Invited Perspective: Key Characteristics as a Starting Point for Improved Hazard Identification of Immunotoxic Agents

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In this issue, Germolec et al.[1] identified "key characteristics" (KCs) for immunoactive substances and described how such characteristics could be applied to improve hazard assessment of potentially immunomodulatory agents. KCs, or the properties associated with potential hazards, have been described for other classes of agents. For example, Smith et al.[2] described 10 KCs for chemical carcinogens that can be used to provide a basis for systematically identifying, organizing, and summarizing mechanistic information as part of the process for evaluating carcinogens. The KCs described by Smith et al.[2] are commonly observed properties of carcinogenic agents that can encompass many different types of mechanistic end points but are not considered outright mechanisms. KCs are the events that cause observed pathologies, not the pathologies themselves (Figure 1). Germolec et al.[1] used approaches similar to Smith et al.[2] for applying the KC concept to immunotoxic agents. This established approach addresses an important need because the effects of exogenous agents on the immune system can affect disease risk across multiple systems and across an organism's life span.[3]

Germolec et al.[1] considered data from agents that are known to induce either immune suppression or inappropriate immune stimulation. Immunosuppression is a reduced ability of the immune system to respond to a challenge regardless of whether clinical disease is present, whereas activation of the immune system may result in a hypersensitivity response or autoimmune reaction.[4] A range of effects—including cancer, reduced resistance to infectious pathogens, allergies, asthma, and autoimmune diseases—can arise due to exogenous agent-mediated immune dysfunction.[3] One feature of the KCs identified by Germolec et al. is critical to highlight, given these definitions of immunotoxicity. The authors concluded that immunoactive agents do not directly cause immunosuppression, inappropriate inflammation, or immune enhancement. Instead, these immunotoxic outcomes are the results of the aftermath of disruption of specific immune cell functions such as cell–cell communication and antigen presentation. Thus, the KCs that Germolec et al.[1] identified for immunotoxic agents are properties of substances known to cause immunotoxicity rather than a listing of immunotoxic outcomes themselves.

The development of KCs for immunotoxicity is especially important because a 2012 report by the International Program on Chemical Safety acknowledged that "xenobiotic exposure via virtually any route will result in exposure of some immune system components."[7] This report indicated that from a risk assessment perspective, "[T]he issue is not whether immune exposure occurs following a chemical exposure, but whether a given exposure is likely to produce an adverse immunotoxic outcome among susceptible populations."[7] Yet, as of June 2022, few agencies have established official specific immunotoxicity testing requirements. Thus, continued efforts to investigate and characterize immunotoxic outcomes and mechanisms by which exogenous agents produce immunotoxicity will be extremely valuable for public health.

Current methods for pharmaceutical immunotoxicity assessments use a weight-of-evidence approach that relies on data from experimental models using a relatively small pool of assays.[5] Differences between model systems and human patients can create challenges in safety and regulatory spaces, leading to uncertainty in translating model data to humans.[5] Although identifying sensitive end points and reliable experimental assays remains a primary focus of immunotoxicology, the lack of standardized testing procedures for immunotoxicity evaluation has spurred a tiered approach to testing for some safety and regulatory approaches.[6] Under this tiered system, each subsequent tier is an opportunity to define a specific target within the immune system.[6] However, traditional tiered approaches are costly and time consuming, and they often require many experimental animals. Furthermore, the complexity of the immune system has hindered the creation of *in vitro* systems that can fully replicate immune actions.[6] To develop advanced approaches to detect immunotoxic outcomes *in vitro*, *in vivo*, and in epidemiological studies, a deeper understanding of the properties of known immunotoxic substances is necessary. The identification of immunoactivity KCs by Germolec et al.[1] is an immense step forward for the field that will enable development of advanced detection and explanatory approaches.

Additionally, the KCs that Germolec et al.[1] developed for immunotoxic substances demonstrated that whether an agent is a pharmaceutical drug or an environmental pollutant, they share immunoactivity KCs in common. This finding suggests that during drug development or during safety testing for pesticides or other chemical substances, agents that exhibit immunoactivity KCs can be identified fairly early in the pipeline. Early identification of potential adverse outcomes not only reduces economic costs but also prevents the potential introduction of unsafe agents into the pharmaceutical space or the environment. To emphasize the benefits of using a KC approach to safety testing, the authors also considered the application of immunoactivity KCs to hazard identification, risk assessment, and clinical practice in the context of the sensitive developing immune system. There are no reliable *in vitro* or rapid assays for evaluating effects of exogenous agents on the developing immune system[8]; thus many tests for the evaluation of developmental immunotoxicity (DIT) are time-consuming and costly. Germolec et al. noted that knowledge and awareness of the KCs would decrease uncertainty in the evaluation of DIT, support development of pharmacological agents with more favorable

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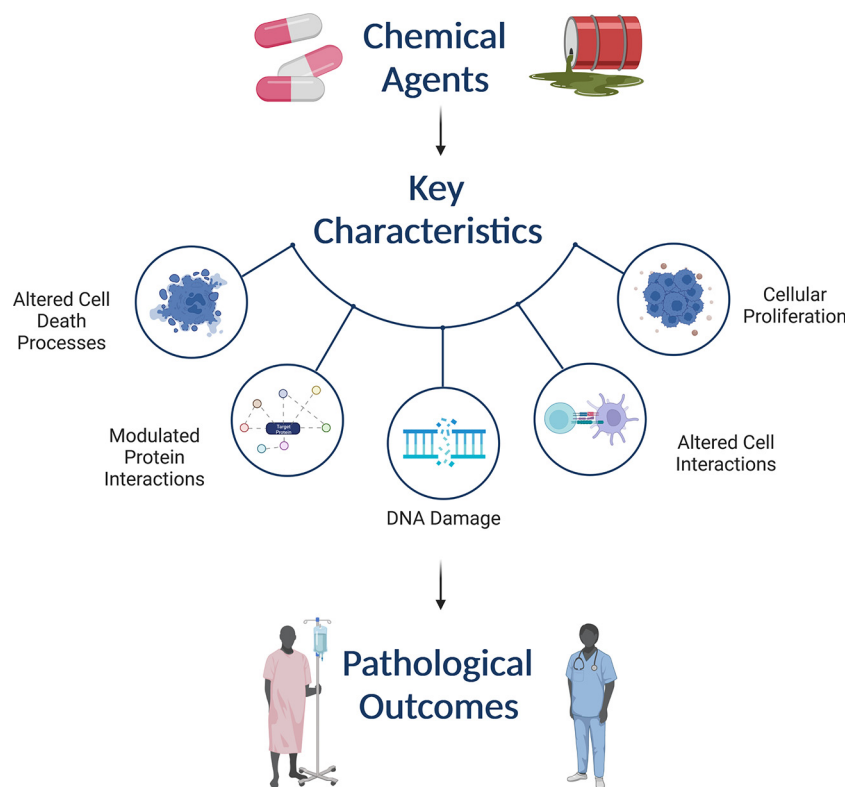
Figure 1. Key characteristics describe how chemical agents cause observed pathologies. Pathological outcomes are considered consequences of hazardous chemical exposures, whereas the disrupted molecular, biochemical, and functional activities are used as key characteristics of chemical agents. Figure Created with BioRender.com.

benefit–risk profiles for developing organisms, and help build improved frameworks for identifying and understanding chemical-mediated immunotoxicity that may occur during development. Therefore, developing *in vitro* or more rapid assays to detect KCs will also be helpful in detecting DIT for enhancing detection of susceptible populations.

Smith et al.[9] criticized KCs developed for human carcinogens as being too broad and nonspecific to be useful as protocols for evaluating potential cancer hazard of chemicals and that high false-positive rates could communicate to the public that everything is a carcinogen. Smith et al.[9] further asserted that tens of thousands of chemicals and pharmaceuticals already in use in commerce would produce a positive finding in one or more of the KCs developed for carcinogens. However, KCs are designed to aid in the identification, organization, and summarizing of mechanistic information as part of the hazard evaluation of various agents and thus can serve as a basis for a structured evaluation of the strength of the mechanistic evidence base and subsequent support of hazard classifications.[2] KCs are not designed to be the deciding factor for an agent's hazard classification but a process for starting the evaluation. Thus a robust attribute of KCs is that they can be a springboard for the development of additional tools, technologies, protocols, and approaches for better exploring the hazard characteristics of agents that produce positive findings under specific sets of KCs. The KCs developed for immunoactivity will therefore provide a stronger basis for the hazard evaluation of agents with immunotoxic potential and facilitate a more streamlined approach for the classification process.

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