaginal introitus. Dermoscopy revealed a classic pattern of multiple, tightly packed glomerular (dotted) vessels and fine, hairpin-like capillaries embedded within whitish, finger-like projections, pathognomonic for viral warts. Application of 5% acetic acid resulted in distinct and rapid acetowhitening. A shave biopsy confirmed the diagnosis of condyloma acuminatum, showing marked acanthosis, papillomatosis, and prominent koilocytes in the upper spinous layers. HPV genotyping of the biopsy specimen was positive for low-risk HPV-6.

Following the standardized protocol, a total volume of 0.3 mL of BCG vaccine (corresponding to the ≈ 6.0 cm^2 area) was injected into the base of the lesion. At the 2-week follow-up, a robust inflammatory response was evident; the lesion appeared erythematous, edematous, and tender. By week 4, a marked reduction in lesion volume of approximately 50% was noted, accompanied by superficial erosion and crusting. The plaque continued to regress steadily, and at the 10-week follow-up, complete clinical and dermoscopic resolution was confirmed, with only faint, resolving post-inflammatory hypopigmentation and no evidence of scarring. She remained disease-free throughout the 12-month follow-up period.

Case 2

A 23-year-old female presented with a two-month history of multiple, pruritic warts around her vaginal opening (Table 1). She had previously been treated with a full 12-week course of topical imiquimod 5% cream and three sessions of cryotherapy, both of which failed to produce any significant improvement. The persistence of the lesions was causing considerable emotional distress and affecting her intimate relationship. Her baseline TST showed 12 mm of induration. Physical examination revealed multiple confluent papules and small plaques with verrucous surfaces distributed around the introitus and extending onto the labia minora. The largest confluent plaque measured approximately 2.5 cm x 2.0 cm (calculated area ≈ 4.0 cm^2), with numerous smaller satellite papules in the vicinity. Dermoscopy showed features consistent with viral warts, and the acetowhitening test was strongly positive across all lesions. A cervical swab for HPV genotyping was positive for low-risk HPV-11.

A single session of immunotherapy was performed. A total of 0.2 mL of BCG vaccine was injected only into the largest confluent plaque; the smaller satellite lesions were deliberately left untreated. At the 2-week follow-up, the injected plaque was intensely inflamed, with prominent erythema, edema, and superficial crusting. Critically, the non-injected satellite lesions, though not directly treated, also appeared erythematous and mildly inflamed. By week 6, the injected plaque had almost completely resolved, and the distant satellite lesions had flattened significantly. At the 8-week follow-up, there was complete clearance of all anogenital warts—both the injected plaque and the untreated satellites—with no residual scarring or pigmentary alteration. No recurrence was noted at her 12-month follow-up visit.

Case 3

A 50-year-old heterosexual male was referred for multiple persistent and pruritic warts in the perianal region that had been refractory to treatment with both topical podophyllotoxin 0.5% and a session of electrocautery two months prior (Table 1). His medical history was significant for late latent syphilis, which had been successfully treated five years prior; his

baseline RPR was non-reactive (titer <1:1). His baseline TST was robust at 18 mm of induration. Examination revealed multiple discrete and coalescing verrucous papules in the perianal region, with the largest lesion measuring approximately 2.1 cm x 1.2 cm (calculated area ≈ 2.0 cm^2). Digital rectal examination confirmed that smaller lesions extended approximately 1 cm into the distal anal canal. Given the recalcitrant nature and the patient's age, a biopsy of the largest lesion was performed to rule out dysplasia. Histopathology confirmed condyloma acuminatum. Unexpectedly, HPV genotyping of the tissue revealed high-risk HPV-16.

The patient was treated with a single 0.1 mL dose of intralesional BCG injected into the largest external perianal lesion. A robust inflammatory response, characterized by erythema and mild edema of all perianal warts, was observed two weeks later. By week 4, a dramatic regression of all warts, including the non-injected perianal lesions and, by patient report, the intra-anal lesions, was apparent. At the 6-week mark, all perianal warts had completely resolved. A follow-up anoscopy at 3 months, performed by a proctologist, confirmed the complete clearance of the intra-anal lesions as well. The patient remained disease-free after 12 months of follow-up.

Table 1. Clinical case summary.

A detailed comparison of three cases with recalcitrant anogenital condylomata treated with single-dose intralesional BCG immunotherapy.

CHARACTERISTIC	CASE 1	CASE 2	CASE 3
Patient Demographics			
Age / Sex	♀ 19 / Female	♀ 23 / Female	♂ 50 / Male
TST Induration	15 mm	12 mm	18 mm
Clinical Baseline			
Location	Posterior fourchette	Introitus, labia minora	Perianal region, anal canal
Duration	1 month	2 months	2 months
Total Area	~6.0 cm^2	~4.0 cm^2 (confluent)	~2.0 cm^2 (largest)
Prior Failed Therapies	Podophyllin resin (2x); Cryotherapy (4x)	Imiquimod 5% cream; Cryotherapy (3x)	Podophyllotoxin 0.5%; Electrocautery (1x)
HPV Genotype	Low-risk HPV-6	Low-risk HPV-11	High-risk HPV-16
Intervention			
BCG Dose (Volume)	0.3 mL	0.2 mL	0.1 mL
Outcomes			
Time to Complete Clearance	10 weeks	8 weeks	6 weeks
Recurrence at 12 Months	✓ No	✓ No	✓ No
Adverse Events	Mild local pain, erythema, edema	Moderate local pain, erythema, edema, superficial crusting	Mild local erythema, edema, pruritus

4. Discussion

This case series provides compelling, albeit preliminary, evidence for the profound efficacy of a simplified, single-dose intralesional BCG protocol in achieving complete and sustained remission of recalcitrant anogenital condylomata. In all three

patients, who had previously failed a multitude of standard first- and second-line therapies, this low-cost intervention resulted in the rapid clearance of not only the injected index lesions but also distant, untreated warts. The absence of any recurrence over a rigorous 12-month follow-up period, coupled with the clearance of a lesion harboring high-risk HPV-16, underscores the potential of this modality. The following discussion will explore the detailed immunopathological mechanisms that we hypothesize underpin these remarkable clinical observations.[11]

The remarkable clinical success of a single intralesional administration of Bacillus Calmette-Guérin (BCG) in eradicating extensive, treatment-refractory anogenital condylomata, as documented in this series, invites a deep and thorough exploration of the underlying immunopathological mechanisms. The observed outcomes—complete, durable, and systemic clearance—are far too profound to be attributed to a simple non-specific inflammatory reaction.[12] Instead, they strongly suggest the orchestration of a sophisticated, multi-stage immunological cascade that effectively overcomes the virus's intricate defenses and re-establishes host immune dominance.

The fundamental success of this intervention is hypothesized to lie in its function as a powerful immunological "adjuvant," capable of shattering the state of local immune tolerance that the Human Papillomavirus (HPV) masterfully establishes to ensure its persistence and propagation.[13]

To fully appreciate the impact of BCG, one must first understand the hostile immunological landscape it is introduced. HPV has co-evolved with its human host for millennia, perfecting a subtle and highly effective strategy of immune evasion.[14] The virus primarily infects the basal keratinocytes of the squamous epithelium, a location that provides a distinct advantage. This site is characterized by a state of relative immune privilege, with limited surveillance by circulating immune cells. Within this sanctuary, HPV executes its life cycle in lockstep with the differentiation of the host keratinocyte, expressing its most immunogenic proteins only in the most superficial layers of the epidermis, far from the reach of dermal antigen-presenting cells (APCs). This strategy minimizes the exposure of viral antigens to the host's immune system, preventing the initiation of a robust adaptive immune response (Figure 1).

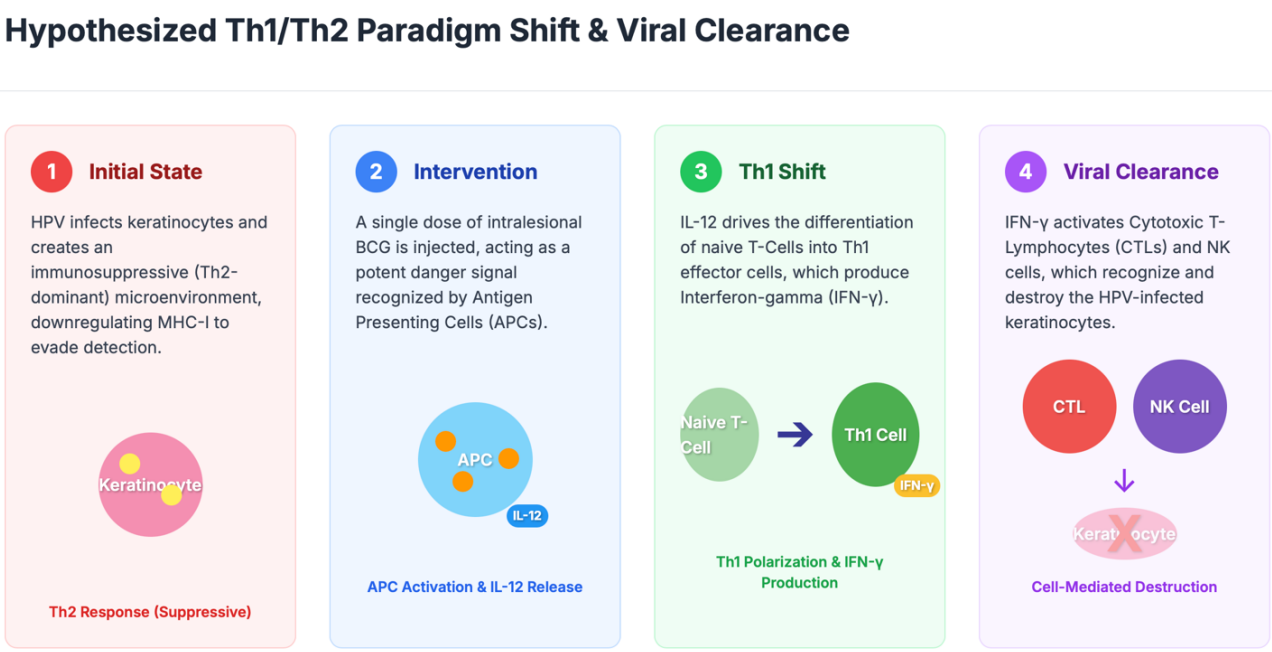

Figure 1. Hypothesized Th1/Th2 paradigm shift and viral clearance.

However, HPV's most sophisticated defense is not passive concealment but active immunomodulation. Its oncoproteins, particularly the E6 and E7 proteins renowned for their role in carcinogenesis, are also master manipulators of the host's cellular machinery for immune recognition. A primary tactic is the active downregulation of Major Histocompatibility Complex (MHC) class I molecule expression on the surface of infected cells. MHC class I molecules are essential platforms that display fragments of intracellular proteins—including viral peptides—to the immune system. Patrolling CD8+ cytotoxic T-lymphocytes (CTLs), the primary soldiers of antiviral cellular immunity, rely on recognizing these viral peptides presented on MHC class I to identify and eliminate infected cells. By actively interfering with the transcription of MHC class I genes and disrupting the antigen processing and presentation pathway, E6 and E7 effectively render the infected keratinocyte "invisible" to these CTLs.[15]

Furthermore, the virus actively cultivates a local microenvironment that is profoundly immunosuppressive. It orchestrates a cytokine milieu skewed away from the potent antiviral Th1 (T-helper 1) response and towards a Th2 (T-helper 2) dominant profile. This Th2 environment, characterized by the presence of cytokines such as Interleukin-4 (IL-4) and Interleukin-10 (IL-10), actively suppresses the development of cell-mediated immunity. IL-10, for instance, is a potent anti-inflammatory cytokine that can inhibit the function of APCs and downregulate the production of key Th1-polarizing signals. In essence, HPV does not merely hide from the immune system; it actively tranquilizes it, creating a state of localized immune tolerance that allows for its unchecked replication and the subsequent formation of clinical lesions.

It is into this carefully controlled, immunosuppressive milieu that the intradermal injection of live, attenuated BCG acts as a profound and disruptive "danger signal." The mycobacterial cell wall is a complex mosaic of potent pathogen-associated molecular patterns (PAMPs), including peptidoglycans, lipoarabinomannan, and mycobacterial DNA. These molecules are immediately recognized by the innate immune system's sentinel cells, primarily the resident Langerhans cells of the epidermis and the dermal dendritic cells (collectively, APCs). This recognition is mediated by a suite of germline-encoded pattern recognition receptors (PRRs), most critically Toll-like receptor 2 (TLR2) and Toll-like receptor 4 (TLR4).[16]

The engagement of these TLRs by mycobacterial PAMPs triggers a rapid and powerful intracellular signaling cascade, predominantly through the MyD88-dependent pathway. This leads to the activation of the master inflammatory transcription factor, Nuclear Factor-kappa B (NF-κB). The activation of NF-κB is a pivotal event, acting as a master switch that turns on the transcription of a vast array of genes encoding pro-inflammatory cytokines. This unleashes a localized cytokine storm, fundamentally altering the immunological narrative from one of tolerance to one of aggressive defense. Among the cytokines released, the most critical for this process are Interleukin-12 (IL-12), Interleukin-1β (IL-1β), and Tumor Necrosis Factor-alpha (TNF-α). We hypothesize that the robust and immediate production of IL-12 is the single most important event that redirects the local immune response from the ineffective, HPV-promoted Th2 pathway to the potent, antiviral Th1 pathway.

IL-12 is the master regulator of Th1 differentiation. It acts on naive CD4+ T-cells that have been activated by APCs presenting mycobacterial (and potentially HPV) antigens, driving them to differentiate into Th1 effector cells.[17] These newly minted Th1 cells become prolific producers of the signature Th1 cytokine, Interferon-gamma (IFN-γ), the cornerstone of effective cell-mediated immunity against intracellular pathogens. The intense local inflammation, erythema, and edema observed clinically in all our patients approximately two weeks after the injection is the macroscopic, clinical manifestation of this powerful cytokine cascade and the influx of activated immune cells to the site.[18]

We propose that IFN-γ then orchestrates the complete clearance of the viral lesions through several potent and synergistic mechanisms. First and foremost, IFN-γ directly counteracts HPV's primary immune evasion strategy. It is a powerful inducer of MHC class I expression on a wide variety of cell types, including keratinocytes. By bathing the local tissue in IFN-γ, the BCG-induced immune response effectively forces the HPV-infected cells to reveal themselves, upregulating their MHC class I machinery and presenting the viral peptides they were previously hiding. This "unmasking" of the infected cells is an absolute prerequisite for their recognition and elimination by the adaptive immune system.

Second, IFN-γ is a potent activator of innate immune cells, particularly Natural Killer (NK) cells. NK cells are capable of recognizing and killing cells that have low levels of MHC class I expression—a state of "missing self"—making them an important first line of defense against viruses like HPV.[19] Finally, and most importantly, the combination of the Th1 environment and IFN-γ promotes the differentiation, clonal expansion, and cytotoxic function of HPV-specific CD8+ CTLs. These CTLs, now able to "see" the unmasked, HPV-peptide-presenting keratinocytes, are the ultimate effector cells. They bind to the infected cells and eliminate them with surgical precision by releasing cytotoxic granules containing perforin, which punches holes in the target cell membrane, and granzymes, which enter the cell and trigger a cascade of programmed cell death, or apoptosis. This orchestrated switch from a permissive Th2 response to a lethal Th1 response is the central mechanism we hypothesize is responsible for the complete regression of the warts.

A critical and particularly telling finding of this case series is the complete clearance of distant, non-injected warts, a phenomenon observed definitively in Cases 2 and 3. This is not merely an interesting side effect; it is the clinical proof of a systemic, HPV-specific T-cell response. It is highly plausible that the HPV-specific T-cells activated and expanded within the intense inflammatory milieu of the BCG-injected lesion do not remain localized. They enter the circulation via draining lymphatic vessels and traffic throughout the body. These circulating effector T-cells are now programmed to recognize HPV-infected cells wherever they exist. They patrol other anatomical sites, and upon encountering other warts, they execute their cytotoxic functions, leading to pan-lesional regression. This "bystander effect" is a hallmark of successful immunotherapy and represents its most fundamental advantage over all lesion-directed destructive modalities, which can only treat what is visible and can never address the entire subclinical viral field that is the source of recurrence.

The remarkably rapid and complete response to a single injection in our patients provides further insight, pointing towards the powerful immunological concept of an anamnestic, or memory, response. As residents of a tuberculosis-endemic country with a mandatory neonatal BCG vaccination policy, all three patients had strongly positive Tuberculin Skin Tests, indicating a pre-existing, robust pool of mycobacteria-specific memory T-cells established decades prior. Immunological memory is a cornerstone of adaptive immunity. Memory T-cells persist for long periods, have a lower threshold for activation, and respond much more quickly and vigorously upon re-exposure to their cognate antigen compared to naive T-cells.[20]

Upon re-exposure to the BCG antigens via the intralesional injection, this pre-existing cohort of memory T-cells likely underwent rapid and massive clonal expansion. This would lead to an accelerated and greatly amplified Th1 cytokine storm, producing far more IFN-γ far more quickly than would be expected in a BCG-naive individual. This pre-existing sensitization is the most compelling explanation for why a single dose was sufficient to overwhelm HPV's immunosuppressive strategies. This observation compares favorably with multi-dose BCG regimens reported in other populations, which often require four to six sessions to achieve clearance. While our 100% clearance rate in a small series is not statistically comparable to larger studies, the profound efficiency of this single-dose approach in a sensitized population

is a key point of novelty and suggests a powerful avenue for future therapeutic protocol optimization.

Finally, while extreme caution is paramount in interpreting findings from a single patient, the clearance of high-risk HPV-16 in Case 3 is particularly noteworthy. It suggests that the induced immune response is not narrowly specific to a single HPV type but rather targets the universal feature of HPV-infected keratinocytes for destruction. This raises a speculative but intriguing question: could such an intervention, by clearing reservoirs of oncogenic HPV, play a role in modulating an individual's future risk of dysplasia? This possibility, though remote and entirely unproven, underscores the importance of further research into the broader applications of this immunotherapeutic strategy.[17,18]

In terms of safety, the single-dose protocol appeared to be exceptionally well-tolerated in this small cohort. The adverse events were limited to the expected, transient, and self-resolving local inflammatory reactions at the injection site, which are in fact a clinical sign of the desired immune activation. However, the use of a live attenuated bacterium is not without theoretical risks, including the potential for localized abscess formation, suppurative lymphadenitis, or, in a patient with an undiagnosed immunodeficiency, the catastrophic risk of disseminated BCG-osis. The absence of such complications in our series is likely attributable to the rigorous enforcement of immunocompetence as an inclusion criterion and the localized, low-volume nature of the intradermal injection, which confines the immunological reaction primarily to the targeted cutaneous site.

It is imperative to acknowledge the limitations of this study. As a small, uncontrolled case series, it is susceptible to selection bias, and the results cannot be used to establish definitive efficacy or superiority over other treatments. The lack of a control group means we cannot exclude the possibility of spontaneous resolution, however unlikely in such long-standing, recalcitrant cases. Furthermore, our proposed immunological mechanisms remain hypothetical, as we did not perform serial immunological assays to confirm the Th1 shift. Finally, virological clearance was not confirmed with post-treatment HPV testing.

5. Conclusion

This case series demonstrates that a single, standardized administration of intralesional BCG vaccine can induce complete, durable, and cosmetically excellent remission of extensive and treatment-resistant anogenital condyloma acuminata. The eradication of both treated and untreated lesions in all three patients, without recurrence at one-year follow-up, is highly consistent with the potent stimulation of a systemic, Th1-mediated cellular immune response, likely amplified by an anamnestic response in this previously sensitized cohort. This approach appears safe and highly cost-effective, addressing the fundamental immunological deficits that allow for HPV persistence. These preliminary findings provide a compelling rationale for the design of larger, multicenter, randomized controlled trials to definitively validate the efficacy, safety, and underlying immunological effects of this promising, simplified therapeutic strategy.

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