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The role of lymphocyte proliferation tests in assessing occupational sensitization and disease

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Abstract

Purpose of Review—Lymphocyte proliferation testing (LPT) is used in diagnosing occupationally-acquired delayed-type hypersensitivity. It has been used in beryllium-health effects, and it role is expanding in metal allergy. It may find application in diagnosis of other sensitizers.

Recent findings—Use of the beryllium LPT (BeLPT) in medical surveillance identifies beryllium sensitization at low exposure with chronic beryllium disease (CBD) that leads to physiologic impairment and need for immunosuppressive medications. New studies indicate that both beryllium exposure and genetic variation are associated with increased risk of CBD. Borderline positive BeLPTs warrant inclusion into diagnostic algorithms. Furthermore, use of LPTs to diagnose metal allergy is being proposed in diagnosis of chromium allergy and hypersensitivity to surgical implants. New occupational sensitizers continue to be identified including metalworking fluids, the sterilizing agent ortho-phthalaldehyde and the solvent parachlorobenzotrifluoride. Use of LPT in occupational surveillance to these agents, and other known sensitizers may play expanding roles.

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Summary—Lymphocyte proliferation testing serves a valuable role in diagnosing occupational sensitization, as demonstrated with beryllium-health effects, as cases continue to be found at low exposure levels. The use of LPTs in diagnosing contact allergy is expanding, and new applications may be identified in human and animal studies.

Keywords

lymphocyte proliferation test; beryllium; chronic beryllium disease; contact allergy; sensitization; occupational surveillance

Introduction

Numerous occupations and environments expose workers to allergens/antigens that can cause sensitization and disease. Traditional methods of determining sensitization include prick skin testing, intradermal skin testing, IgE radioallergosorbent tests, and patch testing, although each of these methods has their drawbacks. The lymphocyte proliferation test (LPT), measuring cell-mediated T cell responses to specific antigens, serves as a useful tool in the clinical evaluation and medical surveillance of exposed workers. Use of LPTs is effective in the early identification of disease risk, and in secondary prevention of disease. This review will examine the utility of the LPT in medical surveillance and focus on recent updates in the use of LPTs to identify responses to occupational sensitizers.

Background

Lymphocyte proliferation testing assesses delayed type hypersensitivity reactions *in vitro* wherein an antigen interacts with an antigen presenting cell (APC), which then activates antigen specific T cells to proliferate. In vivo this process results in a cascade of immunologic events, cytokine release and disease [1]. Lymphocyte proliferation testing has been used to assess hypersensitivity to various materials, but most prolifically with beryllium.

As lymphocyte proliferation testing has evolved, so has LPT methodology as is evidenced by the changes in the analysis and interpretation of the beryllium LPT [2–4]. In general, cells are grown in culture for several days with and without antigen at varying concentrations. After a specified time, tritiated thymidine, a radiolabeled DNA precursor, is added to cell culture. Proliferation is assessed by the degree of cellular uptake of radiolabeled thymidine. Results are expressed as a stimulation index (SI), a ratio of radiolabeled thymidine uptake in the stimulated, antigen-exposed cells compared to uptake in the unstimulated cells [1]. A positive test is generally identified by two or more SIs exceeding a specific threshold of abnormal. In the case of the BeLPT, the threshold of abnormal typically either exceeds a specific cut-point (eg in some labs an SI ≥ 3.0) or relies on statistical determination of a mean peak SI among unexposed, nonsensitized subjects [3,4]. With regards to BeLPTs, a test may be interpreted as normal, if no SI is above the cut off level. When the SI of only one of the concentrations of beryllium is elevated above the cut off, the test is considered “borderline” (BL). Two or more elevated SI’s is considered abnormal.

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The animal model correlate of the LPT, the Local Lymph Node Assay (LLNA), relies on dermal exposure as a route leading to systemic sensitization [5]. Briefly, an antigen is topically applied to an animal's ear – typically a mouse or rabbit- for several days, followed by ear thickness assessments to evaluate irritancy. Subsequently, the animal is injected with tritiated thymidine and the draining lymph nodes and their lymphocytes are harvested. The exposed animal's lymphocyte suspensions are evaluated for incorporation of tritiated thymidine, and compared to unexposed animals to determine stimulation indices. An SI is calculated for the proliferation of lymphocytes in the stimulated mice compared to control mice [6,7]. Identification of sensitizers via LLNA targets exposures that may result in sensitization and warrant further investigation in human populations.

Review of LPT uses to assess biologic responses to various agents

LPTs have been utilized in occupational medical surveillance and clinical diagnosis of diseases induced by several agents to be reviewed here.

Beryllium

To date, the LPT has had greatest clinical application in its use screening workers for beryllium related health effects. Exposure to beryllium can lead to beryllium sensitization (BeS) and chronic beryllium disease (CBD) in susceptible individuals. BeS is the immunological precursor to CBD and is diagnosed by demonstration of a beryllium stimulated immune response via the BeLPT. CBD manifests as a granulomatous lung disease and is generally diagnosed based on the demonstration of sensitization to Be manifest either by lymphocyte proliferation responses of the peripheral blood or bronchoalveolar lavage (BAL) cells in response to Be stimulation, and the presence of non-caseating granulomas on lung biopsy [1]. To determine if an individual has CBD, a bronchoscopy is usually required as the BeLPT does not differentiate between CBD and BeS. The diagnostic criteria noted above emerged following epidemiologic investigations of the use of the BeLPT [8–12]. Most recent studies demonstrate that the positive predictive values (PPV) of two abnormal tests to diagnose BeS range from 96.8% to 99.7% [13–15]. Six to eight percent of BeS patients progress to CBD annually [16]; although the clinical severity of CBD detected by workplace surveillance has varied [17].

Updates on Beryllium Sensitization—Studies by Stange and Middleton provided the most recent probability characterizations for one versus two positive BeLPTs to diagnose BeS [13–15]. The Middleton algorithms to diagnose BeS include the use of BL tests, with the presence of one abnormal (AB) plus one borderline (1 AB + 1 BL), but some diagnostic algorithms and compensation directives do not recognize the value of the borderline test as a measure of abnormality [2,14,15]. Because the predictive value of (1 AB + 1 BL) approaches that of 2 abnormals (AB) over a range of BeS prevalences, cases of CBD could be missed if evaluations are limited to workers with at least 2 AB results [14]. In most surveillance programs, an initial AB or BL is followed by a "split" sample sent to two labs, ultimately leading to 3 tests. Using data from the Stange 2004 study, Middleton demonstrated that the post-test probabilities for the 3-result combinations possible of suggesting sensitization at 2% prevalence were: 3 AB (100%), 2 AB+1BL (100%); 1 AB + 2

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BL (99%); 2 AB + 1 NL (95%); 3 BL (91%); 1 AB + 1 BL + 1 NL (72%) [13,18]. These results suggest that BL results do have meaning and that the combination of 3 BL has a sufficiently high predictive value to refer patients for diagnostic evaluation.

Updates on BeLPT in diagnosis of CBD or CBD Severity—Critics of the LPT have questioned its significance in workplace surveillance, arguing that it does not detect clinically severe CBD. However, longitudinal follow-up of workers identified using workplace surveillance demonstrates a clinically important rate of progression from BeS to CBD and to more clinically severe disease [17]. Mroz and colleagues showed that 19.3% of CBD cases who presented for clinical evaluation because of abnormal workplace surveillance BeLPTs developed clinical abnormalities requiring oral immunosuppressive therapy an average of 1.4 years after diagnosis [18]. At 30 years from first exposure, CBD patients had significantly more gas exchange impairment, manifest as higher resting and peak exercise alveolar-arterial gradient, lower rest and peak exercise arterial oxygen content, and lower diffusion capacity (DLCO) and total lung capacity. Over the course of the study, 8.8% of workers originally diagnosed as BeS developed CBD. Similarly, Duggal and colleagues found that after a mean of 7.4 years of follow-up, workers from a beryllium processing and production plant who had positive BeLPTs had average decreases in DLCO of 17.4 percentage points among both workers with BeS and CBD [19]. Whereas only one of 50 workers with BeS at baseline had abnormal lung function at follow-up, seven of 22 workers with biopsy proven CBD demonstrated abnormal lung function. Together, both studies demonstrate that workplace surveillance BeLPTs identify workers who have or progress to CBD, and that this disease, detected using the BeLPT in medical surveillance, is associated with significant functional impairment.

Updates on associations between BeLPT and exposure response—Cases of BeS continue to be detected through workplace BeLPT medical surveillance even in environments where exposure to beryllium is low, raising the questions as to whether a truly “safe” exposure level exists. In one beryllium processing facility where airborne Be levels were well below the 2.0 mcg/m^3 OSHA PEL, a baseline medical survey revealed 7% BeS and 4% CBD prevalences and estimated a sensitization incidence rate (IR) of 3.8/1000 person-months [20]. After implementing an enhanced multidisciplinary Be exposure prevention program including more frequent BeLPT surveillance, the sensitization IR decreased to 1.9/1000 person-months. Once a high-risk area was enclosed, the IR decreased further to 1.4/1000 person-months. Results from this study suggest that a multidisciplinary approach at exposure control, including more frequent BeLPT surveillance to identify areas of higher exposure and sensitization risk, is able to reduce sensitization in facilities even with low airborne concentrations of Be [21,22].

Mikulski and colleagues examined both Department of Energy (DOE) and Department of Defense (DOD) workers employed at a nuclear weapons facility where exposure largely occurred through machining and grinding of 1–2% beryllium copper-beryllium alloy tools [23,24].. Although only 6% of the DOE workers were considered to have higher exposure, 2.3% of them had BeS, (OR=4.58 compared to lowest exposure workers). Among the DOD workers, prevalence of BeS was 1.5%. The 2.3% and 1.5% sensitization rates in this “low

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exposure" population, where the highest exposures were from refinishing copper-beryllium tools, were higher than expected. Similarly, Nilsen and colleagues found that 0.28% of employees of an aluminum smelter were sensitized to Be using the BeLPT [25]. In this workplace, the BeLPT was useful in identifying low rates of BeS in a population thought to have very low levels of Be exposure as an incidental presence in the processing of aluminum. Arjomandi and colleagues found a similar prevalence of BeS among current and former workers from Lawrence Livermore National Laboratory (LLNL), a nuclear weapons research and development facility [26]. The Be exposures at this facility were significantly lower than those at Rocky Flats, the most well-characterized DOE site, and well below 0.2 mcg/m^3. Nonetheless, the BeLPT detected cases of BeS among these low exposed workers, although the prevalence of CBD was five times lower than at Rocky Flats. Taken together, these studies support the notion that lower exposures are associated with lower rates of BeS and CBD.

Previous studies have shown that a glutamic acid (E) at amino acid position 69 (E69) of HLA-DPB1 Class II on APCs results in the more effective presentation of Be as an antigen, and increased risk of BeS and CBD in workers with this variant [27–37]. Van Dyke and colleagues studied associations between quantitative Be exposure and this genotype to better define exposure-related risk for BeS and CBD [38]. Whereas the E69 variant significantly increased risk for BeS, the development of CBD, but not BeS, was associated with higher Be exposure. Carriage of an E69 significantly increased odds of CBD, and each unit increase in lifetime weighted average Be exposure increased CBD odds twofold. This study showed that **both** increasing exposure and genetic susceptibility increased CBD risk, with no significant gene-by-environment interaction. Interestingly, after adjusting for E69 genetic risk factors, the study showed an exposure response relationship for Be exposure and CBD, but not for BeS. Cases of CBD were still detected even at extremely low Be levels, suggesting that a protective threshold exposure level has not yet been demonstrated.

BeLPT use in research to define therapies, understand disease progression, and develop new diagnostic modalities—Dobis and colleagues incorporated the BeLPT to demonstrate therapeutic effect in potential new treatments for CBD [39]. The authors tested the hypothesis that effective treatment of CBD patients with sulfasalazine or mesalamine, both antioxidants, could be measured by the inhibition of Be-stimulated peripheral blood mononuclear cell proliferation and cytokine production. They found that the BeLPT was significantly decreased in Be-stimulated CBD and BeS PBMCs treated with either drug. Based on these investigations an LPT may be used as the end point in the search for new investigational therapies.

In the quest to find non-invasive ways to diagnose CBD, Fireman and colleagues compared the ability of induced sputum (IS) CD4/CD8 ratio combined with positive blood BeLPTs against the use of biopsy to diagnose CBD [40]. They concluded that with the specificity and sensitivity of IS+ BeLPT+ reaching 92% and 100%, and excellent agreement between both methods (K=0.89, [0.74–1.0]), the use of IS CD4/CD8 ratio plus peripheral blood BeLPT may be an option for diagnosing CBD without need for bronchoscopy. Martin and colleagues studied current and former workers of a Be-machining facility to clarify the role of IFN-γ ELISPOT in diagnosing BeS and CBD [41]. Using a cut-point of 10 or more spot

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forming units (SFU) of IFN-γ, ELISPOT yielded sensitivities and specificities of 85% and 100% for CBD, with PPV and NPV of 100% and 81%. IFN-γ ELISPOT was more sensitive in detecting BeS, as the test was positive in 10% of workers, compared to 4.2% measured by the BeLPT *(p<0.0001)*. ELISPOT was also useful in detecting CBD, as all 14 CBD subjects identified at the time of clinical evaluation had significantly increased production of IFN-γ detected on ELISPOT (27 SFU) compared with those BeS subjects who had not progressed. Advantages of the ELISPOT over the BeLPT to diagnosis sensitization include shorter duration of incubation, lack of radioactivity, and use of fetal bovine serum compared to human albumin [41]. Thus peripheral blood IFN-γ measurement via ELISPOT provides a potential new test that may provide additional data to the BeLPT or be able to identify additional workers with BeS. This may also help provide additional information regarding decisions on who should undergo biopsy to confirm CBD, thereby avoiding low-yield bronchoscopies, although additional study is needed.

Metal contact allergens

Occupationally-acquired dermal sensitization and allergic contact dermatitis from metals present opportunities for use of the LPT in better understanding underlying pathophysiologic mechanisms and in providing accurate and safer diagnostic methods.Martins and colleagues recently studied optimal conditions for performance of chromium LPT to detect allergy [42]. Those with chromium allergy, defined as dermatitis and positive patch tests, were compared to those without Cr dermatitis and negative patch tests. Six-day cultures yielded the best growth, and incubation with CrCl3 (Cr[III]) yielded the most consistent results. The most predictive LPT conditions for identifying allergic versus non-allergic individuals came from the use of nonfiltered Cr[III] solution, yielding sensitivity of 65%, specificity of 95% and accuracy of 80% in identifying dermatitis compared to presence of dermatitis with positive patch test.

Recent studies have compared the use of a tri- or hexavalent chromium LPT, chromium patch testing, and a chromium-specific ELISPOT in understanding occupational chromium allergy. Lindemann and colleagues studied whether each modality could identify chromium-sensitized individuals (CrS), and discriminate between those with and without clinical allergy [43]. Subjects with CrS and clinical allergy had significantly higher LPTs expressed as counts per minute compared to controls with no clinical allergy or positive patch tests, whereas CrS individuals without clinical allergy did not. Allergic subjects also had higher IFN-γ on ELISPOT testing than did controls. In this cohort, while the Cr LPT seemed to identify subjects with clinically-manifest allergy better than patch testing, positive LPT responses were not presented as values exceeding a stimulation threshold. These studies indicate that although the CrLPT may present a more accurate and safer diagnostic method to detect chromium allergy in patients with occupational exposure (because there is lower risk for inducing sensitization compared to patch testing), further refinements of testing methodologies are likely needed.

Updates on use of metal LPTs to evaluate surgical prosthetic implant allergy

—One of the recent advances in the use of LPT has arisen in the assessment of dysfunction of surgical prostheses. This field represents a new area of toxicologic assessment of metal

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allergy with potential occupational surveillance applications and explores relationships with metal ions released as breakdown products into tissue and circulation.

Previously, most orthopedic implants were composed of nickel (Ni)-containing stainless steel, a metal associated with high rates of contact dermatitis. Currently, many are made of cobalt, chromium, and molybdenum alloys containing 0.5 to 1% nickel, or from a titanium/aluminum/vanadium alloy [44,45]. Several studies have demonstrated lymphocytic responses to metals in patients with loosened prosthetic joints, hypothesized due to hypersensitivity to wear-and-tear products of the prosthesis. Some patients have developed local skin reactions and pain in the tissue surrounding the prostheses, suggesting immunologically active antigens from the prosthesis itself [46].

One central question that the LPT may help elucidate among patients with failing surgical implants is whether hypersensitivity to different metals on LPT testing is associated with sensitization diagnosed on patch testing, and ultimately with graft dysfunction. Summer and colleagues found unique patterns of LPT and cytokine responses among subjects with and without implants, positive Ni patch tests, and implant dysfunction [47]. Those with Ni allergy and graft dysfunction showed strong lymphocyte proliferative responses and strong IL-17 expression, but virtually no IFN-gamma expression based on Ni-exposed PBMCs. Cobalt did not induce hypersensitivity or cytokine expression.

Some patients with metal-on-metal (MOM) implants following hip resurfacing arthropathy have developed peri-prosthetic pseudotumors, masses around the implant site that demonstrate lymphocytic infiltration on biopsy thought to be induced by a delayed type hypersensitivity reaction [45]. Most MOM implants are primarily composed of a Co-Cr-Mo alloy, with ≤ one percent Ni. Kwon and colleagues compared patients with pseudotumors and MOM Hip Resurfacing Arthroplasty (MOMHRA) to similar patients without pseudotumor, and to controls with no implants and no history of metal allergy. Subjects with MOMHRA had higher serum ion levels of Co and Cr compared to controls, but did not have positive LPTs to these metals, while those with pseudotumors had higher serum concentrations of Co and Cr compared to those with MOM implants only without pseudotumors. In contrast, some patients with MOMHRA had positive LPTs to nickel, but low serum concentrations of Ni. Notably, this study did not quantify metal ion levels in joint effusions or in the synovium, which may be more important.

If delayed-type hypersensitivity reactions to metals in implants does play a role in graft dysfunction, then pre-operative patch testing or LPT may identify individuals already sensitized and perhaps more likely to develop complications. Frigerio and colleagues found that self-reported history of metal allergy significantly under-identified metal-allergic patients compared to patch testing [44]. Metal LPTs added additional identification of subjects with sensitization, but the number of patients providing LPTs was too small to draw any conclusions from the study.

Non-orthopedic implants may serve as another risk for hypersensitivity. A systematic review examined hypersensitivity reactions to titanium in dental implants [48]. One article utilized an LPT in a case report of a woman who developed facial eczema after implantation of a

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99.64% purity titanium implant. She had positive LPTs to TiCl3, NiSO4, and HgCl2. Her implant was removed, and her eczema resolved without medical treatment [49]. Another case report described the use of an LPT to the fibrin component of a glue used in arachnoid plasty to verify hypersensitivity as a complication in a patient undergoing neurosurgery [50]. In addition, case reports have identified Be as a source of gingivitis, although patch testing has usually been used clinically [51]. We are aware of cases of gingivitis related to Be-containing dental prostheses with demonstrated BeS using the BeLPT (Maier, personal communication). In summary, these reports suggest that further development of LPTs to surgical and dental implants may play increasingly important roles in understanding adverse immunologic outcomes, which may have collateral roles in the demonstration and knowledge of occupational allergy.

Use of novel LPT methodology in evaluation of occupational disease

Exposure to epoxy resin system chemicals has been associated with occupational asthma and allergic contact dermatitis [52,53]. Hines and colleagues found no significant differences in LPT positivity between epoxy-exposed versus unexposed manufacturing populations, despite significant differences in symptom reporting [54]. Two-part glues are also used extensively in orthopedic and dental procedures, including epoxy resins, cyanoacrylates, and methyl methacrylates. These are known potent sensitizers, although establishing the diagnosis remains difficult. LPT testing may provide an approach, although these tests are not yet clinically available.

Metalworking fluid (MWF) exposure is known to cause hypersensitivity pneumonitis (HP). While thought to represent a reaction to microbial contamination of the metalworking fluids, HP may also be related to sensitization from the MWF mixture itself. Anderson and colleagues studied three different types of MWFs and their ability to induce sensitization via LLNA [55]. All soluble and semi-synthetic MWFs induced notable increases in lymphocyte proliferation, whereas only one synthetic MWF induced proliferation. “TRIM VX,” the most irritating soluble MWF, is composed of oleic Acid and 4-chloro-3-methylphenol, which were sensitizers in LLNA assays. While worker surveillance LPTs were not utilized here, data from the LLNA studies suggests that MWFs themselves pose risks for sensitization. Development and use of an LPT in occupationally-exposed populations may be of benefit in risk assessment of MWF.

The healthcare field boasts high rates of occupational illnesses from various sensitizing agents. Ortho-phthalaldehyde (OPA) is replacing the use of glutaraldehyde as a sterilizing agent and is thought safer due to less volatility and no need for activation [6]. However, cases of anaphylaxis and occupational asthma have been reported in exposed workers [56]. Anderson and colleagues found a dose-dependent increase in draining lymph node proliferation and Th-2 cytokine response in LLNA based on expected workplace OPA concentrations [6]. Subsequently, Johnson and colleagues studied the potential for inhalation of OPA to cause respiratory sensitization in mice, demonstrating a concentration-dependent increase in total lymphocytes in draining lymph nodes [57]. Cytokine gene expression and lymphocyte phenotyping in the respiratory mucosa and draining lymph nodes of mice suggested that OPA can be a respiratory sensitizer. As OPA finds increasing use in

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healthcare settings, an OPA LPT may play a role in medical surveillance of workers at increased risk for sensitization.

Parachlorobenzotrifluoride (PCBTF) is used in production of a wide range of products, but is primarily used as a solvent in commercial surface finishes [58–60]. Franko and colleagues found dose-dependent increases in draining lymph node proliferation in LLNA after treatment with PCBTF, along with increases in IFN-γ production [60]. This study demonstrated the sensitizing capacity of PCBTF in animal models at concentrations used in occupational settings. Worker populations who routinely use this solvent may benefit from avoidance of dermal contact and medical surveillance for sensitization by LPT.

Future tools for assessment of sensitization

The LPT is a highly specific tool to assess sensitization to particular occupational agents, although, it has limitations, including length of time needed to run the assay, exposure to radiation for workers performing the test and potential for variability in methodology [41]. Some investigators have examined flow cytology methods to assess lymphocyte proliferative responses to beryllium. *The carboxyfluorescein diacetate succinimidyl ester* (CFSE) labeling method identifies beryllium-specific proliferative responses and immunophenotyping without the need for radioactive agents, while using a single time point, with the ability to be run on commercial clinical flow cytometers [61]. Further study on CFSE-based flow cytometry methods may identify a safer and more efficient tool to diagnose BeS. Similarly, the studies above suggest that ELISPOT may also provide an alternative or adjuvant to BeLPT testing [41, 43].

Summary

The LPT currently has validated uses in the assessment of occupationally-acquired sensitization to Be, and holds promise for the assessment of sensitization to other contact allergens and emerging sensitizers such as metalworking fluids, hospital sterilizing agents and solvents. The well-standardized methodology of the BeLPT model permits extension to other agents to define sensitization and disease. As noted in the case of beryllium, LPTs may help us understand the natural history of sensitization and disease, define exposure-disease response relationships, and develop new therapies and new diagnostic modalities. The LLNA may find similar application in identifying agents as potential sensitizers that will require additional evaluation and testing with the LPT. Finally, future generation of tests may allow detection of hypersensitivity and determining the type of hypersensitivity response by using safer and more efficient methods.

Acknowledgments

Conflicts of interest:

Drs. Maier and Pacheco evaluate patients clinically using the BeLPT and other LPTs and interpret the tests, and NJH provides this testing commercially for these and other patients. In addition, Dr. Maier has received NIH grant funding to evaluate the immune response to beryllium, and the impact of potential therapeutics on this response and the BeLPT. Dr. Hines received NIH and CDC-NIOSH pilot grand funding to study epoxy resin LPT responses.

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evaluation at National Jewish Health after referral following positive workplace surveillance BeLPT. They found that 19.3% of CBD cases required use of immunosuppressive therapy an average of 1.4 years after initial diagnosis, and that they had significantly lower resting and exercise arterial oxygen content and higher A-a gradient than did BeS patients. This study supports that CBD detected via initial workplace BeLPT surveillance identifies workers with clinically significant disease. [PubMed: 19681064]

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Key points

- LPT continues to be useful in occupational medical surveillance for beryllium-exposed workers at risk not only for sensitization, but for clinically significant disease with functional impairment and need for immunosuppressive medication.
- LPT results may correlate with quantitative exposure assessment and contribute to our knowledge of dose-response relationships, threshold levels for health effects, and genetic predisposition to disease in beryllium-exposed workers.
- LPT use may help understand unusual conditions like surgical implant dysfunction, that may be a manifestation of hypersensitivity reactions to sensitizing metals and adhesives, with parallel correlations to occupational contact allergy.