



# Commentary: Granuloma Annulare and Possible Relation to Purified Protein Derivative Administration: A Case Report

Ernest C. Lee, MD, MPH

Principal Investigator, Scottsdale Clinical Trials, Harvard Medical Consultant, 8997 East Desert Cove FL2, Scottsdale, Arizona 85260.

## Article Info

### Article Notes

Received: September 12, 2024

Accepted: March 29, 2025

### \*Correspondence:

\*Dr. Ernest C. Lee, Principal Investigator, Scottsdale Clinical Trials, Harvard Medical Consultant, 8997 East Desert Cove FL2, Scottsdale, Arizona 85260; Tel: 602.565.4633; Email: [ernest.lee.007@post.harvard.edu](mailto:ernest.lee.007@post.harvard.edu).

©2025 Lee EC. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License.

In this case report, the authors observed a temporal correlation between the administration of a purified protein derivative (PPD) of *Mycobacterium tuberculosis* (*M. Tuberculosis*) administered 6 weeks prior to the clinical manifestation of granuloma annulare (GA). Although the term “purified” is utilized, the composition of PPD is highly complex. It can be made from proteins extracted from cultures of *M. tuberculosis*, *Mycobacterium bovis* (*M. Bovis*), or *Mycobacterium avium* (*M. Avium*). The PPD formulation used in the presented case was PPD-S2 extracted from *M. tuberculosis*.<sup>1</sup>

PPD-S2 was formulated after many years of research and clinical studies. In 1890, Robert Koch extracted tuberculin from the tubercle bacilli, which causes tuberculosis. He noticed that tuberculin caused a reaction in guinea pigs with tuberculosis, but not in healthy animals. In humans, tuberculin caused a severe reaction in patients with active tuberculosis, but only a mild reaction in healthy people.<sup>2</sup> In 1907, Clemens von Pirquet developed the first cutaneous tuberculin test by putting a small amount of tuberculin under the skin and measuring the body’s reaction.<sup>3</sup> In 1908, Charles Mantoux updated the skin test by using a needle and syringe to inject the tuberculin.<sup>4</sup>

In 1934, Florence Seibert, PhD developed a process to create a more stable and consistent testing fluid known as PPD of tuberculin, which would become the foundation of the modern-day TB skin test.<sup>5</sup> Seibert produced PPD by steaming cultures of *M. tuberculosis* in an Arnold sterilizer and purifying the proteins by repeating precipitation with ammonium sulfate.<sup>6</sup> Compared to previous tuberculin reagents, this method of PPD preparation was protein-rich, with drastically reduced polysaccharide, nucleic acid, and lipid content. In 1944, a large lot of this improved PPD, renamed PPD-S (PPD-Standard) was provided as the reference product in the United States. It was comprised of approximately 92.9% protein, 5.9% polysaccharide, and 1.2% nucleic acid.<sup>6</sup> In anticipation of the eventual depletion of PPD-S, PPD-S2 was developed and is currently the U.S. standard for PPD skin testing.<sup>7</sup> PPD-S2 is an ideal screen for prior *M. tuberculosis* exposure since it is extracted from *M. tuberculosis*. Utilizing PPD preparations derived from non-*M. tuberculosis* species would decrease test sensitivity for detecting *M. tuberculosis* infection due to protein variation amongst PPDs derived from different *Mycobacterium* species.<sup>8</sup>

Different PPD preparations also differ in carbohydrate and nucleic acid content. One study comparing three PPD preparations found that PPD-RT23 and PPD-KIT differed considerably from PPD-S2. The relative compositional variation correlated to differences in

the histopathology of the delayed-type hypersensitivity response to the PPD preparations, but not in the measurement of erythema. PPD-S2 is comprised of 93% protein, 1% nucleic acid, and 6% carbohydrate. It contains at least 240 proteins and has a molecular weight of 9,700 Daltons, which indicates it is a relatively low molecular weight protein compared to many other proteins in the body. PPD-S2 also contains a disproportionate abundance of heat shock proteins, which significantly influences its biological activity.<sup>8</sup>

Out of 354 proteins from *M. tuberculosis* PPDs, 37 proteins were found common with *M. avium* PPD and 80 were common with *M. bovis* PPD. 18 proteins were common in PPDs obtained from *M. tuberculosis*, *M. bovis* and *M. avium*. 35 proteins were exclusively found in *M. avium* PPD, 19 proteins were exclusively found in *M. bovis* PPD, and 255 proteins were exclusively identified in *M. tuberculosis* PPD.<sup>9</sup>

Histopathology of skin biopsies from infected animals sampled 72 hours after PPD injection revealed severe inflammatory reactions characterized by edema, fibrin transudation, vasculitis, infiltration of neutrophils, monocyte-macrophages and lymphocytes, and tissue necrosis regardless of the PPD preparation.<sup>8</sup> Intradermal PPD administration triggers a Type-IV hypersensitivity reaction during which a subset of antigen presenting cells, known as dendritic cells, likely take up the PPD antigen.<sup>10</sup> Dendritic cells reside throughout the body, particularly at environmental interfaces such as the intestine and skin, which is relevant in the case of intradermal PPD administration. They are a prominent feature in GA. These dendritic cells play a key role in T cell recognition of foreign antigens that have been processed and displayed on the cell surface bound to Major Histocompatibility Complex Class II (MHC-II) molecules.

In GA, the predominant immune cell type involved is CD4+ T cells; meaning that a reaction to GA is more likely to be associated with CD4+ than CD8+ markers. Studies show a greater presence of CD4+ cells within the lesion compared to CD8+ cells. Within GA lesions, T cells upregulate Interferon (IFN)- $\gamma$  production by CD4+, which is associated with inflammatory polarization of macrophages and fibroblasts. Macrophages upregulate oncostatin M, an IL-6 family cytokine, which appears to act on fibroblasts to alter extracellular matrix production, a hallmark of GA. IL-15 and IL-21 production appears to feedback on CD4+ T cells to sustain inflammation.<sup>11</sup>

Hypothetically, dendritic cells engulf protein-rich PPD-S2. Intracellular lysosome enzymes such as cathepsins, then break the proteins into smaller peptide fragments. These peptides are then bound to MHC-II molecules and displayed on the cellular membrane. CD4+ T cells recognize

and bind to these complexes.<sup>12</sup> This sequence of events is a subcategory of a Type-IV hypersensitivity reaction. Once activated, CD4+ T cells release cytokines, chemokines, and other enzymes which can initiate a local immune and inflammatory response and recruit other immune cells.<sup>13</sup>

The Type-IV cell-mediated hypersensitivity response results in the formation of small non-caseating granulomas on the skin appearing in a ring-like pattern, a hallmark of GA.<sup>14</sup> Development of non-infectious granulomas can be explained by a loss of immune tolerance at the site of tissue damage, leading to macrophage activation. Skin damaged through various forms of injury such as trauma, thermal burns, radiation, vaccinations, or tattoo inking, induces immune dysregulation that can pave the way for granulomatous disorders. GA, sarcoidosis, granulomatous vasculitis such as Churg-Strauss syndrome and Wegener's granulomatosis, are common granulomatous disorders that may share common pathogenic mechanisms.<sup>14</sup>

Supporting the possibility that GA is an autoimmune disease is the fact that lesions respond to corticosteroid treatment. Additionally, stress makes some cases of GA worse. Such a phenomenon can be observed in many autoimmune diseases. Acute stress can cause different regions of the brain to signal immune cells to migrate to various parts of the body. It can also trigger the release of hormones that alter cytokine production and lead to immune dysregulation. In GA, the Janus kinase (JAK)-signal transducer and activator of transcription pathway is activated, likely in part through the activity of IFN- $\gamma$  and oncostatin M. This would explain why JAK inhibitors appear to be an effective treatment for GA.<sup>15</sup>

Autoimmunity likely plays a role in GA. PPD administration in the dermis causes tissue damage where dendritic cells are ideally located to ingest PPD protein. The protein is fragmented into smaller peptide units, then transported to the membrane presented on MHC-II molecules. The CD-4 receptor on the CD4+ T cell then binds to the complex and releases interferon, which is a protein that stimulates the CD4+ T cell to clone itself and multiply. CD4+ T cells also release interferon signals to recruit cytotoxic T cells and macrophages, the primary cells involved in the formation of granulomas.

In the case of PPD administration, the most likely autoimmune mechanisms would be bystander activation, hybrid antigenicity, or possibly a combination of both. This might explain why other cases of GA are correlated with animal or insect bites, sun exposure, and tattoo inking. An alternative autoimmune mechanism could involve molecular mimicry, which would explain why some cases of GA are associated with bacterial or viral infections and pharmacological agents. Perhaps different pathways can lead to one common destination, which is the manifestation of GA lesions.

One possible causative mechanism of autoimmunity could involve molecular mimicry, an autoimmune response triggered when a host's immune system cross-reacts with a foreign antigen such as a microorganism or drug molecule. This occurs when the antigen and the host tissue share a similar amino acid sequence or conformation. Histologically, GA consists of a granulomatous inflammatory pattern situated within the superficial and mid dermis. Perhaps a PPD-S2 peptide contains an epitope that mimics an autologous dermal amino acid sequence.

A second mechanism for autoimmunity is through bystander activation. This occurs when immune cells that are responding to a specific antigen such as PPD-S2 also activate immune cells that recognize dermal self-antigens. During the immune response, inflammatory cytokines are released that activate immune cells in the vicinity including bystander T cells. Inflammatory cytokines, such as IL-1 and tumor necrosis factor (TNF) signal bystander T cells to proliferate and differentiate into effector T cells. Bystander activation can lead to the loss of immune tolerance, leading bystander T cells to recognize and attack self-antigens. It can contribute to epitope spreading, which is the expansion of an autoimmune response to additional self-antigens. This occurs when inflammatory cytokines released by bystander T cells damage tissue and expose new self-antigens to the immune system.<sup>16</sup>

Perhaps a third possible mechanism for autoimmunity exists, which can be described as hybrid antigenicity. Intradermal injection of PPD with a syringe needle leads to mechanical dermal tissue damage. This brings foreign PPD protein fragments into proximity to fragmented dermal protein self-antigens which may lose self-recognition and

tolerance. An analogy would be a printed photo of a person that is put through a paper shredder. The shredded pieces would no longer be recognized as a familiar person.

A dermal dendritic cell would engulf fragments of both the PPD and foreign-appearing dermal proteins. These fragments would then be broken down into peptide pieces within the dendritic cell lysosome. As the peptide pieces travel through the endoplasmic reticulum and to the dendritic cell surface, they might hybridize to form an antigen that would have components of both the foreign PPD peptide and the dermal self-antigen peptide. This hybridized antigen would then be presented on an MHC-II molecule on the dendritic cell surface. CD4+ T cells would bind to this complex triggering activation.

Activated CD4+ T cells release cytokines like IL-2, which stimulate their own proliferation and the activation of other immune cells such as macrophages, leading to clonal expansion and a robust immune response. CD4+ memory T cells would be created. They would remember the hybridized antigen to provide a faster more robust effective immune response upon re-exposure. Since the hybridized antigen would contain a peptide sequence common to the dermis, the immune response could propagate to dermal areas remotely located from the PPD injection site.

As more specific details of GA's pathophysiology are revealed, more specific targeting of the pathogenic mechanisms should lead to improved clinical outcomes. For example, localized GA typically responds well to topical corticosteroids; however, generalized GA does not respond well to topical corticosteroids or hydroxychloroquine. Corticosteroids suppress the activity of the transcription

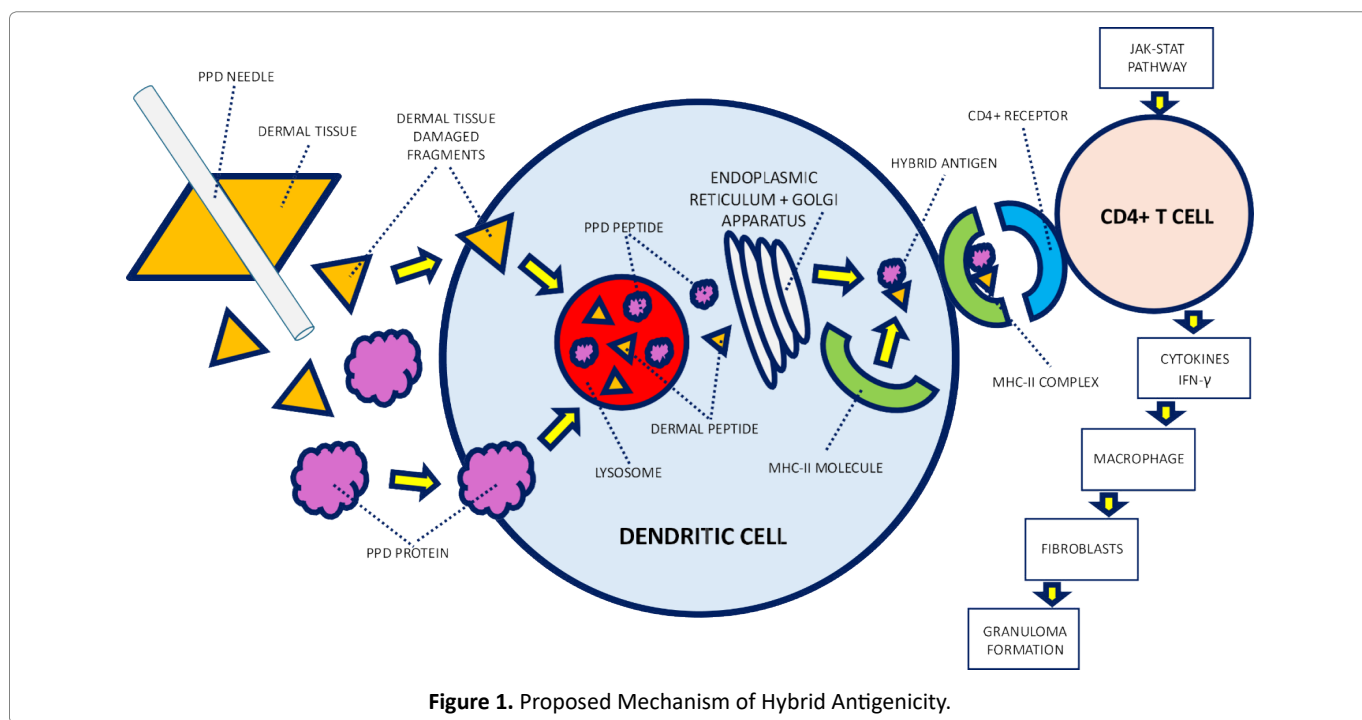


Figure 1. Proposed Mechanism of Hybrid Antigenicity.

factor NF- $\kappa$ B, which leads to sequestration of CD4+ T-cells, subsequently suppressing macrophages. Corticosteroids also suppress the production of inflammatory cytokines by macrophages and dendritic cells, and the production of IFN- $\gamma$  by NK cells. Laser therapy at 308 nm wavelength disrupts the inflammatory process and reduces immune cell activity.

Generalized GA responds well to JAK inhibitors as these block IFN-alpha, IL-3, and IL-4. JAK inhibitors can simultaneously block the signaling of multiple pro-inflammatory cytokines, unlike therapies that target a single cytokine. Hydroxychloroquine mechanism only weakly inhibits immune system targets TNF-alpha and IL-1. Methotrexate is yet another medication utilized to treat GA. It inhibits dihydrofolate reductase, reducing immune cell proliferation, including T lymphocytes.

## References

1. Prasad TS, Verma R, Kumar S, et al. Proteomic analysis of purified protein derivative of *Mycobacterium tuberculosis*. *Clin Proteomics.* 2013 Jul 19; 10(1): 8. doi: 10.1186/1559-0275-10-8. PMID: 23870090; PMCID: PMC3729367.
2. CDC MMWR Weekly March 19, 1982 / 31(10):121-123 [https://www.cdc.gov/mmwr/preview/mmwrhtml/00000222.htm#:~:text=Koch%20continued%20his%20studies%20on,development%20in\\_vitro%20and%20in%20animals](https://www.cdc.gov/mmwr/preview/mmwrhtml/00000222.htm#:~:text=Koch%20continued%20his%20studies%20on,development%20in_vitro%20and%20in%20animals).
3. dgdfg Turk JL. Von Pirquet, allergy and infectious diseases: a review. *J R Soc Med.* 1987 Jan;80(1):31-3. doi: 10.1177/014107688708000113. PMID: 3550077; PMCID: PMC1290630.
4. CDC World TB Day. History of World TB Day. December 5, 2024 <https://www.cdc.gov/world-tb-day/history/index.html>.
5. Seibert, Florence B. "The purification and properties of the purified protein derivative of tuberculin." *American Review of Tuberculosis* 30.6 (1934): 713-720.
6. Seibert FB and JT Glen. "PPD-S was comprised of approximately 92.1% protein, 5.9% polysaccharides and 1.2% nucleic acid." *Am Rev Tuberc* 44 (1941): 9-24.
7. Villarino ME, Brennan MJ, Nolan CM, et al. Comparison testing of current (PPD-S1) and proposed (PPD-S2) reference tuberculin standards. *Am J Respir Crit Care Med.* 2000 Apr; 161(4 Pt 1): 1167-71. doi: 10.1164/ajrccm.161.4.9906050. PMID: 10764307.
8. Cho YS, Dobos KM, Prenni J, et al. Deciphering the proteome of the in vivo diagnostic reagent "purified protein derivative" from *Mycobacterium tuberculosis*. *Proteomics.* 2012 Apr; 12(7): 979-91. doi: 10.1002/pmic.201100544. PMID: 22522804; PMCID: PMC3756804.
9. Prasad TS, Verma R, Kumar S, et al. Proteomic analysis of purified protein derivative of *Mycobacterium tuberculosis*. *Clin Proteomics.* 2013 Jul 19; 10(1): 8. doi: 10.1186/1559-0275-10-8. PMID: 23870090; PMCID: PMC3729367.
10. Romani N, Flacher V, Tripp CH, et al. Targeting skin dendritic cells to improve intradermal vaccination. *Curr Top Microbiol Immunol.* 2012; 351: 113-38. doi: 10.1007/82\_2010\_118. PMID: 21253784; PMCID: PMC4285659.
11. Wang A, Rahman NT, McGeary MK, et al. Treatment of granuloma annulare and suppression of proinflammatory cytokine activity with tofacitinib. *J Allergy Clin Immunol.* 2021 May; 147(5): 1795-1809. doi: 10.1016/j.jaci.2020.10.012. Epub 2020 Dec 11. PMID: 33317858.
12. Kuwabara S. Purification and properties of tuberculin-active protein from *Mycobacterium tuberculosis*. *J Biol Chem.* 1975 Apr 10; 250(7): 2556-62. PMID: 804476.
13. Czarnobilska E, Obtulowicz K, Wsołek K. [Type IV of hypersensitivity and its subtypes]. *Przegl Lek.* 2007; 64(7-8): 506-8.
14. Lo Schiavo A, Ruocco E, Gambardella A, et al. Granulomatous dysimmune reactions (sarcoidosis, granuloma annulare, and others) on differently injured skin areas. *Clin Dermatol.* 2014 Sep-Oct; 32(5): 646-53. doi: 10.1016/j.clindermatol.2014.04.012. PMID: 25160106.
15. The Basics of the Immune System. In [Immunopaedia.org.za](https://www.immunopaedia.org.za). Retrieved March 18, 2025 from <https://www.immunopaedia.org.za/immunology/basics/4-mhc-antigen-presentation>.
16. Pacheco Y, Acosta-Ampudia Y, Monsalve DM, et al. Bystander activation and autoimmunity. *J Autoimmun.* 2019 Sep; 103: 102301. doi: 10.1016/j.jaut.2019.06.012. Epub 2019 Jul 17. PMID: 31326230.