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Immunotoxicity testing using human primary leukocytes: An adjunct approach for the evaluation of human risk

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Abstract

Historically, immunotoxicity testing for chemicals, pesticides and pharmaceuticals has relied heavily on animal models to identify effects on the immune system followed by extrapolation to humans. Substantial progress has been made in the past decade on understanding human immune cell regulation, adaptive and innate immune responses and its modulation. The human immune system is complex and there exists diversity within composition, localization, and activation of different immune cell types between individuals. The inherent variation in human populations owing to genetics and environment can have a significant influence on the response of the immune system to infectious agents, drugs, chemicals and other environmental factors. Several recent reports have highlighted that mouse models of sepsis and inflammation are poorly predictive of human disease physiology and pathology. Rodent and human immune cells differ in the expression of cell surface proteins and phenotypes expressed in disease models, which may significantly influence the mechanism of action of xenobiotics and susceptibility yielding a different profile of activity across animal species. In the light of these differences and recent trends toward precision medicine, personalized therapies and the 3Rs (reduce, replace and refine animal use) approaches, the importance of using 'all human' model systems cannot be overstated. Hence, this opinion piece aims to discuss new models used to assess the effects of environmental contaminants and immune modulators on the immune response in human cells, the advantages and challenges of using human primary cells in immunotoxicology research and the implication for the future of immunotoxicity testing.

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Human immunotoxicity, Immunotoxicology, Risk assessment, Primary human leukocytes.

1. Historical perspective

In the late-1970's there was a growing recognition that the immune system is a sensitive target organ for chemical-mediated modulation, which led to the establishment of immunotoxicology as a sub discipline of toxicology. Examples already existed of chemical-mediated hypersensitivity in the occupational setting. In 1915, Prosser White described contact dermatitis in "The Dermatergoses of Occupational Affections of the Skin" (England). An even greater concern was perceived by chemical-mediated immune suppression, which in the worst case could result in impaired immune surveillance of transformed neoplastic cells and an increased incidence of various types of cancers. With funding from the National Toxicology Program (NTP), a testing approach was developed and validated using several already established immunotoxicants [1]. Specifically, the goal of the NTP funded effort was to develop a simple testing battery of immunological tests that could be conducted in combination with traditional toxicology and carcinogenicity studies.

The initial immunotoxicology testing battery consisted of two tiers of assays. The underlying strategy being that if a compound or agent gave negative results in Tier I assays, there was a presumption that the xenobiotic was unlikely to be an immunotoxicant [1]. In the event of a positive Tier I outcome, additional Tier II assays could be performed to obtain more insights concerning immunotoxic potential. Due to the diversity of the cell-types that comprise the immune system and their broad range of functions, Tier I assays included assessments of antigen-induced humoral (anti-sheep red blood cell IgM antibody response) and cell-mediated immunity (mixed lymphocyte response), proliferation induced by polyclonal stimuli and NK cell function. In addition, hematology, lymphoid organ and body weights, cellularity of spleen and thymus, and histopathology of lymphoid organs (spleen, thymus and lymph nodes) were included. Tier II included evaluation of the anti-sheep red blood cell IgG response, delayed type hypersensitivity, cytotoxic T cell response and host resistance to a live pathogen (bacterial, viral or parasitic).

The animal species of choice for conducting the immunotoxicology tier testing battery and which has widely persisted for over four decades has been the mouse. The mouse was initially selected based on a number of pragmatic factors such as the similarity of the mouse immune system to human, especially from a 1970's perspective. As immunologists embraced the mouse as their model of choice, reagents for studying the mouse immune system continued to be most abundant of any animal species. Also the genetics of the mouse was well characterized with many strains commercially available for experimentation. Conversely, a major drawback of conducting immunotoxicity testing in mice was that the majority of routine toxicity and carcinogenicity testing was and continues to be conducted in the rat. Presently, most of the assays in the original immunotoxicology testing battery have been adapted to the rat.

2. Rationale for immunotoxicology testing in human primary leukocytes

The aim of this opinion piece is not to advocate for replacing the use of rodent models in immunotoxicology with human primary cells, but rather to interject a bit of caution and also to discuss why and under which circumstances utilization of human primary leukocytes could represent a useful adjunct to studies with rodent cells.

A strong argument can be made that during the past 40+ years the immunotoxicology testing battery, as conducted in rodents, has been effective in identifying immunotoxicants and has successfully protected the general population. In fact, one would be hard pressed to identify agents that gave a negative result in the mouse testing battery and were subsequently found to be human immunotoxicants. The converse to this argument is, how do we know that the mouse testing battery has not either missed agents that possess human immunotoxic activity or yielded false positives? To the later point, in comparative studies of three compounds well established to suppress the mouse IgM response, arsenic, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and benzo[a]pyrene-7,8,-dihydrodiol-9,10-epoxide, one out of three, arsenic, did not suppress the human primary B cell IgM response [2]. In the case of agents possessing weak or even moderate immunotoxic activity, in the absence of testing in a human system, how would immunotoxicity be identified? To build on this line of reasoning, in a not so recent opinion piece, the immunologist Mark Davis [3] noted that with the exception of leukocyte counts there are few clinical metrics, even to this day, that are used to assess human immune competence. In other words there is no common standard that defines a "healthy" human immune system. This is in striking contrast for example to the other organ systems such as the cardiovascular system, renal system and pulmonary system, to name a few.

Over the past several decades, there has been an increasing effort to compare the mouse and human immune systems. In light of the fact that the two species diverged approximately 70 million years ago, it is not surprising, that important differences have been identified with respect to immune development, activation, effector responses and transcriptional programs. Many of these differences have been summarized in a number of recent reviews [4,5]. Likewise, in a recent study genomic responses to acute inflammatory stressors were compared between mouse models and in humans. These comparisons showed a remarkably poor correlation between the two species with the authors noting that the changes in differentially expressed genes in response to inflammatory stimuli in human and murine orthologs was close to random. In a recent study of differentially regulated genes in activated B cells by TCDD across three species, time course studies identified 515 human, 2371 mouse and 712 rat orthologous genes over a 24-h period, respectively. Only 28 orthologs were differentially expressed in response to TCDD in all three species with many of those representing what has typically been recognized as genes within the aryl hydrocarbon receptor (AhR) battery; those largely consisting of drug metabolizing enzymes (e.g., cytochrome P-450 1A1) [6]. The overall conclusion from the study was that despite the conservation of the AhR and its signaling mechanism, TCDD elicits species-specific gene expression changes and that the mechanism of B cell IgM suppression is likely different across these three species [6]. Collectively, the above findings raise questions concerning the reliability of solely depending on rodent models for identifying human immunotoxicants.

3. Current approaches to immunotoxicity testing using primary human leukocytes

Immunology as a field has delved into human immunobiology somewhat reluctantly with the mouse still representing the most widely used model. Yet the application of various new technologies has begun to yield insights into the complex biology of the human immune system. Multi-parametric flow cytometry has allowed for independent detection of a large number of proteins simultaneously on single cells thereby generating multi-dimensional data. One of the most common applications of flow cytometry in immunotoxicology has been its use in revealing significant alterations in common and rare immune cell populations in the presence of a xenobiotic. Changes in the relative proportions and absolute numbers of immune cells as markers of immunological integrity or significant deviations from the reference ranges upon impact of a potential immunotoxic agent can be very informative of specific target effects on a given population of immune cells. A recent study used a novel flow cytometry based approach, single cell network profiling (SCNP) to simultaneously