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Immunological and Inflammatory Assessments

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INTRODUCTION

A 30-year-old man was employed for five years in a platinum refinery. Two years after starting this work, he noticed shortness of breath, chest tightness, wheezing, and a persistent dry cough as well as nasal symptoms—such as runny, stuffy nose and sneezing—when at work. These symptoms improved substantially on weekends. His physical examination and chest radiograph were normal. Allergy skin-prick tests (SPTs) to 19 common extracts were negative. His treatment consisted of the use of an albuterol inhaler as needed, budesonide nasal spray 200 μg/day, and antihistamines. When at work, he had severe dyspnea; chest tightness; dry cough; wheezing; runny, stuffy nose; and sneezing, and normal spirometry and airway responsiveness. After three weeks off work, he did not complain of any remaining respiratory symptoms. SPTs with platinum salts could not be interpreted reliably, because the subject was taking antihistamines. Radioallergosorbent test (RAST) to platinum salts showed platinum salt–specific immunoglobulin E (IgE). After two weeks at work, induced sputum cell counts showed eosinophilic inflammation (sputum eosinophils: 10%) that decreased after two weeks away from work (sputum eosinophils: 1%). Serial peak expiratory flow (PEF) monitoring did not show any difference between the periods at work and away from work. Methacholine PC_{20} was greater than 16 mg/mL after two weeks at work.

The next sections discuss the different tests that can be performed to make a diagnosis in this patient. This chapter addresses the current methods for performing an immunological assessment by skin testing and by serological assays, and also describes the role of noninvasive assessment of airway inflammation by sputum cell counts.

IMMUNOLOGICAL ASSESSMENT BY SKIN TESTS

Immunologic assessment of individuals with suspected occupational asthma (OA) by skin testing has been an important tool in the diagnosis of OA, as exemplified by the classic work of Dr. J. Pepys on industrial enzymes in the detergent industry and platinum salts in the refining industry (1,2).

A number of recent excellent reviews on OA and on the epidemiology of asthma in the workplace describe the extensive number and diversity of important occupational allergens (3–9). The examples of occupational agents that have been assessed by skin testing and other diagnostic studies are listed in Table 1 (10–64).

Demonstration of skin test reactivity indicates that the worker has become sensitized to low- or high-molecular-weight allergen in the work environment and is not by itself diagnostic of OA as discussed elsewhere in this volume. The immediate wheal and flare reaction induced by prick or intradermal skin tests to occupational allergens is associated with an underlying IgE antibody–mediated response, which can also be confirmed by specific IgE in vitro assays as discussed below.

Two types of skin tests are employed, the SPT and the intradermal test, with the prick test having safety and specificity and the intradermal test having greater sensitivity, when done with appropriate positive (histamine) and negative (saline) controls. The SPT is usually performed with antigen concentrations between 1 and 10 mg/mL, approximating 10,000 allergy units/mL, while the intradermal tests are conducted at 100- to 1000-fold lower concentrations.

The low-molecular-weight chemical allergens need to be coupled to appropriate carrier molecules to become complete occupational allergens. Although the classic methods of conjugation, in which the reactive chemical is added to a purified protein carrier such as human serum albumin in solution, have served well, newer methods of conjugation, such as the use of isocyanate vapors to haptenize human albumin, may lead to improved diagnostic skin test reagents (65,66).

The acid anhydrides are prototypic, low-molecular-weight, reactive chemical reagents, which have been shown to form bonds in vivo with specific amino acids on the protein carrier molecules in nasal lavage fluid (67). In addition to anhydride hapten specificity, the coupling of anhydrides to human carrier proteins can induce new antigenic determinants (NAD) and elicit specific antibody responses. These new determinants are characterized for specificity with the chemical hapten, but may not be sufficient to completely define the determinant (68). These new determinants are most likely the result of a conformational change in the protein carrier molecule induced by the adducted hapten.

New biochemical techniques allow the detection of anhydride-adducted proteins in human biologic fluids that have added quantitative precision to the assessment of inhalation exposures encountered by workers in the industrial setting (69). These techniques should allow a better understanding of the exposure–response relationships that lead to immunologic sensitization as determined by in vitro and in vivo tests.

IMMUNOLOGICAL ASSESSMENT BY SEROLOGICAL ASSAYS

History

The first evidence that there was a key serum factor important in allergy (found during a blood transfusion) was obtained in 1919, when a case of asthma caused

Table 1 Examples of Etiologic Agents in Occupational Asthma

Agents	Skin test	Immunoassay	Broncho-provocation	References
Arthropods	+	+	+	11
Azodicarbonamide	+	+	+	12
B. subtilis enzymes	+	+	+	13,14
Buckwheat	+	+	+	15,16
Carmine dye	+	+	+	17
Castor bean	+	+	ND	18
Chloramine-T	+	+	+	19
Chromate	+	+	+	20,21
Clam	+	+	+	22
Coffee bean	+	+	+	23
Coriander (nutmeg shell and paprika)	+	+	+	24
DTFB	ND	+	ND	25
Dimethylethanolamine	ND	ND	+	26
Dyes, textile	+	+	+	27
Egg	+	+	+	28,63
Fennel seed	+	+	+	29
Garlic	+	+	+	30
Grasshoppers	+	+	+	31
Hog trypsin	+	+	+	35
HDI	ND	+	+	32
HHPA	+	+	ND	33,34
Laboratory animals	+	+	ND	36
Latex	+	+	+	37
Locusts	+	+	ND	38
Maple wood dust	+	+	+	39
Mealworm	+	+	+	40
MDI	+	+	+	32,41,64
Mite (red spider mite)	+	+	+	42
Mushroom	+	ND	+	43
Nickel	+	+	+	21,44,45
Papain	+	+	+	46
Pancreatic extract	+	ND	+	47
Penicillin	+	ND	+	48
Penicillamine	+	+	+	49
PA, TCPA	+	+	+	50,51
PA	+	+	+	52
Platinum	+	+	+	53,54
Protease bromelain	+	+	+	55
Senna	+	+	+	56
Shrimp	+	+	+	22
Spiramycin	+	+	+	57
TDI	ND	+	+	58,59
TMA	+	+	+	60
Wheat flour components	+	+	+	15,61,62

Abbreviations: DTFB, diazonium tetrafluoroborate; HDI, hexamethylene diisocyanate; HHPA, hexahydrophthalic anhydride; MDI, diphenylmethane diisocyanate; PA, phthalic anhydride; TCPA, tetrachlorophthalic anhydride; TDI, toluene diisocyanate; TMA, trimellitic anhydride; ND, not defined.

by allergy to horse dander was described (70). Prausnitz and Küstner (71) performed their famous passive transfer of a positive skin test, later called the PK test, in 1921. This serum factor was later called reagin. However, despite efforts during the next 40 years, little progress was achieved with regard to isolation and characterization of reagin. In the late 1960s, Ishizaka et al. (72) and Johansson (73) identified the serum factor capable of mediating allergic reactivity as IgE.

Immunoglobulin E

Human IgE is an immunoglobulin of approximately 190,000 Da that circulates in the blood as a monomer. Its concentration in serum is highly age dependent, and it constitutes approximately 0.0005% of the total serum immunoglobulins in adults. The level of total serum IgE is commonly reported in kilo international units per liter (kIU/L) based on the 75/502 IgE standard from the World Health Organization. Conversion of serum IgE levels to mass per volume (μg/L) units is accomplished by multiplying the kIU/L value by 2.4 (1 kIU/L = 2.4 μg/L) (74). The Fc portion of this reaginic antibody attaches to Fc receptors on the surface of target cells, tissue mast cells, and circulating basophils, leaving the F(ab)2 portion of the molecule available to bind with its homologous antigen. The variable region of IgE has an estimated 10^6 to 10^8 antigen-binding specificities (75). Subsequent allergen exposure causes mast cell surface–bound IgE antibody to be cross-linked, leading to an increase in intracellular calcium, and the release of preformed mediators (e.g., histamine and proteases) and newly synthesized lipid-derived mediators (e.g., leukotrienes and prostaglandins) (74). These mediators induce the physiological and anatomical changes ranging from allergic rhinitis, acute allergic urticaria (hives), and extrinsic bronchial asthma, to generalized anaphylactic shock (76).

Measurement of IgE

The discovery of the role of IgE in clinical allergy subsequently resulted in a new generation of in vitro diagnostic assays to measure serum levels of allergen-specific IgE. The first immunoassays (radioimmunosorbent test or paper radioimmunosorbent test, named as such because paper discs were used as the solid support) were developed to quantitate the serum concentration of total IgE and used radioactive iodine ^{125}I as labels. Total serum IgE is currently the only diagnostic allergy test that is regulated under the Federal Clinical Laboratory Improvement Act of 1988 (CLIA-88). Total IgE is almost exclusively measured by a 2-site (capture and detection antibody), noncompetitive immunometric (labeled antibody) assay in which a solid-phase antihuman IgE is used to bind IgE from human serum. Following removal of unbound serum proteins, a second antihuman IgE that is labeled with a radionuclide, enzyme, or fluorophor detects bound IgE. Removal of unbound detection antibody is followed by development and quantitation of the response signal. The quantity of the final assay response signal (counts per minute bound, optical density, fluorescence signal units, etc.) measured is proportional to the amount of human IgE that is bound in the middle of the sandwich between the capture and detection antibodies (Fig. 1). The analytical sensitivity of most total serum IgE assays is 0.5 to 1 μg/L (74). In normal individuals, IgE is usually present at low levels where 130 ng/mL represents the upper limit of the normal range. However, a significant number of asymptomatic normal individuals, patients with parasitic diseases, and patients with depressed cell-mediated immunity exceed this level. Also, some

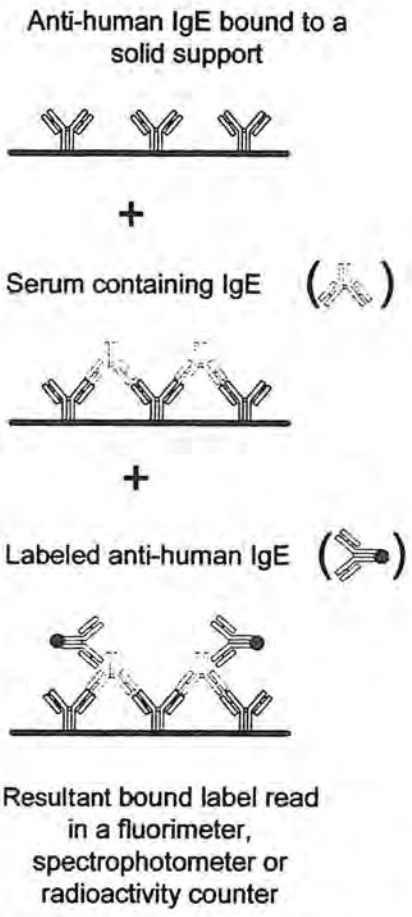

Figure 1 Radioimmunosorbent test (RIST). Antihuman IgE is bound to a solid support, serum is added, and unbound serum proteins are removed. Labeled antihuman IgE is added and the resultant labeled complex measured.

allergic (atopic) persons may exhibit normal total IgE test results in the presence of elevated levels of specific IgE. Although the total serum IgE level is considered useful in the evaluation of an allergic patient, it is more important to demonstrate the presence of allergen-specific IgE in a patient's serum (77).

Measurement of Specific IgE

Allergen-specific IgE was first measured using the RAST in 1967, so called because the secondary antibody was labeled with ^{125}I and an allergen or allergens were bound to an activated (cyanogen bromide) solid support (Sephadex) (77). In brief, a RAST test consists of specific allergen(s) which are bound to a solid-phase support (paper, microtiter well, glass, polystyrene, magnetized beads, or some other surface). Patient serum containing both allergen specific and nonspecific total IgE is incubated with the solid phase material, allowing reaction of the specific IgE in the patient's sample. Excess serum and nonallergen specific IgE is then washed away. Labeled polyclonal or monoclonal antihuman IgE antibody (^{125}I) is added. During this second incubation period, a sandwich complex of allergen-specific IgE and labeled anti-IgE

is formed. A subsequent wash removes unbound labeled antibody. Measurement of the remaining labeled anti-IgE is directly proportional to the patient's allergen-specific IgE (Fig. 2).

The acronym RAST in reality refers only to a test where a radionuclide is used as the label for anti-IgE. Numerous other systems using differing solid supports and differing detection systems (various polyclonal and monoclonal anti-IgE detection antibodies) and labels have been described, such as enzyme allergosorbent test, basically an enzyme linked immunosorbent assay, fluorescence enzyme immunoassay, fluorescence allergosorbent test, etc. Examples of indicator systems (labels) used in RAST are I^{125} for radioimmunoassay systems and alkaline phosphatase, horse radish peroxidase, or urease for enzyme-based immunoassay systems. These assays have been automated to the extent that assay precision and reproducibility have been improved [second-(Pharmacia CAP System) and third-generations (DPC Immulite)] to the level where some IgE antibody assays on autoanalyzers require only singlicate measurements for accurate results (74). In the present chapter, RAST is used to refer to all the specific anti-IgE assays. Systems for measuring specific IgE reactive with

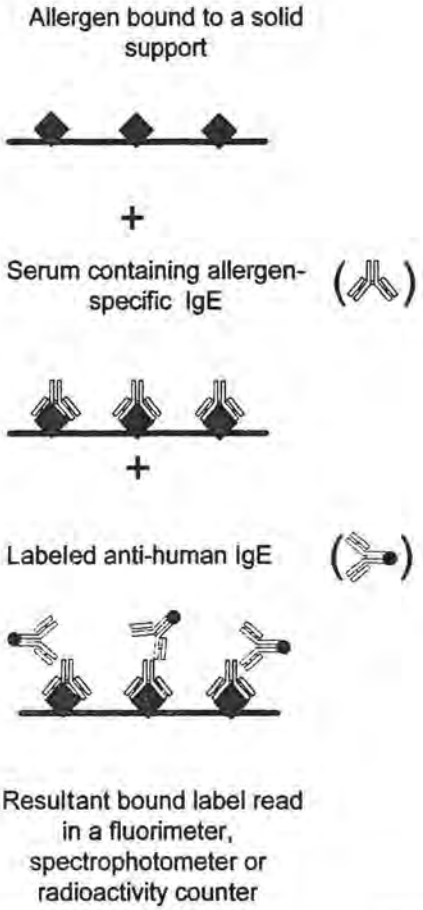

Figure 2 Radioallergosorbent test (RAST). Allergen is bound to a solid support, serum is added, and unbound serum proteins are removed. Labeled antihuman IgE is added and the resultant labeled complex measured.

multiple allergens simultaneously have also been described (78,79). There are also cell based and other technologies for the detection of allergen-specific IgE antibody. These include the measurement of allergen-induced mediator release [histamine and cysteinyl leukotriene C4 (LTC4)], flow cytometric basophil activation assays (CD63 and CD209), etc. These assays, although having great promise, are generally considered as research applications at the present time (80).

The Food and Drug Administration (FDA) defines a RAST test under 21CFR866.5750 as a immunological test system that consists of the reagents used to measure, by immunochemical techniques, the allergen antibodies (antibodies which cause an allergic reaction) specific for a given allergen. Measurement of specific allergen antibodies may aid in the diagnosis of asthma, allergies, and other pulmonary disorders. Some commercial RAST tests are FDA cleared or approved as in vitro diagnostic devices (IVD) through a process known as premarket notification [510(k) program] based on the Medical Device Amendments of 1976 (81). Many RAST tests are 510K cleared as being essentially equivalent to previously cleared assays. The FDA 510K online database (FDA product code "DHB") lists 214 510K cleared RAST tests and/or systems yielding FDA-cleared tests for literally hundreds of environmental, occupational, and food allergens. It should be pointed out that FDA clearance does not guarantee diagnostic performance. In 1996, the FDA introduced a new IVD classification category called analyte-specific reagents (ASR) (81). The FDA defines ASRs as "antibodies, both polyclonal and monoclonal, specific receptor proteins, ligands, nucleic acid sequences, and similar reagents which, through specific binding or chemical reaction with substances in a specimen, are intended for use in a diagnostic application for identification and quantification of an individual chemical substance or ligand in biological specimens." In essence, the FDA recognized ASRs as the active ingredients of in-house tests, which when used in combination with general purpose reagents (such as buffers or reactive materials without specific intended uses) and general purpose laboratory instruments, could be the basis for an assay developed and used by a single laboratory. In addition to those tests to which the regulatory oversight of the FDA applies, laboratories may develop and use in-house tests that are not regulated. Although tests may be useful as a tool in the diagnosis of disease, the responsibility for validation of the test falls to the laboratory developing the test. There are no "rules" for validation of these tests; however, at a minimum such validation should address evaluation of solid-phase binding of antigen or antibody, primary and secondary incubation antibody times, the effect of interfering substances, and nonspecific binding. When developing an assay to determine whether elevated concentrations of allergen-specific IgE are present in a cohort compared with a control population, great care must be exercised in selection of an appropriate control group.

Appropriate comparison groups would generally consist of persons without identifiable excessive exposure to the allergen that is of concern among the "exposed" group. By evaluating clinical samples among the test ("exposed") and comparison ("unexposed") groups, it may be possible to determine whether a statistically elevated concentration of allergen-specific IgE is diagnostically meaningful (82,83).

Many occupational allergens are low-molecular-weight compounds which are not complete antigens (e.g., isocyanates, anhydrides, etc.) (84,85). In vivo, their first interaction is with a native human macromolecule (such as albumin) leading to recognizable epitopes, either on the hapten–protein conjugate or through interaction of the hapten with the constituitive macromolecule leading to the formation of NAD (84). Care must be taken when synthesizing these hapten–protein conjugates for in vitro

serologic testing, as the synthesized hapten–protein conjugates may not be equivalent to those formed in vivo. The National Committee for Clinical Laboratory Standards (NCCLS) is an international, interdisciplinary, nonprofit, standards-developing, educational organization that promotes the development and use of voluntary consensus standards and guidelines within the health care community. The NCCLS has published a guideline that addresses evaluation methods and analytical performance characteristics of immunological assays for human IgE measurements (86).

Interpretation of In Vitro IgE Tests

Determination of specific IgE antibodies to known allergens by SPT or in vitro tests is an important component of an appropriate systematic clinical evaluation for many common medical conditions, including, for example, rhinitis, sinusitis, and reactions related to food allergies (87,88). Contraindications to SPT include generalized skin disease or the inability to discontinue antihistamine use; in these cases, in vitro assays for specific IgE may be useful. In addition, up to 60% of positive SPT results to foods and up to 50% of positive SPT results to latex do not reflect symptomatic allergy (89,90). Clinical accuracy is the basic ability to discriminate between two subclasses of subjects where there is some clinically relevant reason to do so. This concept of clinical accuracy refers to the quality of the initial classification of the subjects based on a diagnostic discriminator. The accuracy of the probing provided by the discriminator is the basis of any comparisons of the usefulness of diagnostic testing. Receiver operating characteristics (ROC) curves is one method to analyze the efficiency of this probing (91,92). ROC plots graphically display the entire spectrum of a test's performance for a particular sample group by demonstrating the ability of a test to discriminate between alternative states of health. The points along the ROC curve represent the sensitivity–specificity pairs corresponding to all possible decision thresholds for defining a positive test result. ROC curves yield a simple graphical method to evaluate the trade-offs obtained between sensitivity and specificity across all test cut offs yielding the possibility to determine, with some confidence, the accuracy of the diagnostic tests used to dichotomize subjects. Choosing the optimal decision is a trade-off between optimizing sensitivity and specificity. For example, sera collected from 311 subjects (131 latex puncture skin test (PST) positive and 180 PST negative) were analyzed for latex-specific IgE antibodies using three FDA-cleared assays. Diagnostic accuracy was evaluated using ROC curves in relation to the subjects' PST status and the results of the immunoassays. ROC areas under the curve (AUCs) based on PST for the three diagnostic tests were 0.858 ± 0.024, 0.869 ± 0.024, and 0.924 ± 0.017. One system had a significantly greater AUC based on PST than those observed for the other two ($p < 0.05$). When the diagnostic tests were probed as to the cutoffs giving maximal diagnostic efficiency compared to PST, two tests yielded values of less than 0.35 kU/L of allergen IgE, while the other yielded 0.11 kU/L. The diagnostic efficiencies based on PST at these cutoffs were 87.1%, 88.1%, and 88.7%, respectively (92). Likelihood ratios of a positive test (LR+) and the likelihood ratio of a negative test (LR–) can also be calculated from diagnostic sensitivity and specificity where LR+ = sensitivity/(1–specificity) and LR– = (1 – sensitivity)/(specificity).

The optimal decision thresholds obtained in this type of analysis are selected assuming that the cost of a false-positive result and the cost of a false-negative result were equal, but this may not be the case in some clinical applications. The optimal decision threshold for a specific clinical application involves a number of factors that

are not properties of the testing system; rather they are properties of the clinical application. These include prevalence, the outcomes and the relative values of those outcomes, the costs to the patient and others of incorrect classification (false positive and false negative classifications), and the costs and benefits of various interventions. These characteristics interact with the test results to affect usefulness. Methods for determining the optimal decision threshold based on the prevalence and the costs of incorrect classification have been developed (92). In general, a higher decision threshold is preferred if the prevalence is low or if the cost of a false positive result is greater than the cost of a false negative result. A lower decision threshold is preferred if the prevalence is high or if the cost of a false negative result is greater than the cost of a false positive result. These data assume that sensitivity and specificity are inherent properties of the test and thus, independent of prevalence. Although this is generally assumed to be the case, sensitivity and specificity may vary among different subpopulations and thus, are dependent on the composition of the population under study (93). Unlike sensitivity and specificity, diagnostic efficiency is dependent on disease prevalence and the prevalence in the study sample may not be representative of the prevalence in the target population in some clinical applications; thus, the diagnostic efficiencies reported for the assays cannot be generalized to other clinical applications. Another disadvantage of comparing diagnostic efficiencies of different tests is that two tests may have the same diagnostic efficiency, but perform quite differently. For example, one test may result in many false positives and few false negatives, whereas another test may result in many false negatives but few false positives. ROC analyses also provide support for the hypothesis that IgE antibody assays can detect different subsets of IgE antibody of a given specificity; possibly as a result, clinical tests intended for disease diagnosis may not perform optimally when used in low prevalence populations for a condition such as IgE sensitization (94). Clinical tests (such as RAST) are optimized for use in evaluating patient populations where there is usually a high pretest probability for the condition of interest. Assuming constant sensitivity and specificity, the higher the true prevalence of a condition, the more accurately a test will identify the prevalence of that condition in a population. If a condition is present at low prevalence, a larger proportion of test positives will be false positives, resulting in poor positive predictive value of the test and overestimation of prevalence (92,95,96).

NONINVASIVE ASSESSMENT OF AIRWAY INFLAMMATION

Use of Sputum Cell Counts as a Noninvasive Assessment of Airway Inflammation

Induced sputum analysis is a reproducible, valid, responsive, and noninvasive method for studying airway inflammation. It comprises two steps: sputum induction and processing. Sputum induction involves inducing sputum production by inhalation of a hypertonic saline solution. A task force composed of experts in the field of sputum analysis have tried to standardize the methods for inducing and processing sputum under the auspices of the European Respiratory Society (97,98). Their conclusions are summarized in a supplement of the European Respiratory Journal (97,98).

Several methods for inducing sputum have been proposed using different types of nebulizers with different outputs. The nebulizer output and inhalation time can influence the sputum cell count and fluid phase measurements (99). An ultrasonic nebulizer with an output of at least 1 mL/min achieves a high success rate (97).

Different methods have been proposed to process induced sputum. Some methods use the entire sample collected, whereas others select denser portions from the expectorated sample using an inverted microscope. Both methods have advantages and disadvantages. The whole selection method is quicker and does not necessitate an inverted microscope, but the reading of the slides may be more difficult especially when there is a high squamous cell contamination. Indeed, the reproducibility of cell counts seems to be lower when the squamous cell contamination exceeds 20% of all recovered cells (100). Although one study reported a higher percentage of eosinophil count on using the method of selecting denser portions of sputum compared with the method using the entire sample, the differential cell counts obtained after processing the entire sputum samples or selected samples have been reported to be similar in others (101–103). One method or the other can be used reliably, but it is important to remember that these two methods are not interchangeable. The same method should always be followed within the same study. The different steps of sputum processing according to the Sputum Task Force are described in Figure 3 (98). The reproducibility of sputum cell count has been clearly demonstrated for differential cell counts in healthy subjects, asthmatics, and smokers. Compared with healthy subjects, asthmatics had increased sputum cell counts for eosinophils, metachromatic cells, and neutrophils as well as for markers of inflammatory cell activation (104). It has been demonstrated in several studies that sputum inflammatory indices such as eosinophils and eosinophil cationic protein are increased by exposure to common allergens or by reduction of steroid treatment whereas treatment with coricosteroids leads to a reduction in these indices (105–112). There is also evidence that the use of sputum eosinophils improves the management of asthma by decreasing the number of asthma exacerbations (113).

Changes in Sputum Cell Counts After Exposure to Occupational Agents in the Laboratory

The changes in airway inflammation have mainly been assessed after exposure to occupational agents in the laboratory. In the majority of cases, sputum eosinophilia has been observed after exposure to occupational agents. Indeed, sputum eosinophils have been shown to increase after exposure to both high- and low-molecular-weight agents such as isocyanates, red cedar, or cyanoacrylate (109,114–117). However, there are examples where exposure to occupational agents can predominantly induce a sputum neutrophilia. Increase in sputum neutrophils has been observed mainly after exposure to isocyanates, but also after exposure to other agents, for example, metalworking fluid or grain dust (118–121). The type of asthmatic reaction and the intensity of airway inflammation induced by exposure to isocyanates can be influenced by the concentration and the length of exposure to these agents (122). Indeed, isocyanate-induced asthma seems to be enhanced when isocyanates are generated at low concentrations for a long period of time, compared to when they are generated at a higher concentration for a shorter duration of exposure.

Changes in Sputum Cell Counts After Exposure to Occupational Agents at the Workplace

Only a few studies have investigated the changes in induced sputum at the workplace. Subjects with OA and asthmatics without OA working in the same environment were investigated during periods at work and away from work (123). Sputum induction

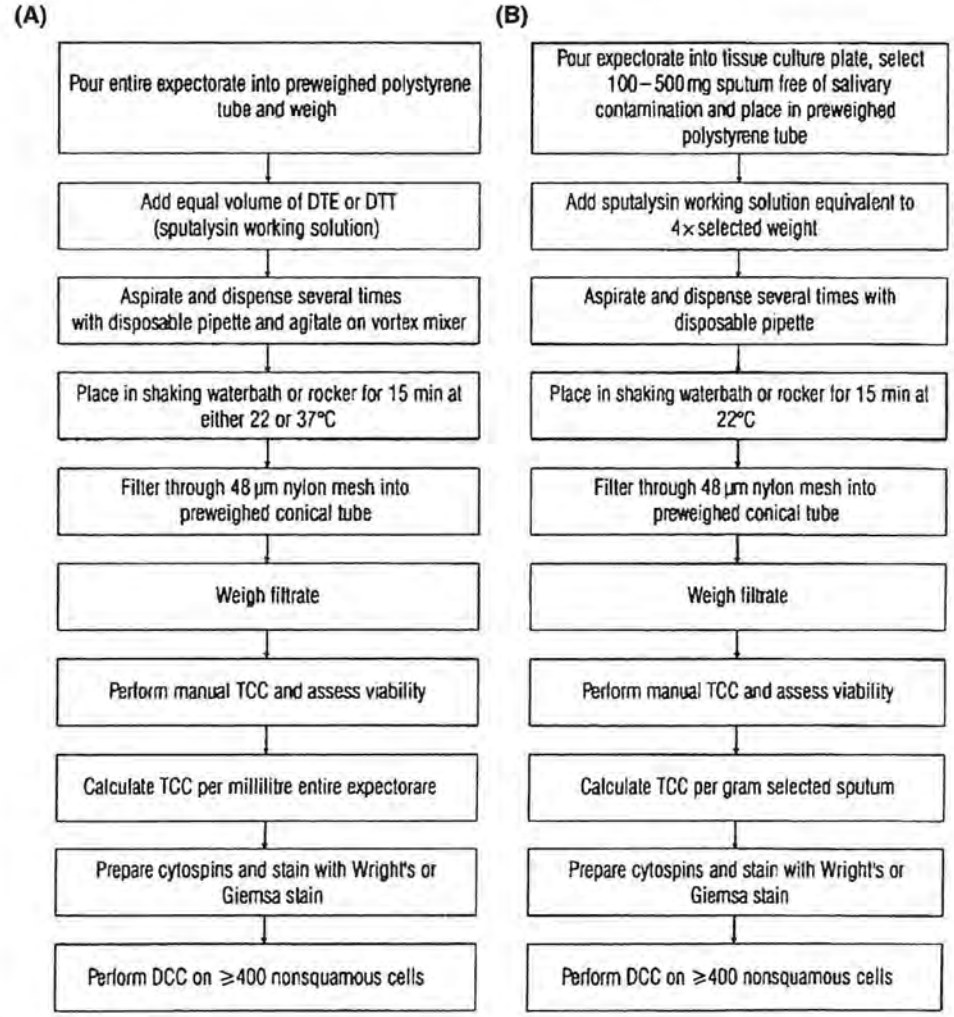

Figure 3 Sputum processing method for: (**A**) entire sputum and (**B**) selected sputum. *Abbreviations*: DCC, differential cell count; DTE, dithioerythritol; DTT, dithiothreitol; TCC, total cell count. *Source*: From Ref. 98.

was performed at the end of these periods. The subjects with OA had an increase in sputum eosinophils when at work, which resolved when they were removed from their workplace, whereas asthmatics, without OA working in the same environment, did not show any change in airway inflammation. Another study investigated the changes in induced sputum in 38 subjects with OA caused by exposure to low-molecular-weight agents while working (124). The diagnosis of OA was confirmed by PEF monitoring in 36 subjects. Twelve of them also underwent specific inhalation challenge (SIC), which were positive. Only 14 had sputum eosinophils greater than 2.2%, when at work. The authors reported that subjects had sputum neutrophilia (59% of neutrophils), but there was no comparison with periods away from work. A subject exposed to metalworking fluid was reported to have marked increase in neutrophils when at work, which resolved after periods

away from work (120). The sputum findings were mirrored by corresponding changes in spirometry and PC_{20} methacholine.

Recently, the addition of sputum cell counts to the monitoring of PEF was shown to increase the specificity of this test compared with SIC by 18% [range (*r*): 13.7–25.5] or 26.8% (*r*: 24.8–30.4) depending on whether an increase of sputum eosinophils greater than 1% or 2% when at work was considered significant (125). When at work, subjects with positive SIC had a significant increase in sputum eosinophils, whereas the group with a negative SIC had higher neutrophils compared with the periods away from work. The mechanisms explaining the neutrophilic inflammation are unclear, but may be due to an irritant effect of agents at the workplace, in asthmatic subjects. Further research is needed to assess to which extent neutrophilic inflammation can be influenced by smoking, inhaled corticosteroid treatment, or exposure to irritant agents in subjects with work-aggravated asthma.

Occupational Eosinophilic Bronchitis

The analysis of induced sputum has led to the identification of a condition causing chronic cough without airflow limitation or airway hyperresponsiveness. This condition, called "eosinophilic bronchitis," was originally described in 1989 (126). The exposure to occupational agents can also cause eosinophilic bronchitis. This condition was initially reported in a subject who developed respiratory symptoms upon exposure to cyanoacrylates and was labeled "occupational eosinophilic bronchitis" (127). Exposure to cyanoacrylate during periods of occupational exposure was shown to induce eosinophilic bronchitis without any change in forced expiratory volume in one second (FEV_1) or PC_{20}; this condition resolved when the patient was away from work and recurred when she returned to the workplace. Sputum eosinophilia was also induced by the exposure to cyanoacrylate in the laboratory. Since this first report was published, eosinophilic bronchitis has been reported after exposure to a number of occupational agents such as latex, mushroom spores, and lysozyme (128–131). This condition is likely to be underestimated, because analysis of induced sputum is not routinely performed in many centers. However, this test should be done when respiratory symptoms, mainly cough, are exacerbated in the workplace in spite of normal airway responsiveness and normal FEV_1. Although it is unknown whether subjects with occupational eosinophilic bronchitis will go on to develop OA if they stay exposed to the offending agent, it would seem medically prudent to remove them from the workplace.

DISCUSSION OF ILLUSTRATIVE CASE HISTORY

The clinical case presented at the beginning of this chapter illustrates the importance of a thorough immunological assessment to confirm the sensitization to an occupational agent. It also emphasizes the importance of suspecting an occupational eosinophilic bronchitis, when there is no evidence of asthma when the subject is working.

The sensitization can be detected by SPTs, which are the most sensitive tests to detect the sensitization to an allergen. However, there are situations where SPT cannot be performed. Indeed, there are no allergenic extracts available for testing the majority of low-molecular-weight agents. In the present case, OA due to platinum salts is clearly IgE dependent and allergenic extracts for platinum salts are available. However, SPT cannot be interpreted reliably when subjects are taking antihistamines

or are experiencing dermographism. In these situations, RAST can demonstrate the presence of specific IgE directed against the suspected occupational agent.

This clinical case also highlights the need for investigating airway inflammation at work and away from work to explore the possibility of an occupational eosinophilic bronchitis when there is no objective evidence of asthma. Indeed, the subject discussed here was sensitized to platinum salts, complained of respiratory symptoms, but did not show any objective feature of asthma—no airflow limitation, no PEF variability, normal PC_{20}—when at work. In these conditions, eosinophilic bronchitis is suspected. Performing sputum induction after periods at work and away from work is the easiest way to confirm this diagnosis.

DIRECTIONS OF FUTURE RESEARCH

- Immunologic testing that assesses IgE-mediated sensitization to occupational allergens depends on availability of well-characterized test allergens. There is a critical need to produce standardized occupational allergens that can be immunologically characterized and then shared between those groups employing in vivo or in vitro tests in assessing OA.
- Laboratories utilize a variety of specific IgE and IgG immunoassay protocols. Multicenter validation studies are needed to determine which assays and antigens exhibit optimal performance in identifying those workers with OA. Adaption of common antigens and protocols by different laboratories would allow for meaningful interpretation of results obtained in individual workers and in large cohorts.

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ASTHMA IN THE WORKPLACE

And Related Conditions

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