



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

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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 its 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 *para*-chlorobenzotrifluoride. Use of LPT in occupational surveillance to these agents and other known sensitizers may play expanding roles.

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

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

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 its 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.

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KEY POINTS

- Lymphocyte proliferation testing (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.

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, a ratio of radiolabeled thymidine uptake in the stimulated, antigen-exposed cells compared with uptake in the unstimulated cells [1]. A positive test is generally identified by two or more stimulation indices exceeding a specific threshold of abnormal. In the case of the beryllium LPT (BeLPT), the threshold of abnormal typically either exceeds a specific cut-point (e.g. in some labs a stimulation index ≥ 3.0) or relies on statistical determination of a mean peak stimulation index among unexposed, nonsensitized individuals [3,4]. With regards to BeLPTs, a test may be interpreted as normal, if no stimulation index is above the cut off level. When the stimulation index of only one of the concentrations of beryllium is elevated above the

cut off, the test is considered ‘borderline’. Two or more elevated stimulation indices is considered abnormal.

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 with unexposed animals to determine stimulation indices. An stimulation index is calculated for the proliferation of lymphocytes in the stimulated mice compared with control mice [6^a,7]. Identification of sensitizers via LLNA targets exposures that may result in sensitization and warrant further investigation in human populations.

REVIEW OF LYMPHOCYTE PROLIFERATION TESTING 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 beryllium manifesting either by lymphocyte proliferation responses of the peripheral blood or bronchoalveolar lavage cells in response to beryllium stimulation, and the presence of noncaseating granulomas on lung biopsy [1]. To determine whether 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]. Recent studies demonstrate that the positive predictive values (PPVs) 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 [16¹¹], although the clinical severity of CBD detected by workplace surveillance has varied [17].

Updates on beryllium sensitization

Studies by Stange *et al.* [13] and Middleton *et al.* [14,15] provided the most recent probability characterizations for one versus two positive BeLPTs to diagnose BeS. The Middleton *et al.* algorithms to diagnose BeS include the use of borderline tests, with the presence of one abnormal plus one borderline indicating BeS, 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 the combination of one abnormal and one borderline approaches that of two abnormal over a range of BeS prevalences, cases of CBD could be missed if evaluations are limited to workers with at least two abnormal results [14]. In most surveillance programs, an initial abnormal or borderline is followed by a 'split' sample sent to two labs, ultimately leading to three tests. Using data from the Stange *et al.* [13] study, Middleton demonstrated that the posttest probabilities for the three-result combinations possible of suggesting sensitization at 2% prevalence were: three abnormal (100%); two abnormal and one borderline (100%); one abnormal and two borderline (99%); two abnormal and one normal (95%); three borderline (91%); and one abnormal and one borderline and one normal (72%) [13,18¹²]. These results suggest that borderline results do have meaning and that the combination of three borderlines has a sufficiently high predictive value to refer patients for diagnostic evaluation.

Updates on beryllium lymphocyte proliferation testing in diagnosis of chronic beryllium disease or chronic beryllium disease 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 *et al.* [18¹³] 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. At 30 years from first exposure, CBD patients had significantly more gas exchange impairment, manifesting 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 *et al.* [19¹⁴] 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. 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 beryllium lymphocyte proliferation testing and exposure response

Cases of BeS continue to be detected through workplace BeLPT medical surveillance even in environments wherein exposure to beryllium is low, raising the questions as to whether a truly 'safe' exposure level exists. In one beryllium processing facility in which airborne beryllium levels were well below the 2.0 $\mu\text{g}/\text{m}^3$ permissible exposure limit, a baseline medical survey revealed 7% BeS and 4% CBD prevalences and estimated a sensitization incidence rate of 3.8/1000 person-months [20¹⁵]. After implementing an enhanced multidisciplinary beryllium exposure prevention program, including more frequent BeLPT surveillance, the sensitization incidence rate decreased to 1.9/1000 person-months. Once a high-risk area was enclosed, the incidence rate 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 beryllium [21,22].

Mikulski *et al.* [23¹⁶,24¹⁷] examined both Department of Energy (DOE) and Department of Defense (DOD) workers employed at a nuclear weapons facility wherein exposure largely occurred through machining and grinding of 1–2% beryllium copper-beryllium alloy tools. Although only 6% of the DOE workers were considered to have higher exposure, 2.3% of them had BeS (odds ratio = 4.58 compared with lowest exposure workers). Among the DOD workers, prevalence of BeS was 1.5%. The 2.3–1.5% sensitization rates in this 'low exposure' population, wherein the highest exposures were from refinishing copper-beryllium tools, were

higher than expected. Similarly, Nilsen *et al.* [25[■]] found that 0.28% of employees of an aluminum smelter were sensitized to beryllium using the BeLPT. In this workplace, the BeLPT was useful in identifying low rates of BeS in a population thought to have very low levels of beryllium exposure as an incidental presence in the processing of aluminum. Arjomandi *et al.* [26[■]] found a similar prevalence of BeS among current and former workers from Lawrence Livermore National Laboratory (LLNL), a nuclear weapons research and development facility. The beryllium exposures at this facility were significantly lower than those at Rocky Flats, the most well characterized DOE site, and well below 0.2 $\mu\text{g}/\text{m}^3$. 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 beryllium as an antigen, and increased risk of BeS and CBD in workers with this variant [27–37]. Van Dyke *et al.* [38[■]] studied associations between quantitative beryllium exposure and this genotype to better define exposure-related risk for BeS and CBD. Whereas the E69 variant significantly increased risk for BeS, the development of CBD, but not BeS, was associated with higher beryllium exposure. Carriage of an E69 significantly increased odds of CBD, and each unit increase in lifetime-weighted average beryllium exposure increased CBD odds two-fold. 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 beryllium exposure and CBD, but not for BeS. Cases of CBD were still detected even at extremely low-beryllium levels, suggesting that a protective threshold exposure level has not yet been demonstrated.

Beryllium lymphocyte proliferation testing use in research to define therapies, understand disease progression, and develop new diagnostic modalities

Dobis *et al.* [39[■]] incorporated the BeLPT to demonstrate therapeutic effect in potential new treatments for CBD. The authors tested the hypothesis that effective treatment of CBD patients with sulfasalazine or mesalamine, both antioxidants, could be measured by the inhibition of beryllium-stimulated

peripheral blood mononuclear cell (PBMC) proliferation and cytokine production. They found that the BeLPT was significantly decreased in beryllium-stimulated CBD and BeS PBMCs treated with either drug. On the basis of these investigations, an LPT may be used as the end point in the search for new investigational therapies.

In the quest to find noninvasive ways to diagnose CBD, Fireman *et al.* [40[■]] compared the ability of induced sputum CD4/CD8 cell ratio combined with positive blood BeLPTs against the use of biopsy to diagnose CBD. They concluded that with the specificity and sensitivity of induced sputum and BeLPT and reaching 92–100%, and excellent agreement between both methods [$K=0.89$, (0.74–1.0)], the use of induced sputum CD4/CD8 cell ratio and peripheral blood BeLPT may be an option for diagnosing CBD without need for bronchoscopy. Martin *et al.* [41[■]] studied current and former workers of a beryllium-machining facility to clarify the role of IFN- γ enzyme-linked immunosorbent spot (ELISPOT) in diagnosing BeS and CBD. Using a cut-point of 10 or more spot forming units (SFU) of IFN- γ , ELISPOT yielded sensitivities and specificities of 85–100% for CBD, with PPV and negative predictive value of 100–81%. IFN- γ ELISPOT was more sensitive in detecting BeS, as the test was positive in 10% of workers, compared with 4.2% measured by the BeLPT ($P < 0.0001$). ELISPOT was also useful in detecting CBD, as all 14 CBD individuals identified at the time of clinical evaluation had significantly increased production of IFN- γ detected on ELISPOT (27 SFU) compared with those BeS individuals 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 with 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 *et al.* [42[■]] recently studied optimal conditions for performance of chromium LPT to detect allergy. Those with chromium allergy,

defined as dermatitis and positive patch tests, were compared with 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 nonallergic 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 with presence of dermatitis with positive patch test.

Recent studies have compared the use of a trivalent or hexavalent chromium LPT, chromium patch testing, and a chromium-specific ELISPOT in understanding occupational chromium allergy. Lindemann *et al.* [43²²] studied whether each modality could identify chromium-sensitized individuals (CrS), and discriminate between those with and without clinical allergy. Individuals with CrS and clinical allergy had significantly higher LPTs expressed as counts per minute compared with controls with no clinical allergy or positive patch tests, whereas CrS individuals without clinical allergy did not. Allergic patients also had higher IFN- γ on ELISPOT testing than did controls. In this cohort, although the Cr LPT seemed to identify patients with clinically manifested 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 of inducing sensitization compared with patch testing), further refinements of testing methodologies are likely needed.

Updates on use of metal lymphocyte proliferation testings 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 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-containing stainless steel, a metal associated with high rates of contact dermatitis. Currently, many are made of cobalt, chromium, and molybdenum alloys containing 0.5–1% nickel, or from a titanium/aluminum/vanadium alloy [44²³,45²⁴]. Several studies have demonstrated lymphocytic responses to metals in patients with loosened prosthetic joints, hypothesized because

of 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 *et al.* [47²⁵] found unique patterns of LPT and cytokine responses among individuals with and without implants, positive nickel patch tests, and implant dysfunction. Those with nickel allergy and graft dysfunction showed strong lymphocyte proliferative responses and strong IL-17 expression, but virtually no IFN- γ expression based on nickel-exposed PBMCs. Cobalt did not induce hypersensitivity or cytokine expression.

Some patients with metal-on-metal (MOM) implants following hip resurfacing arthropathy have developed periprosthetic pseudotumors, masses around the implant site that demonstrate lymphocytic infiltration on biopsy thought to be induced by a delayed-type hypersensitivity reaction [45²⁴]. Most MOM implants are primarily composed of a Co–Cr–Mo alloy, with 1% nickel or less. Kwon *et al.* [45²⁴] 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. Individuals with MOMHRA had higher serum ion levels of Co and Cr compared with controls, but did not have positive LPTs to these metals, whereas those with pseudotumors had higher serum concentrations of Co and Cr compared with those with MOM implants only without pseudotumors. In contrast, some patients with MOMHRA had positive LPTs to nickel, but low serum concentrations of nickel. 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 do play a role in graft dysfunction, then preoperative patch testing or LPT may identify individuals already sensitized and perhaps more likely to develop complications. Frigerio *et al.* [44²³] found that self-reported history of metal allergy significantly underidentified metal-allergic patients compared with patch testing. Metal LPTs added additional identification of patients with sensitization, but the number of patients providing LPTs was too small to draw any conclusions from the study.

Nonorthopedic implants may serve as another risk for hypersensitivity. A systematic review [48] examined hypersensitivity reactions to titanium in dental implants. One article utilized an LPT in a case report of a woman who developed facial eczema after implantation of a 99.64% purity titanium implant. She had positive LPTs to $TiCl_3$, $NiSO_4$, and $HgCl_2$. Her implant was removed, and her eczema resolved without medical treatment [49]. Another case report [50] 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. In addition, case reports have identified beryllium as a source of gingivitis, although patch testing has usually been used clinically [51]. We are aware of cases of gingivitis related to beryllium-containing dental prostheses with demonstrated BeS using the BeLPT (L.A.M. 12/1/2011, personal communication). In summary, these studies 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 LYMPHOCYTE PROLIFERATION TESTING 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 *et al.* [54] found no significant differences in LPT positivity between epoxy-exposed versus unexposed manufacturing populations, despite significant differences in symptom reporting. 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. Although thought to represent a reaction to microbial contamination of the metalworking fluids, hypersensitivity pneumonitis may also be related to sensitization from the MWF mixture itself. Anderson *et al.* [55¹¹] studied three different types of MWFs and their ability to induce sensitization via LLNA. All soluble and semisynthetic 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. Although worker surveillance LPTs were not utilized here, data from the LLNA studies suggest 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 because of less volatility and no need for activation [6¹¹]. However, cases of anaphylaxis and occupational asthma have been reported in exposed workers [56]. Anderson *et al.* [6¹¹] found a dose-dependent increase in draining lymph node proliferation and Th-2 cytokine response in LLNA based on expected workplace OPA concentrations. Subsequently, Johnson *et al.* [57¹¹] 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. 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 healthcare settings, an OPA LPT may play a role in medical surveillance of workers at increased risk for sensitization.

Para-chlorobenzotrifluoride (PCBTF) is used in production of a wide range of products, but is primarily used as a solvent in commercial surface finishes [58,59,60¹¹]. Franko *et al.* [60¹¹] found dose-dependent increases in draining lymph node proliferation in LLNA after treatment with PCBTF, along with increases in $IFN-\gamma$ production. 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 [41¹¹]. 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 [41¹¹,43¹²].

CONCLUSION

The LPT currently has validated uses in the assessment of occupationally acquired sensitization to beryllium 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.

Acknowledgements

None.

Conflicts of interest

L.A.M. and K.P. evaluate patients clinically using the BeLPT and other LPTs and interpret the tests, and N.J.H. provides this testing commercially for these and other patients. In addition, L.A.M. has received NIH grant funding to evaluate the immune response to beryllium, and the impact of potential therapeutics on this response and the BeLPT. N.J.H. received NIH and CDC-National Institute for Occupational Safety and Health pilot grant funding to study epoxy resin LPT responses. S.E.H. reports no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 211–212).

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On the basis of current testing algorithms, workers undergoing surveillance BeLPT, this study demonstrated the value of borderline BeLPT results in confirming BeS. At 2% prevalence, posttest probabilities for three-result combinations including borderline tests ranged from 72 to 100%, with the likelihood of increasing as prevalence increases. This suggests that borderline results truly do have value in diagnosing BeS and potentially CBD.

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This study evaluated all patients who were found to have CBD upon clinical 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 at 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.

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This study challenged the hypothesis that CBD detected via workplace surveillance using LPT is not severe. They found that workers with positive BeLPT had significantly lower diffusion capacity by 17.4% of predicted on pulmonary function testing. One third of those found to have CBD at initial examination developed clinical findings suggestive of CBD by the time of follow-up. This tends to suggest that workers identified as having BeS and CBD on workplace surveillance do indeed have clinically relevant disease.

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A multidisciplinary exposure control program was instituted at a beryllium processing facility that included enhanced engineering and administrative controls, including more frequent BeLPT testing. Despite most exposures being below the OSHA exposure limit, use of more frequent BeLPT surveillance was an important factor in reducing the number of BeS cases detected, demonstrating the utility of the BeLPT as part of a disease prevention program.

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