1
The Evolution of Immunotoxicology*

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1.1
Introduction

The origins of immunotoxicology surprisingly date back to the seventeenth century when Bernardino Ramazzini, an Italian medical professor, described lung disease associated with various occupations including baking, grain handling, and mining [1]. It was not until the early 1900s, however, that the immune system was implicated and the causative agents first identified. Since then various pharmaceutical, occupational, and environmental agents have been shown to potentially influence many facets of immune-mediated diseases including allergy, immunosuppression, autoimmunity, and chronic inflammation. The following is a brief historical perspective of what we now refer to as immunotoxicology.

1.2
Immune-Mediated Environmental Lung Diseases

The most studied environmentally induced lung disease is occupational asthma, which was first described by Henry Slater in 1866 as “hyperresponsiveness provoked by exposure to chemical and mechanical irritants, as well as to particular atmospheres” [2]. It was Ehrlich, however, who described the presence of eosinophils in the sputum of workers, which is now considered a hallmark of immune-mediated asthma (reviewed by Hirsch et al. [3]). In the mid-twentieth century, it was shown that occupational asthma can be caused by two distinct groups of agents. The first group consists of proteins such as alanase, an enzyme found in soap detergent, latex, and flour, the cause of baker’s asthma [4]. The second group represents small molecular weight, highly reactive chemicals

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that behave as haptens, such as various anhydrides and isocyanates [5]. Our understanding of how allergic responses can occur from low molecular weight chemicals originated from the pioneering studies of Landsteiner and Jacobs [6] who showed that when these chemicals covalently bind to host proteins they become antigenic (i.e., act as haptens). Late in the twentieth century, based initially on epidemiological observations of increasing asthma rates in industrialized cities and shortly after on experimental animal studies, it was shown that many common air pollutants do not cause allergic asthma but can exacerbate existing asthma by acting as adjuvants [7]. This seminal finding was followed by epidemiological studies suggesting that co-exposure to bacterial endotoxins early in life leads to a reduced likelihood of developing asthma, often referred to as the hygiene hypothesis [8] and suggested that early stimulus of the immune system is important for its normal maturation.

Immune-mediated environmental lung diseases also exist that are not Type 1 (IgE) reactions. For example, chronic beryllium disease (CBD), first described by Sterner and Eisenbud in 1951 [9], is a granulomatous lung disease representing a Type 4 immune reaction. CBD occurs most often in beryllium workers who possess the HLA DPB1 genotype with glutamic acid at amino acid position 69 [10]. This was an important observation as genetic testing is now often conducted in workers in industries that use beryllium to help identify those individuals that may be at high risk of developing the disease. Hypersensitivity pneumonitis, caused by microbes, animal and plant proteins, and low molecular weight chemicals, leads to Type 3 and 4 immune reactions involving immune complexes and complement. First identified in the 1930s [11], it produces noncaseating lung granulomas and is the cause of pigeon breeder’s lung, among others.

A number of immune-mediated lung diseases have been described in which innate immunity, rather than adaptive immunity, is primarily responsible for pathology. Byssinosis (aka brown lung disease) is believed to be caused by exposure to the endotoxin in cotton dust and often occurs in inadequately ventilated working environments [12]. The severe inflammatory response that occurs, if exposure persists, can result in narrowing of the airways and scarring of the lung. Chronic inflammatory lung disease can also be induced by inhalation of various amphiboles and silica resulting in asbestosis and silicosis, respectively. Both of these fibers can interact with the nucleotide-binding domain leucine-rich repeat containing (NLR) inflammasome, causing it to function abnormally [13]. The pathology that results is caused by long-term interplay between free radicals and expression of cytokines and growth factors, which ultimately leads to the release and deposition of collagen and other extracellular matrix components by mesenchymal cells [14]. While asbestosis was observed in early mine workers in ancient Egypt, it was not until the 1920s that studies from the United Kingdom unequivocally demonstrated a link between asbestos miners and asbestosis and demonstrated a high prevalence (>25%) of the disease among workers [15, 16].
1.3 Immunotoxic Drug Reactions

Many idiosyncratic drug reactions have often been shown to be allergic in nature, either producing autoallergy, in which the immune response is directed to self-tissues, or stimulating a specific immune response against a drug [17]. Much of our early understanding of human drug allergy originated from studies of β-lactam antibiotics, particularly penicillin, which can produce a Type 1 reaction [18]. These early studies helped demonstrate the importance of genetic variability in the development of an immunotoxic drug reaction and in particular genetic variants that control drug metabolism and the HLA gene region that controls epitope recognition [19]. The former is best exemplified by the gene variants that regulate N-acetyltransferase activity in the development of drug-induced lupus erythematosus [20] and the latter in abacavir-induced hypersensitivity, which is so strongly associated with HLA-B*5701 that it can be used as a prescreen for contraindication [21].

1.4 Autoimmunity

Establishing an association between autoimmune disease and immunotoxicology is challenging for a number of reasons. In addition to the fact that there are different types of autoimmune diseases with different organ targets and pathology, there are intrinsic factors (e.g., specific gene polymorphisms, sex-related hormones, and age) and extrinsic factors (e.g., lifestyle, infectious agents) that play varying roles in disease causation. The issue of whether xenobiotics induce disease or simply exacerbate preexisting disease through immunomodulation is often complicated. It is clear that agents such as streptozotocin and the rotenide, pyrinnuron (removed from the US market in 1979), destroy pancreatic beta cells, resulting in type 1 diabetes but this can occur independently of the immune system. Regarding drug-induced autoimmunity, Hoffman in 1945 [22] first observed that administration of sulfadiazine often coincided with the development of systemic lupus erythematosus (SLE), and since then over 35 drugs have been implicated in the onset of autoimmune responses and autoimmune-like diseases [23–25]. Drug-induced autoimmune diseases, however, are different from the classical spontaneous counterpart as they are usually milder, there is minimal organ involvement, autoantibodies to native DNA are seldom observed in the circulation, and disease remission occurs following cessation of drug treatment.

In contrast to these pharmaceuticals, certain environmental chemicals may induce or exacerbate preexisting autoimmune diseases. Since the observation by Pernis et al. in the 1960s [26] of increased prevalence of rheumatoid factor in asbestos-exposed individuals, there has been a growing body of epidemiological and experimental evidence that exposure to fibrogenic fibers including crystalline silica and asbestos as well as to several heavy metals and solvents is associated
with systemic autoimmune diseases [27, 28]. Silica-exposed workers are at an elevated risk for a number of systemic autoimmune diseases, including rheumatoid arthritis (aka Caplan’s syndrome), systemic sclerosis, SLE, and antineutrophil cytoplasmic antibody (ANCA)-related vasculitis/nephritis. Epidemiological studies have also shown a higher than expected risk of systemic autoimmune disease among asbestos-exposed populations [29]. While some consider these adjuvant effects, there is increasing evidence that these diseases are under T-cell regulation, specifically Tregs [30].

Solvent exposure in workers has also been associated with autoimmune disease. For example, a meta-analysis, looking at 10 different epidemiological studies, showed a fairly modest but consistent association between solvent exposure, particularly trichloroethylene, and systemic sclerosis or connective disuse disorders that were likely autoimmune in nature [31]. Epidemiological studies, case reports, and animal studies have also suggested that exposure to mercury contributes to idiosyncratic autoimmune disease in humans. While in most cases these epidemiological studies were underpowered, they have been supportive of experimental animal studies with genetically developed autoimmune-prone rodents [28].

1.5 Immunosuppression

During the 1970s, increasing numbers of studies were published demonstrating that certain agents produce immunosuppression. Initially, the majority of these studies focused on a small set of chemical classes such as heavy metals, halogenated aromatic hydrocarbons, abused drugs (e.g., tobacco smoke and alcohol), and air pollutants, in which case the focus was on the lung rather than on systemic immunity [32–36]. These studies were initially limited to experimental animal models but were soon followed by epidemiological studies that were usually supportive but cross-sectional in nature and often underpowered. The health outcomes most commonly observed were increases in the incidence of certain cancers, such as non-Hodgkin’s lymphoma, or respiratory infections (e.g., [37–39]). Establishing a direct link between immunotoxic exposure and clinical disease in humans was and remains controversial because of the inherent limitations of epidemiological studies in drawing causal conclusions, particularly for common diseases such as respiratory infections.

During this early period, the experimental methods adopted by immunotoxicologists to assess immune function in animals were those common to most immunology laboratories. In addition, the tests that were commonly performed and the experimental design by which they were conducted in these laboratories were ad hoc in nature. Even the experimental species selected varied with the earliest studies, commonly using rabbits and guinea pigs. While the mouse initially became the test species of choice, debates occurred on the use of the mouse versus the rat as those investigators initially trained in toxicology usually preferred the rat to allow comparisons to other toxicology studies, and those
trained in immunology preferred the mouse as the mouse immune system was well studied. Currently, this distinction is usually not of concern for regulatory purposes as subsequent validation studies were conducted in both mice and rats and for the most part the results were comparable [40–42].

To address the lack of standardized testing, a “Tier” approach was suggested with the idea that each subsequent tier provided an opportunity to better define a specific target within the immune system. Subsequently, the National Toxicology Program (NTP) organized a series of workshops composed of experts in immunotoxicology, basic immunology, toxicology, risk assessment, epidemiology, and clinical medicine to help identify the most appropriate tests for immunotoxicology testing [43]. Two major points were agreed on from these workshops. First, the immune system is not fully operational until it is challenged and, thus, the most appropriate strategy would be to incorporate antigen challenge. Secondly, as it may be construed that an inadequate response to antigenic challenge does not represent an “adverse effect,” tests should be included that could be readily identified with disease. The former recommendation highlighted several common assays including measurement of an antibody response following as a measure of humoral immunity and quantification of delayed hypersensitive response (DHR) or cytotoxic T-lymphocyte response (CTL) as a measure for cell-mediated immunity. These assays were based on measurement of a primary immune response, rather than the secondary, since it was agreed that the memory response is less sensitive to inhibition. To address the need to identify a clear adverse effect, a set of tests, usually referred to as host resistance assays was suggested. These tests would also be used to validate the usefulness of other methods. An interlaboratory validation effort involving four laboratories and sponsored by the NTP was conducted using Tier 1 and 2 tests [44]. In addition to demonstrating interlaboratory reproducibility, this effort helped identify the relative sensitivity of the various immune tests and the degree to which they agreed with commonly employed host susceptibility tests. This effort was followed several years later in which the concordance between various histological, hematological, and immune function tests to identify immunotoxicity and host susceptibility changes were determined using a large database [45, 46]. These latter studies were important not only as a validation exercise for tier testing but also to help provide a basis to use immunotoxicology data in risk assessment. The analyses indicated that inclusion of a functional test, most notably the T-dependent antibody response (TDAR) to sheep red blood cells (now often replaced with keyhole limpet hemocyanin), along with a nonfunctional test, such as thymus weights, allowed achieving a high level of concordance, with respect to identifying potential immunotoxic agents.

Tiered screening panels have been the basis for several risk assessment guidelines, and most regulatory agencies in the United States, European Union, and Japan have established or are developing requirements or guidelines [47]. It should be noted that the configurations of these testing panels vary depending on the agency/organization/program under which they are conducted. The most notable
difference is whether a functional immune test (i.e., incorporates antigen challenge) is included in Tier 1 or Tier 2.

There have also been efforts to establish a test panel to assess the immune system of humans for immunotoxicity. The US National Academy of Sciences (NAS) and the International Program on Chemical Safety of the World Health Organization (IPCS/WHO) have proposed a three tier testing scheme to be used for epidemiologic studies of known or suspected immunotoxic agents [48, 49]. Foremost in these various testing schemes is the inclusion of tests in which the immune response following vaccination is assessed.

1.6 Allergic Contact Dermatitis (ACD)

Allergic contact dermatitis (ACD) was first described by Benacerraf and Gell in 1959 following observations that a response that mimics the reactions seen to poison ivy and various industrial chemicals could be induced experimentally by painting hapten on the skin [50]. Over the following 50 years, ACD has received the most attention worldwide within the area of immunotoxicology. This interest originated from observations that cosmetic ingredients including fragrances as well as industrial, therapeutic, and consumer products that come into contact with the skin frequently were often responsible for dermatitis. This stimulated government agencies, industry, and medical professionals to try to limit their exposure through animal testing. The first definition of a real predictive test came from the work of Draize et al. [51]. Since then, numerous protocols have been described whose aims have been, in one way or another, to make improvements to the sensitivity and predictability using guinea pigs as a surrogate for man. All these test protocols followed similar principles; a combination of intradermal and/or epicutaneous treatments is administered to guinea pigs, with or without adjuvant, over a several week period in an attempt to induce skin sensitization, then a 1–2 week rest period to allow for the immune response to mature, followed finally by a topical challenge to assess the extent to which skin sensitization might have been induced. Evaluation of the skin reactions was usually by subjective visual assessments 24–48 h after the challenge application, the main reaction element being erythema. The protocols of Magnusson and Kligman [52] and Buehler [53] were the two most studied and accepted guinea pig methods used for regulatory purposes worldwide [54].

Over the last several decades the Local Lymph Node Assay (LLNA) has replaced traditional guinea pig models and is now routinely used as a validated alternative approach for skin sensitization testing as it provides important animal welfare benefits. The method evokes lymph node cell proliferative responses induced in mice following repeated topical exposure to a test material as a relative measure of sensitizing potential [55]. Not least due to the improved animal welfare benefits, the LLNA has become the preferred method for assessing skin sensitization hazard for various regulatory authorities in most developed countries. The LLNA
was subjected to rigorous independent scrutiny and validated by the International Coordinating Committee on the Validation of Alternative Methods (ICCVAM) [56] with a similar endorsement by the European Centre for the Validation of Alternative Methods (ECVAM) [57]. With the pressure of limiting animal testing, particularly in Europe, *in vitro* tests are currently being developed to replace the LLNA.

1.7

Summary

A brief historical perspective of immunotoxicology is presented. Although the term *immunotoxicology* was first coined in the late 1970s at a WHO sponsored meeting held in Luxembourg, it would appear that the human immune system has been negatively impacted by various xenobiotics for centuries as initially evidenced by impaired lung function in asbestos miners as far back as in ancient Egypt. There is a need for continued vigilance in this area and significant challenges remain. One of these challenges is in the area of biotherapeutics, as exemplified by the well-publicized unexpected adverse events observed in clinical trials with the immunomodulatory molecule, anti-CD28 superagonist mAb (TGN-1412) [58].

References


