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# Immunotoxicants, Immunotoxicity and Immunotoxicity testing: An outline of *in-vitro* alternatives.

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## Abstract

Exposure to drugs and chemicals often results in toxicity to living organisms. The immune system is a tightly regulated complex network, including lymphoid, reticular, dendritic and epithelial cells, interacting by cell-to-cell contacts and communicating via soluble mediators such as cytokines. The immune system can be a target for many chemicals including environmental contaminants and drugs with potential adverse effects on human health and this has raised serious concerns within the public and the regulatory agencies. Modulation of the immune system can lead to either immunostimulation or immunosuppression and can be either intended or unintended. The application of immunotoxicology to the toxicologic assessment of drugs and chemicals is a field of increasing importance. Therefore, successful prevention requires knowledge of pathogenic mechanisms of immunotoxicity and rapid screening procedures by which we can access the potential agents with immunotoxicity. *In-vitro* methods have been invaluable in helping to understand the mechanisms of well-established Immunotoxicants. *In-vitro* methods offer a rapid and economical method of screening specific cell types for specific effects. This insight has also been used to help screen new chemicals for their potential Immunotoxicity. *In-vitro* technologies are advancing rapidly, improving the scientific validity of this approach, and extending their use. The future is therefore one in which more *in vitro* techniques will be used, better to answer questions regarding how to understand disease and improve health for animals and humans.

**Key Words**

Drugs and chemicals Immune system, Immunotoxicity, In-vitro methods, rapid screening.

## Introduction

The immune system is a complex set of cellular, chemical, and soluble mediators (Table No.1) which act in an interrelated manner to protect the host against foreign organisms and chemical substances. The immune system participates in the mechanisms responsible for the maintenance of homeostasis and an altered immune system reflects the adverse changes in both internal and external microenvironments[1]. The immune system can be a target for many chemicals including environmental contaminants and drugs with potential adverse effects on human health .Immunotoxicology is the sub discipline of toxicology, can be defined as the study of adverse/toxic effects on the immune system resulting from exposure to chemicals (including drugs), biological materials and, in certain instances, physiological factors, collectively referred to as agents[3]. Immunotoxicity can be defined as the adverse effect of chemicals or agents on the immune system. The effect may be increased immune activity, manifested as either hypersensitivity or autoimmunity, or decreased immune activity, with increased incidences of infectious diseases or neoplastic diseases, allergy/asthma, or autoimmune diseases, respectively[4]. There is agreement that reliable and readily standardized immunotoxicity methods are needed to address potential adverse effects on a target organ that is as dynamic and complex as the immune system5. During the past two decades, significant progress has occurred in the fields of molecular biology and basic/clinical immunology which promoted the establishment of newer more sensitive methods to assess cell injury or immune system effects in humans and laboratory animals[6]. Sensitive, reproducible and validated *in vitro* assays of immune function are available which allow the evaluation of immunotoxicity as an adjunct

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in the routine safety evaluation of chemicals and drugs. The evaluation of immunotoxicity offers unique possibilities for *in vitro* studies now and in the future because most of the cellular elements can be obtained from peripheral blood. Currently, the majority of immunotoxicity assays are performed *ex vivo/in vitro* using cells or tissues. This review describes the various Immunotoxicants that affects organization and function of the immune system, importance of Immunotoxicity and types of Immunotoxicity and also outlines the various *in vitro* techniques available for rapid and economical Immunotoxicity testing.

**Immunotoxicants / Immunotoxic agents**

Examples of immunotoxic agents, classified by the type of pathology produced, are presented in Table No. 2[7].

**Possible mechanisms for Immunotoxicity**

Exposure to toxicants may have effects on immune system by a variety of mechanisms (Fig No. 1). Not only is the immune system profoundly influenced by other organs or systems of the body, but the complexity of immune responses also provides multiple targets for effects of chemicals[8].

***In vitro* approaches to the assessment of immunotoxicity**

Even though the evaluation of immune functions following *in vivo* exposure to a test material is the most relevant situation, it is increasingly desirable to limit the use of animals whenever possible. *In vitro* tests are also essential to characterize the molecular mechanism of action of direct immunotoxic compounds and to give a better understanding of the immunotoxic effects observed *in vivo*. Furthermore, there are certain situations, for example when a test material is anticipated to be dangerous to handle, or when it is prohibitively expensive, where a totally *in vitro* system would be very advantageous. Finally *in vitro* methods allow direct comparison of rodent and human systems, and can be representative of the *in vivo* situation. *In vitro* methods can be used in immunotoxicology as follows:

- *ex vivo* tests are, *de facto*, *in vitro* methods,
- for mechanistic investigations,
- in the parallelogram approach,
- as an alternative to the use of experimental animals for both immunosuppression and immunoenhancement.

In the screening of potential immunotoxic compounds, one can use a battery of *in vitro* assays measuring, for example, B and T cell proliferation, cytokine production, surface marker expression, T cell cytolytic activity, NK and macrophage functions, etc... A proper functionality of immune cells as assessed by *in vitro* tests should reflect proper host immunocompetence *in vivo*. Thus, alterations in immune cell functions following *in vitro* exposure to chemicals are likely to be relevant for the *in vivo* situation. For studies intended to unravel the molecular mechanism of action, *in vitro* assays should be selected on a case-by-case basis from the 'menu' of available methods.

**Measurement of potential effects on natural immunity**

Innate immune cells include natural killer (NK) cells, granulocytes, monocytes/macrophages, and antigen-presenting cells (APC). NK cells are involved in non-specific immunity and represent a primary immune defense against tumor and virally infected cells[9]. CD3-CD16+ CD56+ cells account for 7-41 % of the lymphocytes in human peripheral blood (absolute counts vary between 130 and 1,000/µl). The measurement of NK cell activity is often recommended as an endpoint in the non-clinical immuno- toxicological evaluation of chemicals. The cytotoxic function of NK cells is usually assayed *in vitro* using 51Cr-labelled target cells, classically K562 erythroleukemia cells for humans, or YAC-1 for rodents, or using flow cytometry assays. Other functional parameters, such as cytokine production can also be evaluated following purification[10]. NK cell activity is exquisitely sensitive to modulation by toxic substances, but the significance is often questioned. Indeed, changes in NK cell activity are not often related to pathological conditions of the immune system and the predictive potential of such assays after *in vitro* exposure is the subject of investigations[11,12]. It is important to mention that rodent spleen-derived NK cell cultures, probably due to loose cell-cell interactions within the splenic microenvironment, rapidly lose their cytolytic function and, in the absence of stimulation, only 25% of cells are still viable after 18 hours[13]. This may actually limit their potential use. The use of other immune cells, such as granulocytes can also be considered. At the moment, however, there is no strong evidence for direct alterations of these cells induced by chemicals. On the contrary, monocytes/macrophages because of their ability to

process and present antigens are key immune cells representing the trait the union of natural immunity with acquired responses, and they are often affected by xenobiotics. Many assays have been developed to test their functions: surface marker expression, phagocytosis, and cytokine production to mention only a few.

**Measurement of potential effects on humoral immunity**

In animals, production of a T-dependent antibody response is considered to be the "gold standard"[14]. However, there are currently no good systems for *in vitro* primary antibody production using human cells. Development of human *in vitro* systems will require optimization of the antigen, culture conditions, and assay endpoints. In addition, there is some concern whether a primary immune response can actually be induced in human peripheral blood leukocytes (PBL). One potential starting point would be an *in vitro* immunization culture system based on the Mishell-Dutton assay, an *in vitro* model for evaluating the humoral immune response of mouse splenocytes to sheep red blood cells[15]. This assay is, however, not considered optimal due to significant variability in results between laboratories including an often complete lack of success. Nevertheless, some authors were able to use this assay to study the immunotoxicity of iron-containing compounds, contributing to explain the decrease in host resistance against infections in workers exposed to iron[16]. As an alternative, non-specific *in vitro* antibody production following stimulation of PBL with pokeweed mitogen (PWM) may be considered. Even though its predictive potential for immunotoxicity evaluation is very low, only 50%, B cell proliferation in response to polyclonal activators (i.e. LPS, PWM) may be considered.

**Measurement of potential effects on cell-mediated immunity**

The activation of specific immune responses involves the proliferation of lymphocytes. For T cells, the stimulatory agent can be a combination of anti-CD3 and anti-CD28 antibodies, or mitogens, such PHA or ConA. Dysregulations of cell homeostasis are likely to cause severe adverse changes in immune functions, increasing susceptibility to infections and some cancers as well as favoring the development of autoimmune diseases.

The mitogen-stimulated proliferative response widely used in clinical immunology and immunotoxicology is an *in vitro* correlate of the activation and proliferation of lymphocytes specifically sensitized by the antigen *in vivo*. *In vitro* stimulation of lymphocyte proliferation is an easy assay. Furthermore, *in vitro* lymphocyte stimulation or transformation could also be performed using the whole blood assay. Both plant lectins (e.g. PHA, Con A, PWM, etc.) and LPS, purified protein derivative of tuberculin (PPD), anti-CD3 and/or anti-CD28 antibodies, etc... can be used to stimulate T or B cell proliferation in the whole blood. *In vitro* antigen-specific and non-specific mitogen-induced activation of lymphocytes can result in a myriad of biochemical events including calcium influx, protein kinase C activation, and phospholipid synthesis, culminating in DNA synthesis and cell division[17, 18]. Thus, xenobiotics interfering with signal transduction pathways are likely to to alter mitogen-induced lymphocyte proliferation, making this assay a possible candidate for an *in vitro* test to identify direct immunotoxicants. The lymphocyte proliferation assay, as described below, is currently in a pre-validation phase. The most obvious disadvantage of the lymphocyte proliferation assay is the requirement of [3H]-thymidine. Efforts, therefore, should also be devoted to find alternative readouts for cell proliferation.

**Measurement of cytokine production**

The activation of any immune response is dependent upon the production and release of cytokines. Cytokines are small molecular weight proteins secreted by many cell types, including immune cells that regulate the duration and intensity of the immune response. For example, Type 1 cytokines (i.e., interferon [IFN-γ], interleukin [IL-12]) mediate the removal of malignant cells and virally-infected cells, whereas Type 2 cytokines (i.e., IL-4, IL-5, IL-13) mediate the removal of soluble bacterial antigens. Clearly, cytokines play key roles in all immune responses and molecular immunotoxicology has indeed focused on analyses of cytokine levels. Cytokines offer important new avenues to explore, both in terms of mechanistic understanding of immunotoxicity and in terms of developing new assays, the immunotoxic potential of novel compounds. Cytokines are released as one of the first steps of immune responses and quantitative alterations can be used as a measure of

immunomodulation. Due to the highly pleiotropic and redundant nature of cytokines, in which a single function may be affected by multiple cytokines simultaneously, it is advisable to include the broadest panel of cytokines possible in any *in vitro* system using this endpoint. A plethora of assay systems are available for measuring cytokines and their receptors including ELISA, flow cytometry, and molecular biology techniques, such as Polymerase chain reaction[19]. Depending on the type of stimulus, human blood cells release different patterns of cytokines originating from several blood cell populations. Whole blood incubations allow to assess either effects on monocytes or lymphocytes employing selective stimuli. This model has been shown to reflect several aspects of immunotoxicity including immunostimulation, priming and inhibitory effects. LPS stimulation for 24 hours leads to the release of interleukin-1b, IL-6, IL-8, TNF-a, and by prolonging the incubation period from 48 to 72 hours, the whole blood model can be extended to determine the release of other cytokines[20], such as IL-2, IL-4, IL-13 and IFN-g, etc... The measurements of Th1 *vs.* Th2 cytokines will be valuable in determining the ultimate result of any cytokine dysregulation. A new *in vitro* system, named "fluorescent cell chip," based on a number of cell lines derived from T-lymphocytes, mast cells, monocytes, each transfected with various cytokine reporter cell constructs for measuring cytokine expression has been developed[21]. Although further refinement of this system by the expansion with other cell type and cytokines is required, this assay holds promises for *in vitro* screening of chemicals for their immunotoxicity. Cytokine production together with lymphocyte proliferation is currently in a pre-validation phase.

**Cell culture models for evaluating the sensitizing potential of xenobiotics**

Many chemicals have the ability to cause skin sensitization and allergic contact dermatitis (ACD) is an important occupational health issue. The assessment of the allergenic potential of chemicals is generally performed using animal models, such as the guinea pig assays and the murine LLNA. At present, no alternative *in vitro* tests to assess the sensitizing potential of chemicals has been validated. Beside its barrier function, the skin has been recognized to be an immunologically active tissue. Keratinocytes (KC) can convert non-specific exogenous stimuli into the production of cytokines, adhesion molecules and chemotactic factors[22]. Second to keratinocytes, Langerhans cells (LC) comprise the most prominent cell type in the skin (2-5% of the epidermal cell population). They are the main APC in the skin[23]. Due to their anatomical location and significant role in the development of ACD, the use of both cell types to evaluate the sensitizing potency of chemicals *in vitro* is amply justifiable. In principle, a test system comprised of KC alone may not be useful in establishing the allergenic potency of chemicals as these cells are devoid of antigen-presenting capacity. However, in addition to chemical processing, LC activation requires the binding of cytokines produced by KC as a result of initial chemical exposure. The irritant potency of allergenic substances can be an additional risk factor as irritant chemicals can be stronger allergens than non-irritant chemicals[24]. In that case, the potency of chemicals to induce cutaneous sensitization can be assessed as a function of cytokine expression by KC. Following the *in vivo* observation that IL-1a expression by KC in the mouse was selectively increased after *in vivo* application of contact sensitizers in contrast to tolerogens or irritants[25], similar *in vitro* results were reproduced using the murine KC cell line HEL30[26]. The same results were obtained by van Och et al[27] and these authors also observed that the ranking of potency was similar to that established with the LLNA. Using human KC, allergens in contrast to irritants or tolerogens were shown to induce IL-12 production[28,29]. Trinitrobenzene sulphonic acid induced the expression of CD40 on KC, whereas SLS did not[30]. Altogether these studies indicate the possibility to identify contact sensitizers in murine or human keratinocyte *in vitro* models. On the other hand, DC form a sentinel network able to detect, capture, and process antigens, such as invading bacteria, viruses, products of tissue damage and haptens [31,32]. Upon antigen capture, the DC undergoes a maturation process leading to the upregulation of co-stimulatory molecules (CD86, CD80, CD40), MHC class II molecules and the CD83 protein[33]. Thereafter, DC migrate to the T cell areas of lymphoid organs where they lose their antigen-processing activity and become potent immunostimulatory cells. These maturing DC acquire the ability to migrate through expression of chemokines and chemokine receptors, and down-

regulation of molecules, such as E-cadherin. In the early 1990s, the knowledge of DC physiology progressed considerably with the discovery of culture techniques supporting the *in vitro* generation of large numbers of DC from hematopoietic progenitors[34]. Two main protocols to generate DC from either monocytes or CD34+ hematopoietic cell precursors (HPC) have been described. Generating DC from murine bone marrow CD34+ HPC has been used as an alternative, but this procedure is time consuming and requires a significant number of animals. The establishment of human *in vitro* models of DC offered the possibility to demonstrate that haptens are able to directly activate cultured DC derived from peripheral blood monocytes or from CD34+ HPC[35-39]. Several studies confirmed these observations by showing the up regulation of maturation markers (CD83, CD80, CD86, CD40) on human DC [40-43]. Cytokine production, such as IL-12p40, TNF-α and IL-1b has also been reported upon hapten stimulation[31, 38]. However, significant differences exist between experimental systems and authors concerning cytokine production. The major drawbacks in the use of primary cells are an important inter-individual variability and the cost, as an alternative myeloid cell lines has been proposed as predictive model[44-46]. In these studies, a selective up-regulation of MHC-II, CD54, CD86 by strong allergens were observed in myeloid cell lines (i.e. KG-1, THP-1), emphasizing the potential of myeloid cell lines to be used as ACD models. Because of their unlimited availability, homogeneity and stability, naïve cell lines represent attractive models for alternative *in vitro* methods.

**The whole blood assay**

The human whole blood cell culture, introduced more than 20 years ago, may also be useful in studying the biological effects of potential allergenic and/or antigenic substances or drugs based on immune cell activation and cytokine secretion. Various clinical uses of whole blood stimulation assays have been suggested, including the assessment of autoimmune diseases, the monitoring of drug and vaccine efficacy, and immunotoxicity[47, 48]. Whole blood assays can be a very useful test due to the easy access of samples from healthy donors and the minimal processing of the sample required. Because the assay mimics the natural environment, whole blood culture may be the best milieu in which to study cell activation and cytokine production *in vitro*. Both plant lectins (e.g., PHA, ConA, pokeweed mitogen, etc.) as well as LPS, purified protein derivative of tuberculin (PPD), anti-CD3 and/or anti-CD28 antibodies, etc. can be used to stimulate T- or B-lymphocyte proliferation in whole blood. Stimulation for 24 hr with LPS leads to the release of IL-1β, IL-6, IL-8, and TNF-α; by prolonging the incubation period from 48 to 72 hr, the whole blood model can be extended to determine also the release of various other cytokines[49] such as IL-2, IL-4, IL-13, and IFN-γ. The potential comparisons to be made between expressions of T-helper lymphocyte type 1 (TH1) vs. TH2 cytokines (i.e., IFN-γ vs. IL-4) will be invaluable in ultimately determining if a test agent can induce cytokinedysregulation. Langezaal group[50] have adapted the whole blood assay for immunotoxicity testing, to permit the potency testing of immunostimulants and immunosuppressants. These Authors proposed the use of LPS-induced IL-1β and staphylococcal enterotoxin B-induced IL-4 release to test the immunotoxic potential of chemicals. This *in vitro* method is capable of determining immunosuppression and immunostimulation, favoring IL-1β release for stimulation and IL-4 release for suppression of cytokines. Thirty-one pharmaceutical compounds, with known effects on the immune system, have been used to optimize and standardize the method, by analyzing their effects on cytokine release. The *in vitro* results were expressed as IC50 values for immunosuppression, and SC (4) (4-fold increase) values for immunostimulation. The *in vitro* results correlated well with the *in vivo* data, so the test appears to reflect immunomodulation. A sensitivity of 67% and a specificity of 100% for the combined endpoints in the test were calculated. Results were reproducible, and the method could be transferred to another laboratory, suggesting the potential use of the test in immunotoxicity testing strategies. As a general strategy, the Langezaal group also proposed that when unknown compounds are tested for their potency to modulate the immune response, conclusions might be drawn from compounds that are showing clear immunosuppression or immunostimulation. Compounds found to be non-immunotoxic will have to be tested for metabolic activation and for effects on additional endpoints such as antibody formation, lymphocyte proliferation, and sensitization, before it can be concluded whether the compound is

immunotoxic or not. The human whole blood assay is also suitable for intracellular cytokine staining, which may be useful to detect low frequency antigen-specific cell responses with respect to clinical significance in assessing immune status in a variety of clinical conditions and determining efficacy or immunotoxicity of drugs and vaccine antigens[51]. Intracellular staining allows one to identify antigen-specific T- or B-lymphocytes at the single cell level with high sensitivity, providing new insights into antigen-specific immune responses of extremely low frequency (events). Overall, these results suggest that *in vitro* assays are able to detect immunosuppression, holding promise for testing these assays using a wide range of chemicals.

**Molecular immunotoxicology**

The *in vitro* testing approach can be expanded beyond simple screening to more mechanistic evaluation. The characterization of specific interferences with cell signaling induced by an immunotoxicant can lead to a better understanding of the molecular mechanism of action. The sciences of immunology and immunotoxicology have evolved to the point where the molecular mechanism(s) of action can be defined. These types of studies are demanding to ensure a good understanding of immunotoxicity profile. Study designs must be "targeted" and should consider the primary cellular target and the specific functional immune parameter affected by the chemical. Many examples can be found in the literature. As an example, some areas of investigation that would naturally follow from a demonstration of overall xenobiotic-induced cytokine modulation might include:

- At what stage is cytokine production affected: transcription, transduction, or release?
- Is cytokine production skewed toward a discrete phenotype (Th1 vs. Th2)?
- Are cytokines overproduced (i.e. immunoenhancement) or under produced (i.e. immunosuppression) in response to a stimulus? Or does the chemical induce cytokine production in the absence of any obvious stimulus?

## Perspective and limitations of *in vitro* immunotoxicity

At present, as in the past, the use of *in vitro* methods in immunotoxicology is mainly limited to the characterization and understanding of *in vivo* observations. It is hoped that in the future *in vitro* methods will also be used to screen the immunotoxic potential of xenobiotics before any animal experimentation. *In vitro* methods using human-derived immunocompetent cells should provide the first step toward establishing a practical and predictive *in vitro* immunotoxicology testing paradigm. The advantages of *in vitro* tests are:

- If human cells are used species differences between humans and animals are avoided.
- Culture techniques are extremely simple.
- In less expensive and less time consuming than *in vivo* testing.
- The same test can be employed *ex vivo* and *in vitro*.
- The number of compounds and concentrations tested can be increased.
- The amount of substance required is dramatically reduced, allowing testing at earlier stages of drug/chemical development.

The validation of an *in vitro* method to detect immunotoxicity must rely on high-quality *in vivo* data. It is essential that a sufficiently large number of positive and negative reference compounds including both drugs and chemicals are tested. To achieve this aim, the establishment of a human database is strongly recommended. This should be accomplished by a coordinated effort from governmental agencies, medical institutions and industry. *In vitro* exposure is most straightforward for direct Immunotoxicants. There are general limitations for in *vitro* methods that also apply to *in vitro* immunotoxicology: materials that need biotransformation would require special culture systems (e.g. culture in the presence of S9), and physiochemical characteristics (i.e. solubility) of the test material may interfere with the *in vitro* system. Such characteristics include the need for serum, effects of vehicle use to solubilize the test compound on cells, and chemical binding to cells. *In vitro* systems do not consider the interactions of different cellular components and it is difficult to reproduce the integrity of the immune system *in vitro*. Finally, *in vitro* systems cannot account for neuro-endocrine interactions with the immune system. Much progress needs to be achieved before *in vitro* tests could indeed replace the use of animals in immunotoxicology. It is hoped that efforts and money will be dedicated to develop alternative *in vitro* tests to assess the immunotoxic potential of chemicals including both immunosuppression and

immunoenhancement. Some *in vitro* tests (i.e. the whole blood assay for immunosuppression; selective cytokine production and co-stimulatory molecule up-regulation for hypersensitivity) have already been proposed and further exploitation is needed before use for hazard identification. All of the promising leads to date are based on observations made from *in vivo* studies conducted in animals and humans, and therefore have a strong mechanistic foundation. Anyway, the future looks promising with continued development of our understanding of the chemical and biological aspects of immunotoxicity and most importantly, with the application of genomics/proteomics to this field on the immediate horizon.

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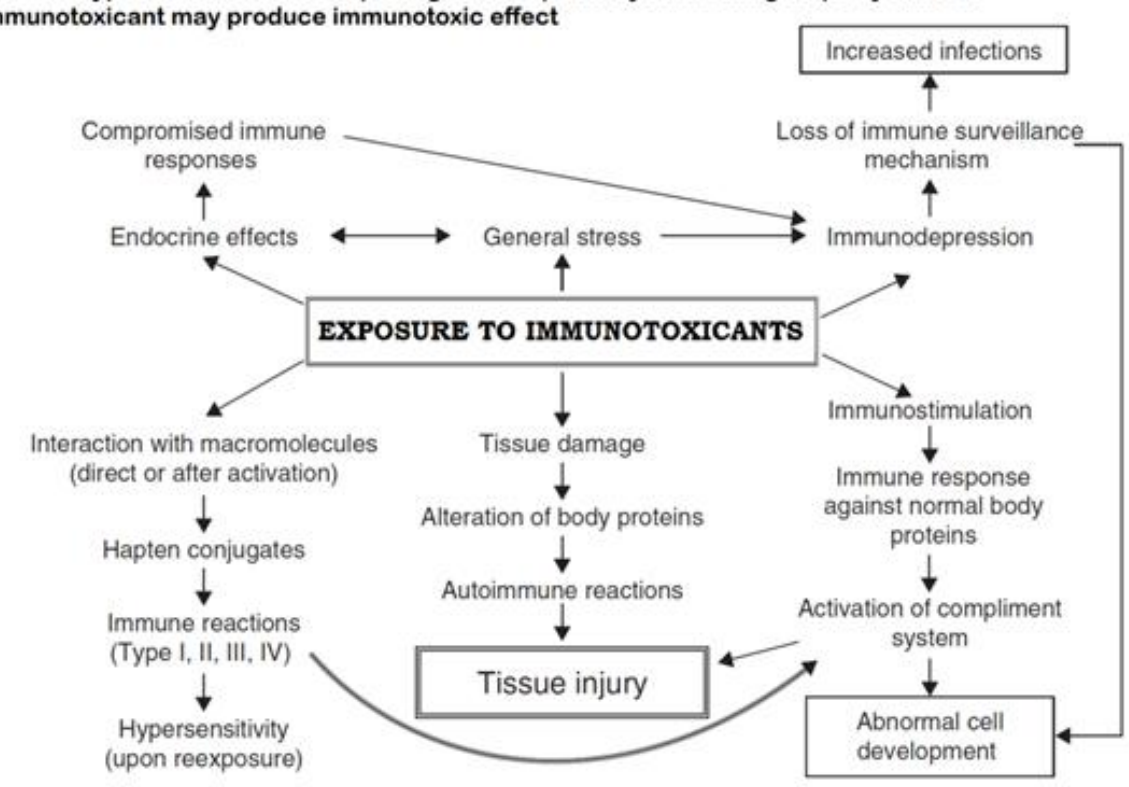
**Table No. 1:** Major components of the host defense system[2].

| Component | Function |
|---|---|
| **Molecular mediators** | |
| Proteins | Viral inactivation; antigen clearance; complement activation; |
| Immunoglobulins (antibodies), Cytokines, | Intercellular signaling |
| Complement (interacting with the kinin, fibrin, and plasmin systems) | Parasite destruction; chemotactic stimulation; acute inflammatory reactions |
| Heat shock | Protein binding and preservation; cross-reactive antigenicity |
| Lipid-derived Prostaglandins, Leukotrienes | Intercellular signaling |
| **Molecular cell surface receptors** | |
| Immunoglobulins; T-cell antigen receptor | Specific antigen recognition on Iymphocytes |
| Immunoglobulin E, Class I histocompatibility proteins and Class II histocompatibility proteins | Specific antigen recognition on mast cells and basophils |
| Immunoglobulin-related proteins (CD4, CD8, $\beta_2$,-microglobulin) | Cell–cell interactions |
| Cytokine receptor proteins | Receptors for the various cytokines |
| Cell adhesion molecules | Cell traffic and migration control |
| **Cell lineages and subsets** | |
| **Granulocytes** | |
| Neutrophils | Phagocytosis and antigen destruction |
| Eosinophils | Parasite destruction; regulation |
| Basophils | Parasite destruction; regulation |
| Monocytes/macrophages | Phagocytosis and antigen destruction; antigen processing and |
| **T-Lymphocytes** | |
| Helper (CD4) cells | Activation of antigen-specific responses |
| Suppressor (CD8) cells | Suppression of antigen-specific responses |
| Cytotoxic (CD8) cells | Destruction of virus-infected and neoplastic cells |
| **B-Lymphocytes** | Antibody production; regulation |
| Plasma cells, Natural killer cells (NK) | Destruction of virus-infected and certain neoplastic cells |
| Dendritic cells | Antigen presentation |
| Platelets | Blood clotting; activation |

**Table No. 2:** Immunotoxic agents.

| Type of pathology produced | Agents reported to cause immunotoxicity in humans or experimental animals |
|---|---|
| **Inhibitors of immune function** | Polyhalogenated aromatic hydrocarbons (dioxins,polychlorinated biphenyls)<br>Polycyclic aromatics (dimethylbenzanthracene,, benzo[*a*]pyrene, methylcholanthrene)<br>Pharmaceuticals (cytoreductives, antibiotics, nucleoside analogues)<br>Aromatic amines (benzidine, acetylaminofluorene)<br>Metals (lead, cadmium, mercury, arsenic)<br>Radiation (ionizing, ultraviolet B)<br>Abused substances (alcohol, opiates, cannabinoids)<br>Pesticides (chlordane, malathion, hexachlorocyclohexane, Trimethyl phosphorothioate, carbofuran) |
| **Implicated in autoimmunity** | Organic solvents (polyvinyl chloride, trichloroethylene, Benzene ,Toluene)<br>Industrial chemicals (silica, poly(brominated/chlorinated)biphenyls)<br>Antibiotics (b-lactams, penicillin, sulfonamides,rifampicin)<br>Antidiabetics (chlorpropamide, tolbutamide)<br>Analgesics (acetaminophen, ibuprofen, phenacetin)<br>Anticonvulsants (phenytoin, carbamazepine)<br>Miscellaneous (gold salts, diphenylhydantoin, digitoxin) |
| **Immediate respiratory sensitizers** | Anhydrides (maleic, phthalic, trimellitic)<br>Proteins (latex, alcalase letic)<br>Dyes (reactive black, rifafix yellow, red BBN)<br>Isocyanates (toluene diisocyanate, diphenylmethane diisocyanate, hexamethylene)<br>Pharmaceuticals (sulfone, pancreatic extracts, antibiotic dusts)<br>Animal products from (laboratory animals, mites, mealworms, pigeons)<br>Wood dusts (western red cedar, California redwood,African maple)<br>Metals (platinum salts, nickel) |
| **Examples of photoallergic contact dermatitis** | Antimicrobial (chlorosalicylanilide, hexachlorophene, fenticlor)<br>Fragrances (musk ambrette, methylcoumarin)<br>Sunscreens (p-aminobenzoic acid, oxybenzones)<br>Pharmaceuticals (sulfanilamide, chlorpromazine, promethazine)<br>Plant derivatives (balsam of Peru, lichen mixture) |
| **Examples of contact allergens** | Mercaptobenzathiazole, p-Phenylenediamine, Formaldehyde, Epoxy resin, Black rubber (PPD mix),Nickel sulfate |
| **Miscellaneous Immunotoxic agents** | Oxidant gases like Nitrogen dioxide, ozone, sulfur, dioxide.<br>Natural products like Selected vitamins, antibiotics, vinca alkaloids, estrogen, plant alkaloids, mycotoxins.<br>Drugs of abuse like Ethanol, cannabinoids, cocaine, opioids.<br>Aromatic amines like Benzidene, acetyl aminofluorene and<br>Others like Nitrosamine, butylated hydroxyanisole. |

**FIG -1.A hypothetical scheme depicting various pathways indicating steps by which a immunotoxicant may produce immunotoxic effect**

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