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Immune System as a Target of Xenobiotics Toxicity

Imunski sistem kao meta toksičnog delovanja ksenobiotika

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Abstract

In addition to physical and chemical insults originating from nature (heavy metals, radiation, electromagnetic field), human beings are exposed to various agents as a result of progress in industry and technology (noise, pesticides, pharmaceuticals, personal care products, food additives, etc.) whose number continuously increases. Some of these agents may have adverse effects on the environment and human health and therefore every new product must be examined for its toxic potential and health risk. The immune system is an important homeostatic system that is included in response to various pathogens, but in other processes as well including wound healing and elimination of damaged cells and tumors. Due to its involvement in various physiological processes, alternation in immune system activity, as a consequence of exposure to xenobiotics, may cause an increase in the incidence of infections, tumors, allergies, and autoimmune diseases. Various tests that exist for the evaluation of immunotoxicity are grouped in two separate sets, tier one (experiments directed to measure the effect of xenobiotics on general immune parameters i.e. basic morphological and functional tests) and tier two(experiments aimed to more specifically define the cellular and biochemical mechanisms of toxicity). Evaluation of potential adverse effects of xenobiotics on the immune system is now incorporated into standard hazard identification and has improved human health risk assessment.

Keywords: *Immune system; Xenobiotics; Direct immunotoxicity; Risk assessment; Developmental immunotoxicity*

Izvod

Pored fizičkih i hemijskih agenasa poreklom iz prirode (teški metali, zračenje, elektromagnetno polje), ljudska bića su izložena raznim agensima kao rezultat napretka industrije i tehnologije (buka, pesticidi, farmaceutski proizvodi, proizvodi za ličnu negu, aditivi u hrani, itd.) čiji se broj kontinuirano povećava. Neki od ovih agenasa mogu imati štetne efekte na životnu sredinu i zdravlje ljudi i stoga za svaki novi proizvod mora biti ispitan njegov toksični potencijal i rizik po zdravlje. Imunski sistem je važan homeostatski sistem koji je uključen u odgovor na različite patogene, ali i u druge procese kao što su zarastanje rana, eliminaciju oštećenih ćelija i tumora. Zbog uključenosti u različite fiziološke procese, promenjena aktivnost imunskog sistema, kao posledica izloženosti ksenobioticima, može dovesti do povećanja učestalosti infekcija, tumora, alergija i autoimunskih bolesti. Postoje različiti testovi za procenu imunotoksičnosti koji su grupisani u dva odvojena seta, prvi nivo (eksperimenti usmereni na merenje uticaja ksenobiotika na opšte imunološke parametre, tj. osnovni morfološki i funkcionalni testovi) i nivo dva (eksperimenti koji imaju za cilj da preciznije definišu ćelijske i biohemijske mehanizme toksičnosti). Procena potencijalnih štetnih efekata ksenobiotika na imunski sistem je sada uključena u standardnu identifikaciju opasnosti i poboljšala je procenu rizika koju ksenobiotici mogu imati po zdravlje ljudi.

Ključne reči: *Imunski sistem; Ksenobiotici; Direktna imunotoksičnost; Prcena rizika; Razvojna imunotoksičnost*

Introduction

Modern human beings are exposed to various chemicals present in the environment or used in industry such as pesticides, heavy metals, pharmaceuticals, personal care products, food additives, etc., that can have a potential impact on health. Regardless of their origin (natural or man-made) or potential use (in industry or medicine), all chemicals foreign to the organism (xenobiotics) should be examined for their toxicity. In general, there are five different sections to be examined for new man-made chemicals: physical and chemical properties, effects on biotic systems (51 relevant testing methods regarding acute or chronic toxicity on various organisms), environmental fate and behavior (23 methods for evaluating bioaccumulation, biodegradability), health effects (in total 82 tests that examine gene mutation, skin sensitization/irritation, eye irritation, inhalation toxicity, reproductive toxicity, etc.) and other tests (mainly for pesticides) [1].

In the general population, the main routes of xenobiotics entering the body include the skin, lungs, and gastrointestinal tract. Although these organs are considered to be a physical barrier between the organism and the environment, the barrier function is not just physical and is tightly regulated. Beside epithelial cells and commensal microbiota on the surface, all barrier tissues are enriched with various types of immune cells. Components of the immune system are actively involved in maintaining barrier function, and any alteration in the activity of the immune system can affect barrier tissue homeostasis.

Immune system

Traditionally it was considered that the physiological role of the immune system is to defend the host against pathogenic microorganisms, but nowadays it is well established that the immune system represents homeostatic mechanisms aimed to respond to both infectious and non-infectious insults (mechanical and chemical injury, cellular dysfunction, etc.). The immune system consists of innate (natural, native) immunity, whose components respond rapidly to microbial invasion and tissue injury, and adaptive (acquired, specific) immunity, which is activated later and is specific for an insult [2]. Despite this division into innate and adaptive immunity, the immune system is an integrated system of host defense in which numerous cells and molecules function cooperatively. The main components of innate immunity are anatomical barrier tissues (such as epithelial surfaces), phagocytic cells (polymorphonuclear leukocytes/neutrophils and mononuclear phagocytes), dendritic cells, innate lymphoid cells (such as NK cells), and soluble factors in the blood (complement system and various inflammatory mediators). There are two types of adaptive immunity: humoral (mediated by antibodies produced by B lymphocytes) and cell-mediated (cellular) immunity (mediated by $CD4^+$ and $CD8^+$ T lymphocytes).

Due to its complexity, the immune system is a sensitive target for a variety of physical and chemical agents that impose the need to examine immunotoxic effects during hazard identification. US regulatives recommend basic morphological tests of the immune system during standard toxicological studies for pharmaceuticals and immune function tests when an indication for immunosuppression exist. On the other hand, European regulatives [3] require conducting more exentisive and sensitive immune function tests on all chemicals (the potential immunotoxic effect of the chemical should be assessed). Although investigators paid more attention to the effects that chemical agents may have on the immune system, physical agents such as vibration stress [4], radiation (particularly ultraviolet) [5], electromagnetic fields [6] and noise [7] can also affect (directly or through the neuroendocrine system) components of the immune system.

Various tests exist for the evaluation of immunotoxicity that are generally grouped into two separate sets, tier one and tier two. Tier one consists of sets of experiments directed to measure the effect of xenobiotics on general immune parameters (basic morphological and functional tests). Set of experiments aimed to more specifically define the cellular and biochemical mechanisms of toxicity (alteration in immune system activity) are gathered in tier two.

Direct immunotoxicity

Animal-based models were widely used in the first days of immunotoxicology as a discipline, but in recent years there has been a tendency to replace animal-based assays with in vitro methods for investigating the immunotoxic potential of xenobiotics. There are commercially available, well-defined primary cells, cell lines, and cells differentiated from induced pluripotent stem cells that can be used in experiments. In addition, cells from human peripheral blood (i.e. monocytes and polymorphonuclear phagocytes/PMN) are also available for research. There are several strategies for evaluating the immunotoxic potential of chemicals. As immune cells originate from bone marrow, any xenobiotic affecting the viability of bone marrow cells or proliferation and differentiation of pluripotent hematopoietic stem cells and progenitors of blood cell lineages will have an impact on the immune system [8]. Apart from myelotoxic effects, xenobiotics can directly affect differentiated (mature) immune cells. Cell viability/death, expression of surface markers and assessment of cell activities (proliferation in response to mitogens, production of reactive oxygen species, or cytokine production) can be analyzed following stimulation of cells with a test chemical in culture in vitro. These assays indicate how the chemicals affect the proper functionality of immune system components but are usually limited to one specific cell type, which raises the question of selecting the appropriate cell type for the investigation. There is no single immune test that can fully predict altered immune function. Similar as for cell types, not all cell functions are equally affected by xenobiotics. Depending on the activity examined, immunosuppressive, proinflammatory, and absence of effect can be observed for the same chemical [9, 10]. Beside proper selection of the cell type and activity to be investigated, there are additional limitations of in vitro models including the absence of cell-cell interactions, the requirement of special culture systems for materials that undergo biotransformation or the inability to examine immunotoxicity of xenobiotics that are mediated by other organ systems (i.e. neuroendocrine-mediated immunosuppression).

As various cell types, that might be affected by xenobiotics, are part of the specific organ, more precise methods for evaluating immunotoxicity are in use including three-dimensional organotypic culture [11], precision-cut tissue slices [12] or the human whole blood cell culture. Mentioned models mimic the natural environment including the interaction between various cell types, both immune and non-immune or resident. The communication between immune cells and tissue-specific cells maintains immune homeostasis, moderates immunity to various insults, and shapes the type of response. In addition, epithelial and endothelial cells in tissue can produce cytokines and chemokines in response to various insults, thus contributing to the immune response in tissue. For example, our examination of pulmonary immune response to prolonged Cd exposure revealed decreased production of proinflammatory cytokines (interleukin-1β/IL-1 β, and tumor necrosis factor/TNF) in lung leukocytes, while overall tissue cytokine content was increased [10]. Using organotypic culture methods cytotoxicity, cytokine, and reactive oxygen species production and expression profile following stimulation with xenobiotics can be measured, but there are still features of the immune system and its responses to chemicals that can not be investigated in these models. Information on the cell migration from blood to tissue or vice versa, activation of adaptive immunity cells in regional lymph nodes which drain from affected tissue, as well as systemic, or effects on distal organs are missing from these models.

The use of animals has several issues such are an expense, ethical concern, and relevance to human risk assessment, but animal-based models provide the most complete insight into immunotoxic potential of chemicals. In immunotoxicological examinations (same as in general toxicology) the most often used animals are laboratory rodents (mice and rats) followed by nonhuman primates and, for some investigations, swines. Alterations in organ weight, peripheral blood cell number, acute phase proteins in serum and histopathology of organs are routinely monitored during standard toxicological investigations as first indices of immunotoxic potential. Although mentioned parameters can be indicative for immunotoxicology, they are not overly sensitive and must be

followed by measurement of immune system function. Animal based-models provide insight into xenobiotic effects at the local (tissue) level, as well as at the systemic level or on distal organs. An important response of the organism to a tissue homeostasis disruption caused by chemical or physical insult is inflammation [13]. It involves the recruitment of inflammatory blood-derived mediators (plasma proteins and fluids, as well as leukocytes) into the affected tissue, a process facilitated by increased local vasculature activities (vascular permeability, vasodilatation, and blood flow). Normally, inflammation aims to restore tissue homeostasis, but chronic inflammation may lead to persistent tissue damage.

Usually, solely one tissue or organ is examined for immunotoxic effects of xenobiotics, but components of the immune system are present in all organs, and xenobiotic through blood might gain access to various tissues. For example, oral Cd exposure affects the intestine [14], skin [15] and lungs [10]. Noted effects might be expected as this heavy metal is deposited in various organs and can induce oxidative stress, but similar was noted for another xenobiotic, warfarin. Following oral exposure of animals to warfarin, immunotoxicity was noted in the intestine [16], as well as in the skin [17]. Distal effects are not limited to orally applied xenobiotics, as both local (skin) and systemic toxicity was noted for warfarin [18, 19] and contact allergen dinitrochlorobenzene [20]. In general, animal-based models provide insight into the immunotoxic potential of xenobiotics on various cell types, local or distal sites and allow us to examine the functional consequences of the effect of xenobiotics on the immune system.

Risk assessment

As a consequence of exposure to xenobiotics, alteration in immune system activity may occur (immunosuppression, immunostimulation, autoimmunity or allergy).

Immunosuppression and/or immunostimulation might result in altered resistance/susceptibility to infectious agents (“host resistance assays”). Among infectious agents, bacteria Listeria monocytogenes, Streptococcus pneumoniae and influenza virus are most commonly used for evaluation of immunostimulatory or immunosuppressive effects of xenobiotics but other infectious models can be used as well, for example nematodes [21] or opportunistic fungi. Opportunistic fungi (and other opportunistic pathogens) might be useful model organisms for investigating the immunosuppressive effects of xenobiotics, as these pathogens do not cause infection in individuals with the functional immune system. In general, the selection of challenge agents is based on their association with significant human disease or well-documented resistance mechanisms, and mortality of animals or load of infectious agents are (in a majority of experiments) end-point measures [22].

Some indices regarding altered immune cell activity might be obtained in vitro following the co-culture of cells with a potential pathogen (phagocytosis and killing of micro-organisms or cytokine production). Epidermal cells [23] and lung leukocytes [10] isolated from animals exposed to Cd produce a higher amount of proinflammatory cytokines in response to commensal bacteria Staphylococcus epidermidis suggesting a breach of immunotolerance to commensals and altered tissue homeostasis. Alteration of the activity of specific cells in response to infectious agents does not necessarily indicate altered resistance/susceptibility to infection as the immune system contains components with overlapping functions (one component might compensate for a defect in another). For the same reason, the absence of xenobiotic effect on selected cell activity, does not indicate the absence of effect in individuals. Our investigations of the immunotoxic potential of Cd revealed similar activity (cytokine production) of lung leukocytes isolated from healthy or Cd-treated animals in response to co-culture with conidia of opportunistic fungus A. fumigatus which might indicate an absence of Cd effect on resistance to infection. However, when animals that were exposed to Cd were infected intratracheally with fungal conidia, more efficient elimination from the lungs and lower degree of infection were noted in Cd-treated individuals (compared to Cd-untreated) [24]. Although this finding suggests a more efficient immune response to infectious agents, more intense lung

inflammation noted following Cd exposure results in higher tissue damage after infection, suggesting that additional tests are required in host resistance assay (not only pathogen load in the organ).
Some xenobiotics might cause gene mutations and be cancer inducers per se, but the effect of these chemicals on tumor development might be mediated by their effect on immune system activity, as the immune system is involved in tumor response. Any alteration of immune activity might result in a higher incidence of tumors and/or their intensive growth and in that regard immunotoxic propensity of xenobiotics could be evaluated based on the estimation of tumor growth, burden or metastatic potential.
Xenobiotics might by themselves interfere with proteins in an organism, alter protein structure or process of antigen presentation, and cause cell and tissue damage thus releasing previously hidden autoantigens [25]. As a result of these processes allergies and autoimmunity could occur. Regardless of the type of allergy (types I-IV), there are two phases necessary for an allergic reaction to occur. The sensitization phase is characterized by the generation of a primary specific immune reaction (antibodies or specific T cells) directed against a xenobiotic. The immunogenic potential of chemicals (their capacity to induce an immune response, to sensitize) can be measured [26], but is not predictable for the clinical manifestation of disease. The elicitation phase occurs after a subsequent challenge with the same chemical (usually at a lower dose) and has clinical manifestation. Beside direct allergic potential, the consequence of xenobiotic exposure may be an increase in the incidence of allergic/autoimmune diseases and/or severity of existent disease and in this regard for evaluation of allergic or autoimmune potential models of diseases should be used. For this type of investigation, well-characterized models of various allergic and autoimmune diseases exist, as well as animal strains (mostly mice) that spontaneously develop the disease.

Developmental immunotoxicology

The majority of toxicological effects on the immune system are examined in adult animals, but the effect on the developing immune system should be also taken into account. The rationale for these investigations is that the immune system, owing to its immaturity, is particularly vulnerable to chemical toxicity, an effect that might not be seen if examined in adults. In general, there is a window in which maturation of the immune system occurs and during which transient or permanent effects on immune system function following exposure to xenobiotics may occur. These critical windows of exposure include in utero, postnatal and lactational exposure, and accordingly developmental immunotoxicology is mainly investigated in rodents [27]. At an early stage of life, basic tests including relative organ weight, cell number and surface antigen expression can be evaluated and have some prognostic value for adult immunotoxicity. Standardized functional tests could not be applied to young animals due to a functionally immature immune system, so analyses of the effect of exposure to xenobiotics during early life on the function of the immune system are mainly conducted following the “recovery period” (i.e. in adult animals).

Conclusion

The examination of the effects of xenobiotics on the immune system is an important part of the toxicological examination of chemicals as components of this homeostatic system are present in every organ and highly sensitive to a variety of physical and chemical agents. Although there are various methods developed for testing immunotoxicity of chemicals at the cellular or tissue level, due to the complexity of the immune system, animal models remain a valuable and useful tool for examining direct immunotoxicity, estimation of risk assessment and developmental immunotoxicity.

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