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Immunotoxicology: Modulation of the Immune System by Xenobiotics

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ABSTRACT

Starting wih a definition of immunity, this review describes general mechanisms by which immune system is modulated and details several immunotoxicological screening methods to assess the immumologic and host resistance alterations following chemical exposure. Among a variety of immuno toxic compounds known, only four representative compounds namely o-chloro benzylidine malononitrile (CS), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), chloroquine, 1,3-bis (2-chloroethyl) 1-nitrosourea (BCNU) have been chosen for an elaborate discussion.

1. INTRODUCTION

The immune system is a surveillance mechanism to protect the host against disease-causing microorganisms such as bacteria, viruses and from parasites. The immune system is also essential to eliminate cancer cells in the body and any perturbation in the immune system can lead to disease conditions called autoimmune diseases. These findings therefore suggest, how crucial it is to have an intact immune apparatus functioning in harmony with other systems of the body.

In recent years, several xenobiotics were found to be toxic to the immune system. This meant that the xenobiotics had the capacity to suppress the body's defence against pathogenic microorganisms and cause increased susceptibility to cancer or autoimmune diseases. These serious repercussions made the toxicologists to realize the importance of including the immune system during the toxicity testing of various xenobiotics and led to the emergence of a new field of science called immunotoxicology.

The definition of immunotoxicology is still open as it is still debatable as to which compounds should be called immunotoxic. Although a majority of the compounds tested are immunosuppressive, there are certain chemicals which enhance the activity of the immune system. Thus, immunotoxicology is a science that deals with the study of substances which alter the normal immune system. The use of the word 'alter' rather than 'toxic' is prefered because use of the word 'toxic' gives the impression that the compound is detrimental to the host by depleting the cells of the immune system. In contrast, a compound may destroy certain cells of the immune system such as the suppressor lymphocytes and thereby amplify the immune system which may be beneficial to the host. Thus, a compound may be 'toxic' but still be useful to the host.

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In recent years, excellent literature reviews appeared, dealing with various aspects of immunotoxicology.[1-6] The present review briefly introduces the immune system, and deals in greater detail with the mechanisms of immunomodulation, immunotoxicological screening methods, immunotoxic compounds particularly referring to the studies on o-chlorobenzylidine malanonitrile (CS) and chloroquine which were carried out at the Defence Research & Development Establishment, Gwalior on 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) at McMaster University, Hamilton, Canada and on 1,3-bis(2-chloro-ethyl)l-nitrosourea (BCNU) carried out at the University of Kentucky, Lexington, KY, USA.

2. IMMUNE SYSTEM

The immune system can be broadly divided into nonspecific and specific immunity.

2.1 Nonspecific Immunity

There are several elements in the immune system which can protect in general against invasion by any foreign organisms or cells, collectively constituting the nonspecific defence mechanism. This system includes external skin which provides a passive barrier to the penetration of pathogens and cells such as blood monocytes and neutrophils or tissue macrophages which can phagocytose and digest a majority of the invading organisms. In addition, there are natural killer (NK) cells and lymphokine activated killer (LAK) cells which can elliminate a variety of parasitized cells and cancer cells. The phagocytes are also helped by a variety of humoral or circulating factors belonging to the nonspecific immune system such as lysozyme, C-reactive protein, properdin, complement, etc.

2.2 Specific Immunity

The immune system also consists of specialized cells called lymphocytes which can specifically recognize and selectively eliminate the pathogens or foreign antigens. This component of the immune response is known for its exquisite specificity and for displaying a long-term memory of earlier exposure to the specific foreign antigen.

Specific immunity can be divided into two components – humoral and cellular. Humoral immunity is provided by proteins called antibodies which circulate in the body. Antibodies are secreted by plasma cells which originate from lymphocytes called *B* cells. Humoral immunity defends primarily against infectious agents when they are not inside the host cells. Cellular immunity, in contrast, is effective against organisms which grow inside the cells and also against cancer cells. It is mediated by lymphocytes called *T* cells, since they need the thymus for maturation. Functionally, at least 3 main types of *T* cells can be described – *T* helper cells which provide help in the form of various factors called lymphokines. These *T* cells can interact with other *T* cells or *B* cells and help them to differentiate and grow. The second type of *T* cells are called cytotoxic *T* lymphocytes (CTL). These cells can recognize cells bearing foreign antigens and they can lyse such cells. The CTL are believed to play important role in protection against viral infections and against cancer. They are also the cause of major frustration

for transplantation immunologists since the hosts CTL recognize the foreign antigens present on the transplanted grafts and cause their rejection. The third type of lymphocyte is called *T* suppressor cell (*Ts*). These lymphocytes help to down-regulate the ongoing immune response thereby making sure that the immune response triggered by an antigen comes back to the original resting state. Recently yet another subset of *T* cell called contrasuppressor cell which down-regulates the *Ts* activity has been described.

Morphologically all lymphocytes look alike. However, they differ in several of their surface properties, receptors and functions. The *B* cells have distinct surface antigens called Lyb while *T* cells have Lyt antigens. The *B* cells also carry class II major histocompatibility (MHC) antigens called la antigens (which these antigens) are generally absent on *T* cells. *B* cells recognize an antigen through its surface immunoglobulin or antibody molecule which serves as its receptor, while *T* cells have a distinct receptor and the *T* cell receptor can see a foreign antigen only in association with the MHC molecules. Thus, the *T* helper cells generally recognize a foreign antigen in association with class II MHC molecules while the CIL respond to a foreign antigen in association with class *T* MHC molecule. There are a number of antigenic determinants on *T* cells closely associated with the *T* cell receptor functions. These antigens are essential for the recognition and activation of the *T* cells. *T* helper cells have one such antigen called L3T4 while the CTL carry an antigenic determinant called Lyt2. The *Ts* cells carry *I-J* epitopes which are encoded by a locus called *I-J*. *I-J* has been defined as a locus mapped in the murine MHC which encodes serological markers found primarily on the surface of *Ts* and soluble suppressor factors (*TsF*). The *I-J* epitopes are known to play a major role in the functioning of *Ts*.

3. GENERAL MECHANISMS OF IMMUNOTOXICITY

Some of the possible mechanisms by which xenobiotics can modulate the immune response is shown in Table I. The toxicity of a compound may depend on several

Table 1. General Mechanisms of Immunotoxicity

Direct Effects
1 Cytotoxic to lymphoid organs such as bone marrow, thymus, lymph nodes and spleen.
2 Cytotoxic to stem cells, *T* cells, *B* cells, macrophages or other circulating cells.
3 Effect on the antigen recognition, phagocytosis, processing and presentation.
4 Alterations in the *T* and *B* cell receptor functions.
5 Effect on the production, release or action of mediators such as lymphokines, interleukins, interferrons, etc.
6 Effect on immunoregulatory circuits such as on helper *T* cells, suppressor *T* cells or contrasuppressor *T* cells etc.
Indirect Effects
1 Mediated through the hormones which influence the immune response such as thyroid hormones, corticosterone and gonadotropins, thymic hormones etc.
2 Mediated through nutritional deficiency, e.g. proteins, vitamins, minerals such as zinc etc.

factors. All species of animals may not respond to a particular xenobiotic in the same manner. Thus, genetic factors may influence the outcome of immunotoxicity. Secondly, the route of administration of the xenobiotic may be an important factor determining the immunotoxicity. Other factors include the amount and the duration of exposure. The concentrations in experimental animals of the compound to be screened should be close to simulate natural exposure in humans so that the extrapolation of the data from experimental animals to man is better appreciated. Lastly, a compound may not be directly cytotoxic to the cells of the immune system but can modulate indirectly its function and therefore certain xenobiotics may be immunosuppressive at low doses unaccompanied by clinical signs of the disease.

4. METHODS FOR SCREENING THE IMMUNE RESPONSE

Testing a chemical for immunotoxicity has been found to be much more difficult than testing its toxicity on other systems. The reason is that a single or a few tests cannot reveal whether the immune system is intact or not, especially since the immune system consists of a network of milions of interacting lymphocytes. A number of assays utilized in the Immunology Program at the National Institute of Environmental Health Sciences, U.S.A., to assess the immunologic and host resistance alterations following chemical exposure[7] are listed in Table 2.

It should be noted that most of these assays are direct without dealing with the complex cellular interactions involved in the immune system. Some of these tests may be extremely useful to assess whether a chemical has an overall immunotoxic effect. However, more research needs to be carried out to evaluate at the cellular and molecular level, the exact mechanism by which the chemicals alter the immune response. Thus, tests need to be standardized to evaluate whether a xenobiotic affects the individual functions of cells such as *Th, CTL, Ts* or *B* cells. Furthermore, if it does affect, whether it is due to its direct action on cells or through indirect action-such as its action to inhibit the synthesis or release of lymphokines, like the interleukins.

Any immunological alterations brought about in one cell population can affect several other lymphocytes with varied functions. For example, a suppressed *T* helper cell response can lead to diminished CTL activity or *B* cell function. Suppression of *T* suppressor cells may, in contrast, lead to enhanced CTL activity and antibody production by *B* cells-a situation which can be beneficial or deleterious to the host. For example, it may be beneficial to deplete *T* suppressor cells specific to M. leprae in diseases like lepromatous leprosy, thereby permitting the effector *T* cells to act against the pathogen and control the infection. However, depletion of *T* suppressor cells regulating the *B* cells specific to self antigens, can lead to development of autoimmune diseases. Also, in organ or tissue transplant recipients, it may be beneficial to spare the *T* suppressor cells while selectively depleting the effector *T* cells which destroy the graft.

Thus, the tests employed to screen chemicals for immunotoxicity may not only lead to useful information on the potential risk of the exposed individuals to susceptibility to infections, cancer and autoimmune diseases, but if the chemicals are relatively nontoxic to other systems' they may constitute important

immuno-modulating agents which can be used particularly in the transplant program. Studies in this direction, may be regarded to come under the subdiscipline of immunopharmacology.

Table 2. Assays for Detecting Immune Alterations

Parameter	Procedure performed
Pathotoxicology	Hematology profile (hemaglobin, red blood cell count, white blood cell count, differential)
	Liver chemistries (SGPT, triglycerides, cholestrol)
	Serum proteins (albumin, globulin, A/G, total proteins)
	Weights (body, spleen, thymus, liver, kidney, heart, lung, brain)
	Histology (liver, thymus, lung, kidney, spleen)
Host resistance	Tumor assays (tumor cell challenge TD_{10-20}, and radiometric tumor mass)
	Listeria monocytogens LD_{10-20}, challenge
	Endotoxin hypersensitivity LD_{10-20}
	Expulsion of *Trichinella spiralis*
Delayed hypersensitivity	Radiometric assay with *T* cell-dependent antigen
Lymphocyte proliferation	One-way mixed leukocyte culture
	Mitogens (PHA, Con A, LPS)
Humoral immunity	Immunoglobulin levels (IgG, IgM, IgA) Antibody response to *T*-dependent and *T*-independent antigens
Macrophage function	Resident peritoneal cell numbers and nonspecific esterase staining
	Phagocytosis of ^{51}Cr-SRBC
	Lysosomal enzymes (5'-nucleotidase, acid phosphatase, leucine amino peptidase)
	Cytostasis of tumor target cells
	Cytolysis of tumor target cells
	RES clearance using 125 I-trioleion
Bone marrow colony forming units	CFU-S (multipotent, hematopoietic stem cells)
	CFU-GM (granulocyte/macrophage progenitor)
	CFU-E (erythrocytes progenitor)
	Cellularity
	^{59} Iron incorporation in bone marrow

5. IMMUNOTOXIC COMPOUNDS

Table 3 summarizes various chemicals/xenobiotics, which have been known to alter the immune response and are therefore immunotoxic.

It should be noted that although a majority of the compounds listed in Table 3 are immunosuppressive, there are a few compounds which are known to enhance the

Table 3. Immunotoxic compounds

Environmental contaminants – such as insecticides, pesticides, herbicides and heavy metals. Specifically halogenated biphenyls (PCB, PBB), TCDD, vinyl chloride, hexachlorobenzene.
Pesticides – DDT, parathion, Lindane, Methylnitrophos, Hepatochlor, Carbamine, paraquat, chlorophos, etc.
Dusts – silica, carbon.
Heavy metals – lead, cadmium, mercury, selenium, arsenicals, cobalt, nickel, silica, chromium, platinum
Addictive substances – alcohol, heroin, cigarette smoke etc.
Food additives – pyrogallol, vanillin, tartrazine, carrageenan, saccharin, etc.

immune response. For example, not all metals are immunosuppressive. Selenium enhances the immune response by increasing the number of antibody-forming cells.[8-9] Similarly, although some investigators have shown that hexachlorobenzene (HCB) suppresses humoral immune response, Vos 10 et al. found that HCB in fact stimulated the humoral immune response in the rat, enhanced the mitogenic responseiveness of the spleen cells and did not alter cell-mediated immunity. In addition, Levamisol, an antihelminthic drug has been found to enhance the immune response.[11]

5.1 Immunotoxicity of o-chlorobenzylidenemalanonitrile (CS)

O-chlorobenzylidenemalanonitrile (CS) is one of the most commonly employed sensory irritants used in the control of riots.[12] CS is an alkylating agent and many alkylating agents have been reported to be immunosuppressive due to their action on nucleic acids. However, CS differs from other alkylating agents in that it does not interact with nucleic acids in biological systems. Investigation of effect of CS on the immune system revealed that CS when used in sublethal doses in mice caused a decrease in the weight and cellularity of the thymus, suppressed the antibody response to thymus dependent and independent antigens and suppressed the DTH response to Sheep Red Blood Cells. It was also found that the effect of CS in lower doses was mediated directly on the lymphoid cells since the nutritional status and corticosterone levels were not altered significantly in CS-treated mice[13,14].

5.2 Immunotoxicity of TCDD

TCDD is one of the most potent toxic chemicals known. It is found as an impurity during the production of 2,4,5-trichlorophenoxyacteic acid (2,4,5-T), used as a defoliant. Several studies suggested that TCDD is immunosuppressive [1,6].

TCDD has been shown to bind to a cytoplasmic protein, the synthesis of which is regulated at a locus called [15] Ah. This binding results both in the induction of mixed

function oxidase enzyme activities and some of the toxic effects[15] of TCDD. Inbred mouse strains can be divided into 'responsive' strains such as C57BL/6(Ah^b/Ah^d) where there is marked induction of the enzymes such as aryl hydrocarbon hydroxylase (Ahh) and 'nonresponsive' strains such as DBA/2 (Ah^d/Ah^d) in which there is low or no induction of the AHH. The genetically determined variation in susceptibility to the effects of TCDD is not linked to the H-2 locus since it has been shown that certain $H\text{-}2^d$ (Balb/c) and $H\text{-}2^k$ (CBA/J) strains are susceptible while others are more resistant to toxicity caused[16] by TCDD.

We found that low doses of TCDD (4 ng/kg) suppressed the generation of allospecific cytotoxic *T*lymphocytes (CTL) from 'susceptible' C57BL/6 mice but not from 'resistant' DBA/2 mice[17] To determine if TCDD acted directly on the 'susceptible' lymphoid cells, the immune response of C57BL/6 DBA/2 and DBA/2 C57BL/6 allogeneic radiation bone marrow chimeras was also measured. We found that the susceptibility to suppression in chimeric mice was determined by the Ah genotype of the host and not by the genotype of the grafted lymphomyeloid cells. Experiments demonstrated that suppression of CTL generation by TCDD was due to *T* suppressor cells. The frequency of CTL precursors was not affected by TCDD. These results suggested that the Ah locus may play an important role in the immunotoxicity of certain carcinogens and haloaromatic hydrocarbons.

5.3 Immunomodulation by chloroquine

Chloroquine is a 4-aminoquinoline derivative used extensively in the treatment and prophylaxis of malaria. It has also been used to treat autoimmune diseases. The use of chloroquine as an anti-inflammatory drug and its ability to stabilize the lysosomal membranes suggested that chloroquine may have some immunomodulating effect *in vivo*. We therefore undertook a systematic analysis of the *in vivo* and *in vitro* effects of chloroquine on surface receptors of human peripheral lymphocytes[18]. Chloroquine was administered orally to twenty normal individuals. The total number of circulating lymphocytes or leucocytes in the blood did not change significantly after chloroquine administration. However, there was a significant fall in the percentage and number of lymphocytes with erythrocyte (E) and complement (C'3) markers and an increase in the cells lacking both these markers. Lymphocytes treated with chloroquine *in vitro* failed to show any change in their expression of *E* or *C'3*receptors. The sera from the chloroquine-treated individuals also failed to demonstrate any factor inhibiting the E and EAC rosette formation. These findings suggested that chloroquine may indirectly affect *in vitro* the normal expression of these receptors on lymphocytes.

It has been demonstrated that treatment of macrophages with chloroquine leads to decreased antigen catabolism and such macrophages cannot process the foreign antigen and present[19] it to the *T* cells.

5.4 Immunotoxicity of Nitrosoureas

Nitrosoureas constitute one of the extensively used classes of anticancer agents. They have clinical application especially because they are active against a range of

solid tumors and lymphomas. The cytotoxicity of these compounds is due to their metabolites, chloroethyl diazohydroxide and isocyanate.

We have been particularly interested in BCNU 1,3-bis(2-chloroethyl 1-nitrosourea), since this compound cures a high percentage (> 90 percent) of mice bearing syngeneic tumors and more interestingly 100 percent of the cured mice are resistant to rechallange with the same tumer.[20] These finding suggested to us that BCNU may act on the immune system, in addition to its tumoricidal property. We observed that tumor-bearing mice cannot generate cytotoxic *T* cells (CTL) due to the fact that these mice develop greater numbers of *T* suppressor cells (*Ts*) which down-regulate the CTL, response against the tumor. However following BCNU-treatment, these tumor-bearing mice generate high levels of CTL activity and so Ts-cell activity. This suggest that BCNU selectively acted on tumor-specific Ts thereby permitting the antitumor CTLs to destroy the tumor cells. In addition, we have also found that treatment of tumor cell-targets *in vivo* or *in vitro* with BCNU increased their susceptibility to lysis caused by macrophages but not by the CTL or natural killer cells. Thus, nitrosoureas in addition to the tumoricidal activity, seem to act in a variety of ways leading to an overall enhanced anti-tumor immunity.[20]

6. IMMUNOTOXICOLOGY IN DEFENCE SCIENCE

Although immunotoxicology is relatively a new field, a considerable amount of data accumulated during the past few years on immunotoxicity of certain xenobiotics. The majority of the research thus far carried out has been on environmental contaminants. Thus, considerable work is required to study the immunotoxicity of food additives, commonly used drugs, smoking and industrial solvents, pollutants and gases. From the Defence point of view there are several chemicals and some bacterial and fungal toxins which may be potential chemical warfares agents. Furthermore, there are several chemicals used in the Defence industry to which the Defence industrial workers may be constantly exposed. These chemicals following low level exposure to humans and animals may cause immunological alterations. Immunotoxi studies on such chemicals are essential to understand the potential risks of such exposure on the hosts defense mechanism.

It is possible that some of the toxic compounds may be antigenic, thereby evoking an antibody response *in vivo*. Alternatively, some may act as haptens and it is possible to couple these to larger protein molecules for *use in vivo* to produce antibodies. If these antibodies can bind to the determinant on the parent molecule which is responsible for causing the toxicity, then it can lead to the biological inactivation of the parent molecule and thereby prevent the toxicity. This may constitute an immunological antidote-approach to neutralize the toxicity of certain compounds. Thus, passive administration of the antibodies may be enough to prevent the toxic effects of the specific compound and this approach may be useful in biological or chemical warfare to protect against the toxicity of known chemicals or toxins used. The antibodies can also be used to protect industrial workers against the toxic effects of known chemicals or gases during an accidental exposure.

Although this approach seems simple, we like to caution that practically it will involve elaborate and time consuming research first to identify the site of the parent molecule responsible for causing toxicity, to chemically link the molecule with a large protein molecule which should be immunogenic but not toxic and then screen various antibodies raised, for their capacity to prevent the toxicity of the compound.

7. CONCLUSION

The field of immunotoxicology is new and is in the early stages of rapid development. Although several assays have been described to screen the immune system, these do not cover the entire immune system which is very complex involving the interaction of lymphocytes and accessory cells at the cellular and molecular level. In addition to determining whether a chemical is immunotoxic or not, by including the immune system during routine toxicity studies, attempts must also be made to conduct basic research to address at the cellular and molecular level to the mechanism of immunomodulatory action of various xenobiotics. Screening the chemicals for immunotoxicity is not only useful to predict the susceptibility of the host to infections, cancer and autoimmune diseases, but may also be useful in the pharmaceutical industry to evaluate new compounds for the treatment of transplant recipients, cancers, autoimmune disorders and immunodeficiency diseases.

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