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## IDENTIFICATION OF IMMUNOTOXIC EFFECTS OF CHEMICALS AND ASSESSMENT OF THEIR RELEVANCE TO MAN

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## Contents

Page NOS

## A. SUMMARY AND CONCLUSIONS

1. Immunotoxicity is defined as the adverse effects of foreign substances (xenobiotics) on the immune system. Two types of effects are possible : immunosuppression (which may result in an increased susceptibility to infection or to the development of tumours) and immunopotentiation (which may manifest as allergy or as autoimmunity).

2. Two classes of foreign substances have been discussed as potential immunotoxins: medicines and industrial chemicals, which include environmental chemicals, agricultural chemicals and food additives.

3. A critical review of the literature on the potential immunotoxic effects of medicines and industrial chemicals indicates that there is, as yet, little evidence to substantiate any belief that well controlled occupational exposure to industrial chemicals has led to clinically significant immunosuppression. A number of medicines have been specifically designed to suppress the immune system in organ transplant recipients or are immunosuppressive due to their cytotoxic action during cancer chemotherapy, but there is little to suggest that other medicines have inadvertently caused immunosuppression.

   In contrast, a number of industrial chemicals and medicines have been shown to cause immunopotentiation in exposed populations, producing occupational asthma, dermatitis, drug induced allergy and autoimmunity.

4. Immunotoxic effects have been produced by industrial chemicals in experimental models. In only a minority of such studies has immunosuppression been monitored by assessing overall immune competence; in most studies it has been examined using in vivo or in vitro immune function tests, the results of which are difficult to interpret in terms of effects on human health. Although industrial chemicals and medicines have been studied extensively in experimental models, there are few reports of inadvertent immunosuppression leading to increased susceptibility to infection. Similarly, there is no clear evidence that medicines or industrial chemicals have induced tumours in animals as a direct consequence of an immunosuppressive effect.

5. A strategy for assessing immunotoxicity in experimental models has been discussed. It is considered that sub-acute and chronic toxicity studies (provided

# FOREWORD

Over the past few years the European Chemical Industry Ecology and Toxicology Centre (ECETOC) has published a number of Monographs in which it has attempted to clarify, and express its views on, some of the more important problems in toxicology. This Monograph is a further addition to the series.

The possibility that chemicals could interact with, and modify, the responses of the immune system is well recognised. The successful use of medicines to inhibit the rejections of organ transplants, the present research in the pathogenesis of AIDS, and the widespread use of chemicals with a possible effect on the immune system of man have increased the research in immunotoxicity.

In this Monograph a critical review of potential immunotoxic effects of chemicals is given, and a strategy for assessing immunotoxicity in experimental models and in exposed human populations is discussed. A number of recommendations for further research are also made.

I therefore recommend this Monograph to all those who are responsible for, or concerned about, the health protection of potentially exposed human populations.

R.R. Knowland
Chairman ECETOC Board

they include a full gross and histopathological assessment of the lymphoid organs) together with skin sensitisation tests are capable of providing considerable insight into the integrity and activity of the immune system of a treated animal. Should such studies indicate that a substance has affected the immune system, an assessment of immune function, using function tests, should follow. These detailed studies should be performed at dose levels below those which cause frank toxicity.

6. A strategy for assessing the occurrence of immunotoxicity in exposed human populations has also been discussed. There is a need for reliable clinical assessment, accurate medical record keeping, environmental and biological monitoring for levels of contaminating chemicals and the judicious use of validated immune function tests.

7. A number of recommendations are made. The reliability of toxicity studies in determining immunotoxic effects should be further assessed. In addition it is recognised that there is a need for fundamental research in this area.

## B. INTRODUCTION

The possibility that chemicals and medicines could interact with and modify the responses of, the mammalian immune system is well recognised. The successful use of medicines to modulate the immune system, e.g. to inhibit rejection of organ transplants, the research into the pathogenesis of AIDS and recognition that chemicals used for a wide variety of purposes may have a potential to produce effects on human and animal immune systems, have stimulated discussion on the subject. There is clearly a need to assess whether substances cause immunomodulating effects and whether these effects present a risk to human health. A number of national and international organisations are now showing an active interest in immunotoxicity. It has been the subject of several major scientific conferences which have been extensively reported (NIEHS, 1980; BTS, 1983). The EEC, UNEP, ILO and WHO (IPCS) also discussed the problem at a seminar in 1984 (IPCS, 1984) and in a Technical Review and Working Group (IPCS, 1986)

In the USA the National Toxicology Programme (NTP) considered the types of test which could be used to screen for specific effects on the immune system during the toxicological evaluation of chemicals.

This increasing interest and experience in immunotoxicology and the promotion of immune function tests for incorporation into routine toxicological test schedules was considered to be of particular importance to ECETOC and should be the subject of a critical review since no clear guidance is presently available on how to address immunotoxicity.

A TF was therefore set up with the following terms of reference :

1. To review to what extent conventional toxicological testing can provide information of value in the detection of immunotoxicity.
2. To comment whether supplementary tests are necessary for the detection of immunotoxic effects.
3. To review critically the reliability of tests from which the effects of chemicals on the human system can be predicted.
4. To advise whether it is possible at present to set out strategies for assessing the immunotoxic effects of chemicals and the assessment of likely hazard to human health.

Definitions of some terms specifically related to immunotoxicity as well as the meaning of some abbreviations are given in Appendix 1.

## C. THE IMMUNE SYSTEM AND IMMUNOTOXICITY

### 1. The Immune System

The immune system is concerned with recognising and defending against infective micro-organisms and neoplastic cells. It also responds to transplanted organs or tissues and food and chemical materials which have been ingested, inhaled or applied to the skin. Many foreign materials are prevented from entering the body or are rapidly eliminated by non-specific, non-immune (e.g. mucous secretions and phagocytosis by macrophages) and immune mechanisms. With some substances, individuals may develop an immune response which is specific to the substance and which imparts immunological memory so that the body is able to react more quickly and effectively to a future attack by the substance. This adaptive immune system may be considered in simple terms to consist of three specific elements : the foreign substance which is called the antigen; lymphocytes which are cells of the blood and lymphoid system and antibodies (cf. Appendix 1 for definition of the underlined terms). Interactions between

these three elements and other specialised cells (e.g. antigen presenting cells) or other biological systems (e.g. the complement system) form the basis of the activity of the immune system. A response against an antigen mediated directly by lymphocytes is termed cell-mediated immunity. Responses involving antibodies are referred to as humoral immunity.

A generalised reduction in the capacity for either type of response is known as immunosuppression and may result in an increased susceptibility to infection by micro-organisms or to the development of tumours as seen for example in AIDS.

A generalised increased immune responsiveness is known as immunopotentiation. This report considers only two antigen specific manifestations of this phenomenon, namely hypersensitivity (allergy) and autoimmunity. Appendix 2 describes in further detail the mechanisms of immune hypersensitivity.

Detailed reviews of the immune system and its components can be found in Roitt (1984), Paul (1984), Staines et al.(1985) and Roitt et al.(1985). Figures 1 to 3 depict the basic elements of the immune system.

2. Immunotoxicity

Immunotoxicity is defined as the adverse effects of foreign substances on the immune system. Two types of effects are possible : immunosuppression and immunopotentiation. Foreign substances may be of synthetic, natural or biological origin and include medicines as well as industrial chemicals.

## D. IMMUNOTOXIC EFFECTS OF CHEMICALS IN HUMANS

1. Introduction

Using experimental models a large number of medicines and industrial chemicals have been shown to have various effects on the immune system (see Chapter E). With the exception of hypersensitivity reactions and the effects of those medicines specifically designed to modify the immune response, evidence

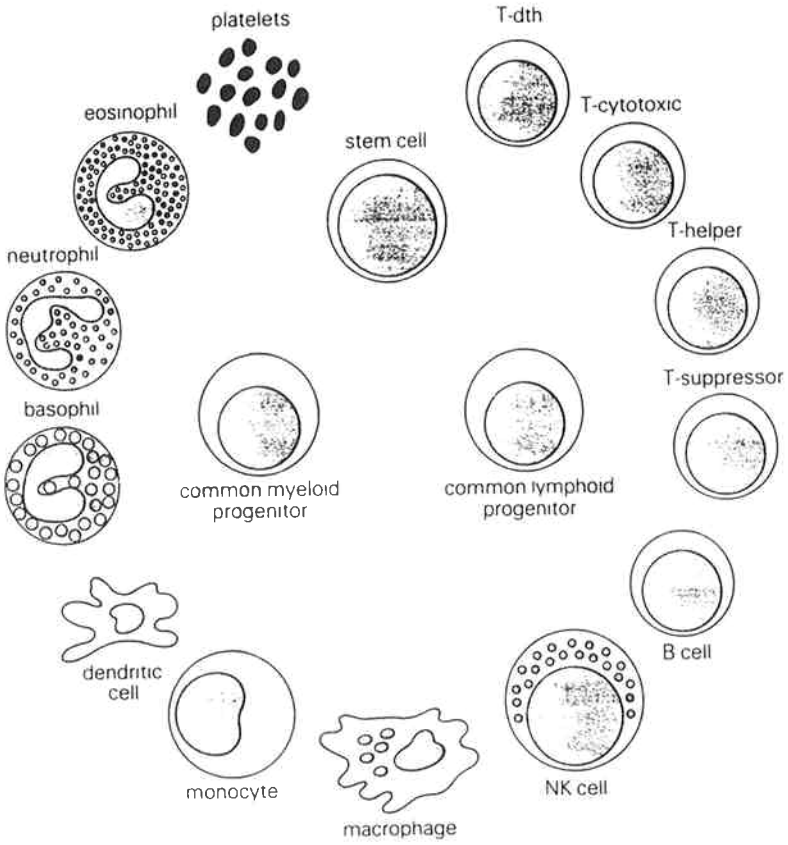


Figure 1. The cells of the immune system. Two major categories of cells are derived from the common stem cell precursor: lymphoid and myeloid. A "third population" known as natural killer (NK) cells may be lymphoid but their origin is uncertain. (Cells not drawn to scale).

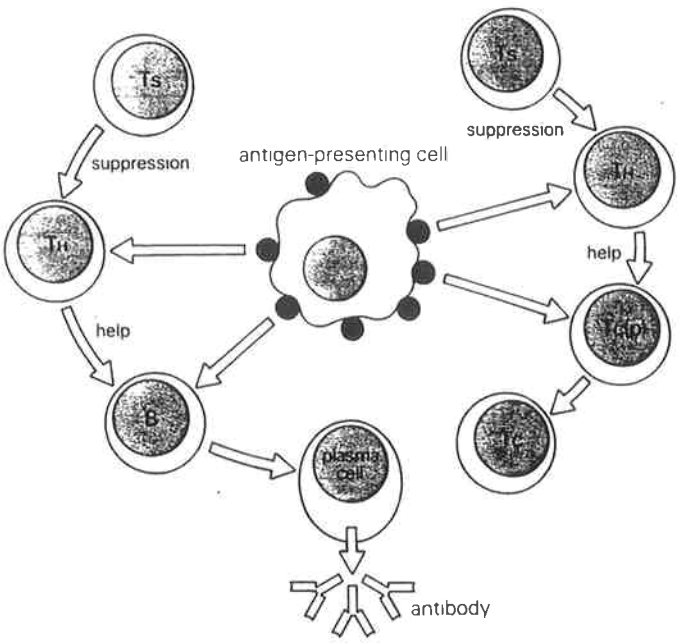


Figure 2. The interaction of cells in an immune response. Antigen is presented to B cells, T-helper cells and precursors of T-cytotoxic cells by antigen-presenting cells. The activation of T-cytotoxic cells requires a signal from the T-helper cell, which may be suppressed by T-suppressor cells. The transformation of B lymphocytes into antibody-producing (plasma) cells usually requires both the presentation of antigen and a help signal.

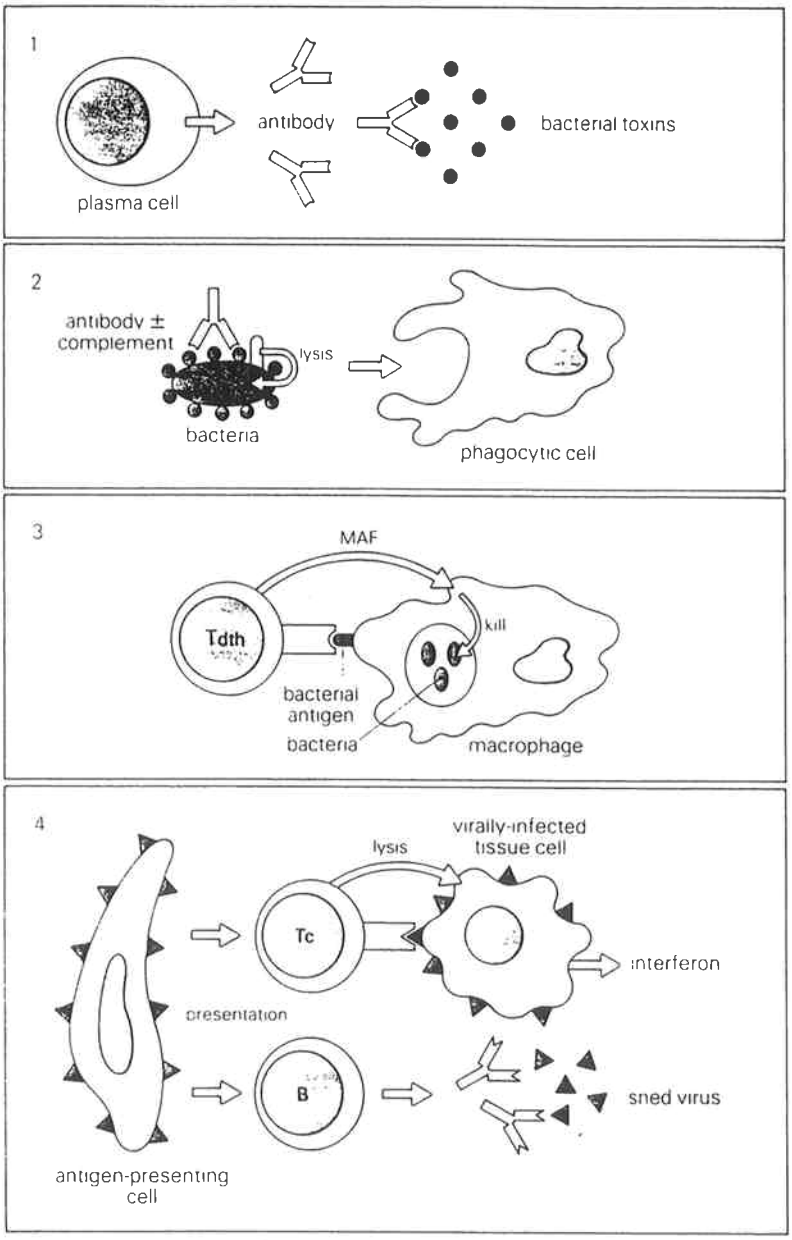


Figure 3. Immune responses to infectious agents. Antibody protects against bacterial damage by combining with and neutralising toxins (1) and binding to the bacterium itself, thus facilitating phagocytosis and activating complement (2). Macrophages which are unable to kill the phagocytosed bacteria present bacterial antigens on their surface (3). These are recognised by a sub-set of amplifier T cells known as Tdth cells which secrete lymphokines, a heterogeneous group of chemicals, one of which, macrophage activating factor (MAF), stimulates the macrophage to kill the intracellular bacteria. Virus-infected cells are lysed directly by T-cytotoxic cells (Tc) following presentation of viral antigens by antigen-presenting cells (4). Virus infected cells release interferons which limit viral replication in other tissue cells. Antibody performs two important functions by binding to virus which has been shed by infected cells : it promotes phagocytosis and prevents the virus attaching itself to other tissue cells.

Fig.1, 2, 3 reprinted from : Introducing Immunology. Eds. N. Staines, B. Brostoff, K. James (1985). By permission from Gower Medical Publishing, London.

that medicines and industrial chemicals produce unexpected immunotoxic effects in humans is scarce. Examples of immunotoxic effects described here are limited to a few medicines and industrial chemicals for which evidence of an effect on the immune system has been found.

## 2. Immunosuppression

The normal immune response can be suppressed by factors such as aging, acute and chronic infections, stress, malnutrition and neoplastic disease. Assessment of the possible immunosuppressive effects of medicines and industrial chemicals must take account of these confounding factors.

### 2.1. Medicines

When considering the immunosuppressive effects of medicines it is essential to differentiate between those which are intended to interact with and suppress the immune system and those which are not expected to have immunosuppressive effects.

There is evidence that medicaments which suppress the immune response predispose to infections, aggravate existing infections or reactivate latent infections. An increased incidence of infections is seen for example in patients undergoing therapy with corticosteroids and other medicines used in organ transplantation or to treat cancer (Cupps and Fauci,1982; Heise, 1982). In cancer patients, the frequency and severity of infections are greatly increased; this arises not only from the immunosuppressive effect of anticancer medicines but also from the disturbed immune function caused by the neoplastic disease itself.

As corticosteroids and cytotoxic medicines (e.g. azathioprine, cyclophosphamide, chlorambucil, thiotepa) influence almost all components of the immune system (Bach, 1975), the exact mechanism by which resistance to infections is reduced is unclear.

An increased incidence of neoplasia is a serious problem in patients treated with immunosuppressive medicines. Further malignancies, mainly leukaemias and lymphomas, develop frequently following chemotherapy for a variety of neoplastic diseases. With the increase in the survival time of patients treated with chemotherapy there is an increased probability of the development of a second cancer (Penn, 1985). This complication seems to be

associated particularly with prolonged use of alkylating immunosuppressive agents. However treatment with high doses of the medicine Ciclosporin A, which is not an alkylating agent, has also been associated with an increased incidence of lymphomas. These may, however, result from a decrease in resistance to Epstein Barr (EB) virus, as a consequence of the immunosuppressive action of the medicine, and they regress when the dose of Ciclosporin A is reduced or when treatment is discontinued. (Elves, 1983; Starzl et al., 1984; Penn, 1985). On the other hand reports indicate that antimetabolite immunosuppressants such as methotrexate and azathiophrine can also increase the risk of cancer as can long term treatment of organ transplant patients with mixtures of non-alkylating immunosuppressive medicines (Penn, 1985; Bennett and Norman, 1986). Thus it is not clear whether the increased cancer incidence seen in patients treated with immunosuppressive medicines is caused directly by the genotoxic action of the medicine used, or whether it is a consequence of the immunosuppressed condition of the patient. It seems likely that the development of malignancies in the patients is determined by complex interactions of a number of factors, such as reduced immunosurveillance, individual susceptibility, graft-versus-host reaction, co-carcinogenic effects and oncogenic viruses.

2.2. Industrial chemicals.

Over the last 10-15 years there have been a number of incidents in which aromatic halogenated hydrocarbons such as polychlorobiphenyls (PCBs), polybromobiphenyls (PBBs) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) have been released into the environment leading to direct human exposure and more commonly to indirect exposure from contaminated food or soil. These chemicals have been shown to have a potential to induce immunosuppression in a number of experimental models (cf. Chapter E).

Bekesi et al.(1978) investigated the effects of PBBs on the immune system of dairy farmers from Michigan who had eaten 9-10 mg in contaminated food (Fries, 1986). In comparison with control groups, Wisconsin farmers and New York residents, the Michigan farmers showed a decreased number of circulating T-lymphocytes and an increased number of circulating lymphocytes without surface markers (null cells). In addition T- and B-cell proliferation was decreased in the exposed group. No correlation was found between these effects and the PBB serum concentration. Although Roboz et

al.(1980) described a correlation between the changes in lymphocyte numbers and the PBB level in the lymphocytes, Strass et al.(1981) could not confirm the findings of Bekesi et al. (1978) in a study of Michigan farmers and PBB manufacturing workers who had serum PBB concentrations of 14 and 48 ppb respectively. There is no clear evidence that the exposure resulted in an increased susceptibility to infection.

Shigematsu et al.(1978) found an increased incidence of respiratory infections in humans who had been exposed to PCB in contaminated rice oil; the estimated average total exposure was 800 ml of oil containing 1000 ppm PCB. In another study Chang et al.(1982-a) found a reduced number of immunoglobulin and complement receptors on monocytes and polymorphs in a population exposed to PCB (serum concentration 15.5 - 98.4 ppb) and concluded that the decreased function of the cells could lead to an increased incidence of infections. In another study, Chang et al.(1982-b) found a significant correlation between the suppression of cellular immunity (assessed by response to recall antigens) and the severity of dermal lesions caused by PCB exposure.

Following an accident in 1976 in Seveso an immunological investigation was performed on 44 children exposed to an estimated total of 7.5 mg TCDD. Concentrations of serum immunoglobulins and complement and in vitro lymphocyte proliferation were all normal (Reggiani, 1978). In contrast, Hoffmann et al.(1986) reported an increased frequency of anergy to recall antigens in people living in a TCDD contaminated area for up to 15 years. The TCDD levels in soil ranged from 39-1100 ppb.

Studies performed on workers exposed to solvents have shown effects on certain immune parameters,e.g. decreased serum immunoglobulin levels (Lange et al., 1973), and decreased numbers of circulating T-lymphocytes (Moszcsynsky, 1981; Denkhaus et al., 1986). However their relationship to solvent exposure is uncertain since quantitative exposure data were lacking. In addition, no increase in infections was described so the clinical significance of these findings remains unclear.

Some heavy metals (Pb, Hg and Cd) have caused immunosuppression in experimental animals as has been demonstrated for example by host resistance tests (Koller, 1980; Lawrence, 1981). Only limited human data

are available and these do not substantiate the findings in animals. Kimber et al.(1986) found no alteration in serum immunoglobulin levels (IgG, IgA, IgM), T-lymphocyte numbers and NK-cell function in workers exposed to lead (mean blood lead concentration 400 $\mu g.l^{-1}$,mean exposure time 10 ± 6 years).

Although alkylating agents are expected to cause immunosuppression, Van Sittert et al.(1985) could find no effects on serum immunoglobulin levels and B- and T-cell proliferation in a group of workers exposed to low concentrations of ethylene oxide (<0.05 ppm).

Experimental data show that animals exposed to $O_3$ or $NO_2$ have a decreased resistance to infection and it has been suggested that this may be due to impaired non-specific defence mechanisms (Gardner and Graham, 1984). There are no well validated human data available to confirm that these agents are immunosuppressive in man although an association has been reported between exposure to $SO_2$, $NO_2$ and $O_3$ as air pollutants, and the incidence of acute respiratory tract infection, especially in children. (Lunn et al., 1967; French et al., 1973; Mostardi et al., 1981). A number of confounding factors such as smoking, and socio-economic status may play an important role in the pattern of disease observed (Thomas et al., 1973; Ferson et al., 1979; Anon., 1985; Van Sittert et al ., 1985); even passive smoking has been connected with an increased incidence of pneumonia and bronchitis in childhood. (Colley et al., 1974).

Numerous epidemiological studies have explored the relationship between occupational exposure to chemicals and cancer incidence. Gozodilovic and Mandrik (1978) found tumours in workers exposed to benzidine, especially those with suppression of cellular immunity as shown by skin tests. Asbestos exposure also suppresses cellular immunity (Doll et al., 1983; Bekesi, 1984) and it has been suggested that the increased cancer incidence in asbestos workers could be due to this immunosuppression. In spite of the immunotoxic effects demonstrated to occur with these and other chemicals no conclusion can be drawn from data at present available on the relationship between immunosuppression and the occurrence of cancer following exposure to industrial chemicals.

Animal data demonstrate that organotin compounds are immunosuppressive and their mechanism of action has been clarified (cf. Chapter E). At present

there is no evidence to suggest that exposure to organotin compounds leads to immunosuppression in man.

In conclusion there are, as yet, few human data available to substantiate the belief that exposure to industrial chemicals can lead to clinically significant immunosuppression.

3. Immunopotentiation

Immunopotentiation arising from exposure to medicines and industrial chemicals may result in the development of allergy and/or autoimmunity.

3.1. Allergy

Allergic reactions most commonly involve the respiratory tract and the skin. The following examples are only a selection of the vast amount of data available in the literature.

3.1.1. Medicines. Antibiotics in particular penicillins, are probably the most common inducers of respiratory allergy which manifests as rhinitis, bronchoconstriction and bronchospasm and occasionally anaphylaxis (Descotes, 1986). Other antibiotics may also cause anaphylactic reactions but more rarely than penicillin allergy. Anaphylactic reactions have also been reported to dextran solutions and medicines containing animal proteins (Brewis, 1981; Assem, 1981).

The most common allergic reaction in the skin following topical exposure is eczema. Allergic skin reactions are also encountered when medicines are administered systemically. The mechanism by which such effects occur is often not understood and the fact that one medicine may produce a variety of skin reactions makes it difficult to decide whether or not any or all of the effects are immunologically mediated. Medicines such as penicillin, imipramine, dextran solution and insulin may induce an urticaria (Felix, 1979) which is mediated by a type I hypersensitivity mechanism (cf. Appendix 2). Allergic contact dermatitis, which is mediated by a type IV mechanism (cf. Appendix 2) is often seen following topical administration of certain medicines. In one multi-centre study, 14% of 4000 patients with eczema were considered to have drug-induced allergic contact dermatitis (Felix and Stevenson, 1981). Topical

medicines containing local anaesthetics (e.g. benzocaine), antihistamines, antibiotics (e.g. neomycin), corticosteroids and certain cream or ointment ingredients (e.g. lanolin, parabens) are well known sensitisers (Fisher, 1986). A particular problem with some allergens is cross-reactivity. For example patients sensitised to neomycin may react also to gentamycin, kanamycin and framycetin (Felix, 1979).

Certain medicines require activation by UV light before they induce allergic contact dermatitis. They are known as photosensitisers and include the sulphonamides, phenothiazines, tetracyclines and nonsteroidal anti-inflammatory agents (Felix, 1979).

3.1.2. Industrial chemicals. Several important industrial chemicals are now recognised as causes of occupational respiratory allergy, for example isocyanates, acid anhydrides, platinum salts and reactive dyes (Parkes, 1982; Chan-Yeung and Lam, 1986).

There are two distinct clinical manifestations of occupational allergic lung disease, asthma (Bernstein, 1981) and alveolitis (Fink, 1984-b). Allergic occupational asthma is mediated by a type I hypersensitivity reaction and extrinsic allergic alveolitis (EAA) by a type III and/or type IV reaction (cf. Appendix 2). In addition, repeated exposure to allergens may increase the bronchial hyperreactivity of sensitised individuals (Cockcroft et al., 1977), making them more likely to react to nonspecific (irritant) stimuli.

Rhinitis and/or asthma have been seen in many subjects exposed to toluene di-isocyanate (TDI) and occasionally in those exposed to hexamethylene di-isocyanate (HDI) and diphenylmethane di-isocyanate (MDI). The mechanism of TDI-induced asthma is not fully understood. Immunological factors, such as the detection of isocyanate-specific IgE in the serum of symptomatic individuals (indicating a type I hypersensitivity mechanism) have been demonstrated in only 15-20% of subjects who showed bronchial hypersensitivity to TDI in inhalation challenge tests (Butcher et al.,1980; Baur, 1983). In the remaining 80-85% it was suggested that non-immunological mechanisms such as $\beta$-adrenergic receptor blockage and/or acetylcholinesterase inhibition may have led to the asthmatic symptoms

(Butcher et al.,1982; Dewair et al.,1983). Both immunological and non-immunological mechanisms can increase non-specific bronchial reactivity. Isocyanate-induced EAA has also been described (Malo et al., 1983; Nielsen et al.,1985).

The acid anhydrides, phthalic anhydride, trimellitic anhydride and tetrachlorophthalic anhydride, also cause occupational rhinitis and asthma. A type I hypersensitivity mechanism has been demonstrated in many subjects who develop symptoms immediately following exposure by the finding of specific IgE in the serum or a positive skin prick test (Bernstein et al.,1983; Howe et al., 1983; Wernfors et al.,1986). Some individuals develop symptoms which begin 4-12 hrs after exposure to anhydrides (Bernstein et al.,1983). This is referred to as late respiratory systemic syndrome and is characterised by the presence of specific IgG antibodies in the serum. It thus resembles a type III reaction, such as that seen in EAA. TMA exposure can also result in an infiltrative lung disease with haemoptysis and anaemia which resembles Goodpasture's syndrome, and may therefore involve a type II reaction (Zeiss et al.,1983).

The respiratory allergenicity of platinum salts is well documented. The immediate onset of symptoms, the presence of platinum-specific IgE antibodies and positive skin prick tests have confirmed that many of the symptoms are caused by a type I hypersensitivity mechanism (Biagini et al.,1985; Murdoch et al.,1986). Other metals and salts such as the well-known skin sensitisers, chromates and nickel salts, can also cause respiratory allergy (Fink, 1984-a). Hard metal lung disease (asthma and/or EAA) can result from exposure to cobalt (Sjögren et al.,1980; Gheysens et al.,1985). Berylliosis is a granulomatous lung disease which possibly arises from a type IV reaction (Parkes, 1982).

Respiratory allergy to reactive dyes was first described by Alanko et al.(1978). Four workers had IgE-mediated hypersensitivity reactions to a small number of reactive dyes as shown by nasal and bronchial provocation tests and by positive skin tests and RASTs. Further cases of respiratory allergy to a wide range of reactive dyes have since been identified in dye-house workers in the UK, the majority being confirmed to be IgE-mediated by skin tests and RASTs (Docker et al.,1986).

Exposure to proteolytic enzymes derived from Bacillus subtilis caused respiratory allergy in the detergent industry. Again, tests confirmed that they were mediated by a type I reaction (Juniper et al.,1977).

Type I and type IV reactions can also mediate allergic skin diseases. Type IV reactions which lead to contact dermatitis can occur following exposure to potassium chromate, nickel sulphate, para-phenylenediamine, cobalt chloride, colophony, ethylenediamine, mercaptobenzthiazole, formaldehyde and epoxy resins (Foussereau et al.,1982). Some of these substances can also cause occupational asthma (Sherwood Burge, 1981).

Diethyltoluamide (Kleinhaus, 1985), parabens (Henry et al.,1979) and chloramine-T (Dooms-Goossens et al.,1983) are examples of allergens which can cause contact urticaria by a type I reaction. It should be appreciated that many cases of urticaria following exposure to industrial chemicals are caused by non-immunological mechanisms (Kleinhaus, 1985).

## 3.2. Autoimmunity

3.2.1. Medicines. A number of medicines induce autoantibodies and/or clinical symptoms indicative of autoimmune disease. The symptoms usually disappear after cessation of drug treatment.

Drug-induced autoimmunity is best seen in the systemic lupus erythematosus (SLE)-like condition associated with the use of certain medicines (e.g. procainamide, isoniazide, hydralazine, penicillamine and thiouracils) (Rothfield et al.,1978; Reidenberg, 1981; Davies, 1981; Jaffe, 1981; Litwin et al.,1981; Assem, 1981; Hughes et al., 1981). Long-term penicillamine treatment has occasionally induced autoimmune responses resulting in skin eruption (pemphigus-like blisters) or a neuromuscular disorder (a syndrome similar to myasthenia gravis) (Felix, 1979). Autoimmune haemolytic anaemia has been reported following long-term treatment with methyldopa, l-dopa, mefenamic acid, chlorpromazine and hydantoins (Girdwood, 1979). Mild to severe proteinuria has been seen in rheumatoid arthritis patients treated with gold salts; immunological tests indicated possible autoimmune pathogenesis (Wooley et al., 1980).

3.2.2. Industrial Chemicals. Asbestos and silica exposure appear to stimulate the humoral immune response as shown by an increased prevalence of auto antibodies, e.g. antinuclear antibodies (ANA). Exposed individuals have an increased frequency of certain autoimmune diseases (e.g. rheumatoid arthritis, SLE, progressive systemic sclerosis) (Doll et al.,1983; Sluis-Kremer et al.,1985). Although the results of some studies suggest a relationship between the presence of autoantibodies and the development of lung fibrosis in asbestosis patients, other studies indicate a relationship between lung fibrosis and suppressed immunity (Boyd et al., 1982; Lange et al.,1986).

An association between exposure to high levels of solvent vapour and the development of glomerulonephritis, especially Goodpasture's syndrome, has been proposed (Ravnskov et al.,1983; Polla et al., 1983; Bernis et al.,1985). The suggested mechanism of action was the production of antibodies against alveolar basement membrane which had been damaged by the solvents and cross-reaction between these antibodies and the basement membrane of the glomerulus (Lauwerijs et al.,1985).

Immunological mechanisms have been suggested in the aetiology of lead and mercury nephropathy (Wedeen, 1984). For example antiglomerular basement membrane antibodies have been reported following exposure to mercury compounds (Druet et al.,1982).

Autoimmunity has been implicated in the aetiology of the "Toxic Oil Syndrome" (Kilbourne et al.,1983). In 1981 this disease occurred in 20,000 Spanish people after consumption of a chemically contaminated rape-seed oil. Symptoms were diverse and included an unspecified neuromuscular syndrome, scleroderma, Raynauds' disease, Sjögren syndrome and pulmonary hypertension. However, evidence suggests that the "Toxic Oil Syndrome" cannot be explained solely by the development of autoimmunity.

Chemical and medicine induced autoimmunity presents a complex problem. Its development is influenced by factors such as age and nutritional state and perhaps most importantly, by genetic predisposition. For example, the induction of SLE-like disease by medicines depends on the rate of metabolism (Reidenberg, 1983) and there may be a link between HLA

type and the development of solvent related Goodpasture's syndrome (Rees et al., 1978).

## E. IMMUNOTOXIC EFFECTS OF CHEMICALS IN ANIMALS

### 1. Introduction

Experimental animal models have been used to study the effects of chemicals and medicines on the immune system. Using a wide variety of experimental techniques, a large number of medicines and chemicals have been demonstrated to affect cell mediated and humoral immunity, and non-specific defence mechanisms. However, apart from sensitisation, many of the findings reported in animals, have not so far been observed in humans. The reasons for the poor correlation are unclear but it may reflect the use of high experimental dose levels (causing frank toxicity), inappropriate routes of exposure or species differences, etc..

Although chemicals and medicines can suppress or potentiate particular aspects of the immune system, it should be recognised that it is the final overall effect that substances have on immune competence which is most important. Demonstration of an effect on an isolated aspect of immune function, e.g. mitogen responsiveness, does not necessarily indicate that the immune system of the animals has been sufficiently compromised to cause adverse health effects.

A comprehensive review of all published data on the immunotoxic effects of industrial chemicals and medicines is not within the scope of this monograph; this chapter will examine only selected examples of chemicals which have been shown to be immunotoxic to animals. The immunotoxic effects of chemicals and medicines have been catalogued by Descotes (1986).

### 2. Immunosuppression

Suppression of the overall immune response in the three immune pathways (humoral, cell mediated and non-specific) is usually reflected by :

a) increased susceptibility to infection;

b) decreased antibody response to immunisation;

c) decreased cell mediated immunity.

These immunotoxic effects are commonly associated with pathological changes such as bone marrow suppression or lymphoid tissue atrophy, which are seen as sensitive indicators of possible toxicity to the immune system (Vos, 1977; Luster et al., 1982).

2.1. Medicines

Cyclophosphamide suppresses all cells of the immune system by acting on bone marrow stem cells (Hersh, 1974). In the mouse this has been shown to manifest itself as increased susceptibility to bacterial (Bradley, 1985) and viral infections (Dempsey and Morahan, 1985), decreased resistance to tumour transplants (Dean et al., 1979-c; Murray et al., 1985) and reduction in cell-mediated immunity (Dean et al., 1979-c). In vitro/ex vivo assays suggest that cyclophosphamide reduces B and T cell proliferation and development of antibody producing cells (Dean et al., 1979-c). It also causes a number of pathological changes such as lymphoid tissue atrophy and alterations in numbers of circulating lymphocytes.

In a comparison of the effects of diethylstilbestrol and cyclophosphamide in mice, both compounds impaired resistance to bacterial infection but no marked depression was seen to parasitic infections (Morahan et al., 1984).

The immunosuppressive effects of corticosteroids are well known (Bach, 1975). Their mechanism of action is complex (Young et al., 1981); they are cytotoxic to lymphocytes and consequently suppress both humoral and cell mediated immune pathways leading to an increased susceptibility to infections and a reduction in delayed hypersensitivity responses. In vitro/ ex vivo experiments have shown that corticosteroids also suppress B and T cell proliferation and their cytotoxic action means that they cause extensive atrophy of lymphoid tissue and a reduction in numbers of circulating lymphocytes. Although corticosteroids seem to have similar effects in a number of species, the mouse and rat appear to be more sensitive than the guinea pig or non-human primates (Frenkel and Havenhill, 1963).

Ciclosporin A is a potent immunosuppressive agent which acts specifically against T cell populations (Elves, 1983). In animals it impairs antibody production to T cell dependent antigens, reduces delayed type hypersensitivity responses, prolongs skin graft survival and causes atrophy

of lymphoid tissue (Ryffel et al., 1983). Following exposure of rats and primates for up to 2 years there was no evidence of increased susceptibility to infection or neoplasia. In another study an increased incidence of lymphomas was found in primates undergoing organ transplants and receiving a combination of immunosuppressive drugs including Ciclosporin A (Reitz and Bieber, 1982). The relevance of this finding remains controversial but is thought to be related to the presence of Epstein-Barr virus in these animals (Elves, 1983).

2.2. Industrial chemicals

The immunosuppressive effects of PBBs are well documented (Faith et al., 1978) and have been reviewed by Fries (1986). Exposure of several animal species to high levels of PBBs results in atrophy of the thymus and T cell dependent areas of the spleen and lymph nodes. Reductions in serum IgM and IgG have also been reported. In rats and mice these findings have been accompanied by decreased mitogen responsiveness, indicative of an effect on T and B cell function, although some contradictory results are reported (Faith et al., 1978). Reduction of a delayed type hypersensitivity response has also been demonstrated. The effects on the immune system invariably occur at dose levels at which there are other signs of toxicity (liver lesions, decreased body weight gain). There appears to be considerable species difference in response, the rat being particularly sensitive (Luster et al., 1978).

Other halogenated aromatic hydrocarbons such as PCBs and TCDD also suppress the immune system of animals. These have been reviewed by Vos (1977), Koller (1979), and Silkworth and Loose (1981). In common with other immunosuppressive agents the effects of TCDD on lymphocyte function appear to vary with the age of the animal; immature T and B cells are particularly sensitive to the suppressive effects of TCDD. In addition, exposure of rabbits to low levels of PCBs or TCDD had either no effect on cell mediated and humoral responses (Street and Sharma, 1975) or enhanced the IgG antibody response to tetanus toxoid (Sharma et al.,1984). Another study in rodents showed that exposure to low levels of PCBs enhanced rather than suppressed mitogen responsiveness (Bonnyns and Bastomsky, 1976). Suppression of T-dependent antibody responses and the thymic atrophy induced by PCBs in mice depends on the planarity of the PCB molecule (Silkworth and Brabstein, 1982). Use of different strains of mice has

demonstrated that PCBs bind to the Ah (aromatic hydrocarbon) receptor to produce altered growth and differentiation responses. Studies of hepatic aromatic hydrocarbon hydroxylase induction have led to the conclusion that immunotoxicity caused by halogenated aromatic hydrocarbons is a consequence of activation of the Ah gene complex (Silkworth et al, 1984).

The immunotoxic effects on animals of organotin compounds, particularly di(n-octyl)tin chloride (DOTC), have been extensively studied. DOTC causes thymic atrophy in the absence of other pathological change on oral adminis tration to inbred rats (Seinen and Williams,1976). A depletion of small lymphocytes in the thymic cortex, without signs of lymphocyte destruction or macrophage activation, is the most prominent feature (Penninks et al. 1985). DOTC exposure led to a reduction of cell-mediated immune responses in the rat (Miller, 1983). Miller et al.(1986) have also shown that DOTC exposure can affect the T-cell dependent humoral immune response at high doses in female Balb/c mice (500 mg/kgbw by gavage at weekly intervals for 8 weeks). However, the delayed hypersensitivity response to oxazolone was not influenced by DOTC treatment.

Inorganic metals (particularly lead, cadmium and mercury) affect the immune system in animals; this subject has been reviewed by Koller (1980). The relevance of the effects is uncertain since both immunopotentiation and immunosuppression can occur depending upon the species studied, the dose administered, the route of exposure and the particular metal salt administered. For example, intravenous injection of lead acetate increased the susceptibility of rats to various gram-negative bacteria (Selye et al., 1966). The resistance of mice to Salmonella typhimurium was also decreased following injection of various lead salts (Hemphill et al., 1971). Using the Jerne plaque assay, Hillam and Ozkan (1986) have shown that aerosolized lead nitrate (estimated exposure 80 µg/d for 14 d) was more immunosuppressive than similar amounts of the same compound administered by gastric intubation (125 µg/d). Exposure of a number of strains of mice to lead even at relatively high doses (10 mM in drinking water for 3 to 4 weeks) did not produce immune suppression (Lawrence et al.,1984) whilst Balb/c mice showed enhanced responses (Koller et al.,1976). These effects were usually obtained after the injection or oral administration of substantially greater concentrations of lead than is normally found in the environment. Conflicting results were reported by Kerkvliet and

Baccher-Steppan (1982), who found enhancement of cell mediated cytotoxicity whereas Neilan et al.(1983) described a reduction of antibody dependent cell cytotoxicity in the absence of overt toxicity in mice administered lead acetate in the drinking water.

Airborne chemicals which affect the human immune response,for example asbestos, silica , coal dust, and $NO_2$ (cf. Chapter D) have also been shown to affect the immune response in animal models (Burns et al.,1980; Jakab, 1987;Gardner and Graham, 1984; Hahon et al., 1985; Hahon and Booth 1986) although the nature of the effect depends on the type of animal model used (Foster et al, 1985). As in man, silica exposure in the mouse resulted in either immune enhancement or suppression (de Shazo, 1982), with low doses generally stimulating and high doses suppressing the response, as assessed by the plaque forming cell (PFC) assay, alveolar macrophage phagocytosis and cytotoxic antibody activity (Burns et al., 1980; Scheuchenzurber et al., 1982, 1985). Similarly, exposure to asbestos resulted in various effects on the immune system (de Shazo, 1982). Exposure of mice to $NO_2$ resulted in increased susceptibility to bacterial infections (Gardner and Graham, 1984). By using different bacteria, it has been demonstrated that both alveolar macrophage phagocytosis and the killing of gram negative bacteria by polymorphonuclear leucocytes are adverseley affected by different concentrations of $NO_2$ (Jakab, 1987).

## 3. Immunopotentiation

### 3.1. Allergy

Animal models of delayed hypersensitivity in skin and lung are more predictive than *in vitro* models. The guinea pig model of skin sensitisation has been widely used and has provided data of value in assessing risk to man (Andersen and Maibach, 1985). This animal has also been proposed as a model for the study of pulmonary hypersensitivity reactions (Doe, 1983; Karol et al., 1985) but as yet no validated procedure is available for prediction of human reactions. Similarly there are no predictive animal models for immediate type hypersensitivity reactions in skin or type II or type III allergic reactions (e.g., haemolytic anaemia, glomerulonephritis).

3.1.1. Medicines. The guinea pig is susceptible to pulmonary anaphylactic reactions (Ratner et al.,1927) and for some groups of medicines (e.g. penicillins) type I lung hypersensitivity can be demonstrated in this animal. Using a variety of guinea pig models, delayed (type IV) hypersensitivity reactions in the skin have been demonstrated with a number of medicines which cause allergic dermatitis in man, for example neomycin, penicillin and benzocaine. (Andersen and Maibach, 1985).

3.1.2. Industrial Chemicals : Isocyanates are a major cause of occupational respiratory allergy; essentially all the features of human disease have been reproduced in the guinea pig (Karol, 1986). Sensitisation to toluene diisocyanate (TDI) by the inhalation route can be achieved in the guinea pig at exposure levels consistent with those in man (Karol, 1983).

Environmental exposure to platinum group metals, which causes an IgE antibody response in humans (Murdoch et al., 1986), has been reported to produce similar type I hypersensitivity reactions in rabbits and rodents (Tomilets et al., 1980); others could reproduce these results in the rat only with a Pt-ovalbumin conjugate (Murdoch and Pepys, 1984). Although many chemicals are thought to cause respiratory allergy in humans it has so far proved difficult to reproduce these effects consistently in animal models. This failure may reflect the difficulties in identifying and preparing immunologically relevant materials (hapten-protein conjugates) for use in experimental studies.

Many industrial chemicals e.g. acrylates, epoxy resins, rubber chemicals, preservatives, dyes (Wahlberg and Boman, 1985) have been shown to be skin sensitisers in the guinea pig and this species represents a sensitive model for the prediction of skin sensitisation potential.

It has been postulated that mercury induced renal damage may be mediated by a type III (immune complex) allergic reaction (Bariety et al.,1971). The presence of immune complex deposits in the kidney can be demonstrated in the Brown-Norway rat but susceptibility to the deposition and the subsequent lesion appears to be particularly dependent upon the strain used (Bigazzi, 1985).

3.2. Autoimmunity

The aetiology of autoimmunity is not well defined. The presence of autoantibodies cannot necessarily be taken as being indicative of autoimmune disease; indeed it has been suggested that autoantibodies may have no direct involvement in the pathogenesis of autoimmune disorders (Russel, 1981). The development of medicine and chemically induced autoimmune disease is influenced by genetically based differences in pharmacological and immune reactions and by other factors such as age and nutritional state. Reliable animal models are therefore difficult to develop and animal data on autoimmune disease are scarce.

Mouse strains spontaneously developing SLE-like syndromes (non-organ specific autoimmune disease) show a variety of T- and B cell abnormalities (Steinberg et al., 1980) but the organ specific autoimmune diseases, for which few animal models are available, remain virtually unexplored. Since conditions such as thymic defects and modification of T- and B lymphocyte activities may lead to autoimmunity (Theofilopoulos and Dixon, 1982), it may be possible to detect autoimmune reactions in animals treated with agents known to affect the immune system. The popliteal lymph node assay (Gleichmann et al.,1984) has been proposed for investigating the autoimmunogenic properties of medicines and chemicals, but it is not yet validated.

3.2.1. Medicines. Some medicines thought to cause autoimmunity in man also induce autoimmune responses in specific strains of experimental animals. Medicine-related SLE-like phenomena have been reported in mice administered procainamide, hydralazine or isoniazide, in guinea pigs given hydralazine and in rats dosed with penicillamine (Davies, 1981). Other studies with the same medicines have failed to induce SLE-like phenomena in animals; these findings therefore remain controversial (Russel, 1981).

3.2.2. Industrial chemicals. In addition to the type III hypersensitivity reaction previously described (see 3.1.2.), antiglomerular basement membrane antibodies and complement deposition have been reported in mercury treated Brown Norway rats (Druet et al., 1978). The presence of

auto-antibodies in a number of species following exposure to mercury salts have been reviewed by Bigazzi (1985) who concluded that chronic administration of mercury salts gives rise to renal damage via autoimmune mechanisms.

## F. THE ASSESSMENT OF IMMUNOTOXICITY IN EXPERIMENTAL MODELS

This chapter reviews the use and value of data generated during conventional experimental toxicological studies and by the use of immune function tests, in assessing the effects of industrial chemicals and medicines on the immune system.

### 1. Conventional Tests

Immunotoxicity has been described in chapter C as the adverse effects of foreign substances on the immune system. The immune system is complex and diverse and there are many possible points where such materials could attack to modulate normal immune mechanisms. The overall effect may be suppression or potentiation of the normal immune response.

"Normality" is difficult to define since the immune system, by its nature and purpose, is a defence system against largely unpredictable and extracorporeal influences. Various physiological and environmental factors may influence the immune system e.g. stress, age, nutritional deficiency, infections (Hudson et al., 1974) and it is important to appreciate this since findings in animal studies may reflect either a direct immunotoxic effect of a chemical or an indirect effect. For example lymphoid tissue atrophy may be due to a direct immunotoxic effect or may be caused indirectly by increased stress induced adrenal corticosteroid secretion. Experimental toxicology is concerned with determining the overall effects of a chemical as judged by its ability to cause structural and functional damage, and reliance is placed upon such information generated during standard toxicity studies to provide an indication of possible immunotoxicity. During acute, subacute and chronic toxicity studies information is gathered which can provide an insight into the integrity and activity of the immune system of the exposed animal. The types of data that could be gathered to assess the possible immunotoxicity of chemicals are shown in Table 1. For example, atrophy of lymphoid organs (as judged by weight, size and histopathology), lymphopenia or an increase or decrease in serum immunoglobulins all suggest that the chemical may be acting upon the immune

system and that there may be an alteration in the ability of the body to effect a normal immune response.

TABLE 1

Toxicological Parameters Indicative of Possible Immunotoxic Effects.

1. Body Weight, mortality, incidence of infections.
2. Haematology profile (determined by red/white cell counts, differential cell counts).
3. Serum proteins (Total Protein, Albumin, Globulins, Immunoglobulins).
4. Organ weights (spleen, thymus, lymph nodes, adrenals).
5. Histopathology (spleen, thymus, lymph nodes, endocrine organs).
6. Cell types and numbers in the bone marrow.

It must, however, be recognised that the current OECD guidelines for "sub-acute" and "repeated dose" toxicity studies do not require routine examination of all of these parameters; for example the only lymphoid tissue examined microscopically in the sub-acute (28 d) dose toxicity study is the spleen (ECETOC, 1985). The reliability of such enhanced testing in identifying immunotoxic effects of industrial chemicals and medicines needs to be further assessed and the possibility of an interlaboratory trial considered.

If observations during initial toxicological studies indicate that the immune system may have been directly or indirectly affected, a detailed assessment of the significance of the experimental findings to human health will be needed. This assessment will include consideration of dose response relationships, the route of exposure used in the tests, species differences in response to the substance, metabolism, distribution and excretion, as well as the potential for human exposure (the likely duration, levels and routes of exposure). This assessment may indicate a need for further and detailed investigation of the mechanisms.

This approach assumes that perturbations of the immune system beyond its normal capacity will eventually manifest themselves as some form of tissue injury or abnormality. This assumption is supported by evidence that those chemicals

shown by function tests to affect immune function in animals also cause systemic damage. Sometimes damage is seen only at higher doses or longer treatment periods than required to alter function test results. For example, TCDD suppresses cell mediated immune function and causes severe atrophy of the thymus in rat, mouse and guinea-pig (Koller, 1979). Di-n-octyltin chloride suppresses cell mediated immunity in the rat and causes lymphoid tissue atrophy (Faith et al.,1978). The fact that an unexpected, increased incidence of immunologically based disease is not apparent in human populations provides some reassurance that the current toxicological screening procedures are adequate. However, it is recognised that few epidemiological studies of immunologically based diseases have been carried out and that it may take several years before a significant change in disease rates in exposed individuals is revealed.

During standard toxicological studies the clinical and pathological consequences of specific hypersensitivity reactions may readily be identified but the fact that they arise as a result of a hypersensitivity reaction will not be so easily recognised. At the present time reliable predictive experimental models to identify hypersensitivity reactions as the cause of human disease are available only for type IV skin reactions. Tests for this type of hypersensitivity are already required by many legislative bodies and have been reviewed previously (ECETOC, 1980; Andersen and Maibach, 1985). The identification of other types of hypersensitivity reactions, particularly respiratory allergy (type I), requires further research. In addition, it is not yet possible to identify those chemicals or drugs which have a potential to cause autoimmune reactions, unless autoimmunity is expressed as overt clinical symptoms or pathological changes in animals.

2. Immune Function Tests

Many experimental models have been developed by immunologists to investigate specific aspects of immune function and, by using them, it is now possible to examine closely the three basic phases of the immune response :

a) recognition of antigen;
b) activation (lymphocyte proliferation and differentiation);
c) expression of immunity.

A variety of these in vivo and in vitro immunological models are now being proposed as tests to investigate routinely the effects of chemicals and drugs

on immune function. Their application to toxicology has been reviewed extensively elsewhere (Dean et al., 1979a-b-c; Silkworth and Loose, 1981; Luster et al., 1982; Norbury, 1985) and examples of the tests that have been used are listed in Appendix 3. Many have been used to examine the effects of medicines and industrial chemicals on specific aspects of immune function in animals. For example, lead has been shown to inhibit antibody synthesis (Koller and Kovacic, 1974) and to affect immunological memory (Koller, 1980) and TCDD and PBB have been demonstrated to inhibit lymphocyte proliferation (Luster et al., 1982). The results of these tests show that they are sensitive and able to detect subtle changes in the functional capacity of isolated components of cell mediated, humoral and non-specific immune mechanisms. However, most of the tests have been performed using specific strains of mice and little data have been generated on other species more commonly used in standard toxicity studies.

The significance of the changes is uncertain since there is insufficient knowledge of the relevance of changes in isolated immunological parameters to overall functional capacity. It is likely that, in common with other organs and systems, the immune system has a considerable reserve capacity and that small, transient fluctuations in activity are unlikely to be of clinical or pathological significance. The degree of functional change (as shown by the tests) which is of significance to health is not yet known. Data on the non-cytotoxic immunosuppressant medicine Ciclosporin A show that, despite clear evidence from function tests of a suppressed immune system, there was no evidence of an increased incidence of neoplasia or infectious disease during long term animal toxicity studies (cf. Chapter E).

Immune function tests may prove useful where there is a need to investigate in detail any finding in conventional toxicity studies which points to immunotoxicity. However the immune system is a fully integrated system, involving interactions between many organs and cell types and this suggests that single ex vivo and in vitro function tests on isolated aspects of the system will be of limited value in assessing overall immune competence. Equally a standard set of tests will be of no value since the choice of function test must depend upon the type of immunity and specific aspects of immune function to be investigated. The design of each experimental protocol is also extremely important since many factors can influence the results obtained, e.g. species differences (rodent lymphocytes are particularly sensitive to corticosteroids)

and metabolic ability (the degree of metabolic activation/ deactivation present in the test system). Any test carried out must take into account the likely route and degree of human exposure and tests should be performed at dose levels below those which cause frank toxicity in animal studies.

The results of immune function assays must be assessed critically and in the context of the overall immune response. The complex, diverse nature of the immune system, the variability of the immune status of individuals and groups and the likely reserve capacity make comparative quantitative measurements of immune function difficult. Hence demonstration of a quantitative change in the results of a function test is not necessarily a reliable indication that the immune system has been significantly compromised, and it is important to correlate results obtained in function tests with findings of pathological significance previously observed in standard toxicity studies.

## G. PREDICTION OF IMMUNOTOXIC EFFECTS IN MAN FROM EXPERIMENTAL ASSAYS

### 1. Introduction

Chemicals and medicines can and do interact with and modify the responses of the human immune system (e.g. use of immunosuppressive drugs and hypersensitivity reactions). As discussed in Chapter D there are few examples, apart from hypersensitivity reactions, of inadvertent immunotoxicity in man despite a wealth of data showing effects in experimental models (cf. Chapter E). This section examines the data on a selection of medicines and chemicals and discusses the correlation between effects observed in humans and in experimental models.

### 2. Immunosuppression

#### 2.1. Medicines

Many medicines,e.g. corticosteroids, cyclosphosphamide and azathioprine, are used clinically to suppress immune function. Prolonged usage at high doses leads to an increase in the frequency and severity of infection and with cytotoxic medicines there is evidence of an increased susceptibility to the development of neoplasia (cf. Chapter D). Such effects are consistent with a profound suppression of immune function produced in patients at therapeutic

dose levels. However, evidence that other medicines have inadvertently produced clinically significant immunosuppression in man has not been found.

Experimental models such as resistance to infections or tumour transplants can be used to examine the overall effects of immunosuppressive medicines. The effects on overall immune response and on specific aspects of immune function (e.g. mitogen responsiveness) have proved useful in the investigation of their mechanism of action (cf. Chapter F). While the effects of medicines such as corticosteroids, cyclophosphamide and Ciclosporin A in man can be clearly demonstrated in experimental models,it is notable that at pharmacologically active dose levels they invariably demonstrate profound effects on lymphoid tissues (e.g. atrophy) in standard toxicity studies.Thus such studies would be sufficient to indicate qualitatively a possible immunosuppressive effect.

2.2. Industrial Chemicals

Examples of industrial chemicals causing immunosuppression in man are rare and have in the main occurred following accidental overexposure (cf. Chapter D). While the immunosuppressive effects of industrial chemicals have been extensively studied in experimental models, no correlation can be made between effects observed in experimental models and those observed clinically, both because of the lack of human data and the fact that experimental studies have often been performed using inappropriate routes of exposure and at dose levels well in excess of the likely human exposure and generally at levels which cause frank toxicity.

The findings in a population exposed to PBBs (Michigan incident) are discussed in Chapter D. Similar findings (reduction of T and B cell proliferation and numbers of circulating lymphocytes) were demonstrated in experimental models (Fries, 1986) but generally the effects were observed only at dose levels which caused overt toxicity. There was also considerable species variation in response, rats being particularly sensitive (Luster et al., 1978).

Accidental exposure to PCBs has been associated with an increased incidence of infectious diseases (Shigematsu et al.,1978), suppression of cellular immunity and reduction of immunoglobulin receptors. The effects of PCBs on immune function using experimental models have been extensively reviewed

(Vos, 1977; Faith et al.,1978; Koller., 1979). The main effects were reductions in T-cell dependent antibody responses and in delayed hypersensitivity reactions. These studies were often performed at dose levels which also cause overt toxicity. There is some evidence that low exposure to PCBs does not result in effects on humoral or cell mediated immune responses in animals (Street and Sharma, 1975).

Other industrial chemicals (e.g. lead, some organotin compounds, TCDD) suppress immune function in specific tests. There is no evidence at present that these effects occur in man. Studies of workers exposed to lead (Kimber et al.,1986) and ethylene oxide (van Sittert et al.,1985) support the view that immunosuppressive effects do not occur with those chemicals which have been shown to be immunosuppressive in experimental models when exposure levels are below present TLV's.

## 3. Immunopotentiation

### 3.1. Allergy

Both medicines and industrial chemicals can induce allergy in man. Although allergy is a well defined clinical problem it has proved difficult to develop reliable, predictive experimental models. While guinea pig models for allergic contact dermatitis (Maurer, 1983; Anderson and Maibach, 1985) have been used successfully to identify those materials with a potential to cause delayed (type IV) skin reactions in man, it is not possible to identify with confidence those materials likely to cause type I, II or III allergic responses in exposed populations. Structure-activity relationships may be of some some help in prediction (Edwards, 1983; Goodwin and Roberts, 1986).

### 3.2. Autoimmunity

A number of medicines and industrial chemicals have been associated with the induction of autoantibodies and/or an incidence of autoimmune-like pathology (cf. Chapter D). Chemically induced autoimmunity is a complex issue since its development is greatly influenced by age and nutrition and especially genetic predisposition and this makes it difficult to establish a cause-effect relationship in humans. Correlation with experimental findings is of little value since, although autoimmune effects can be shown in specific experimental models, the above mentioned factors make the development of a reliable model difficult.

## H. STRATEGY TO ASSESS THE IMMUNOTOXIC EFFECTS OF CHEMICALS IN HUMANS

### 1. Introduction

A medicine or industrial chemical should be suspected of having the potential to cause immunotoxic effects in man if :

a. it has been shown to produce health effects in man which relate to immunotoxicity (e.g. increased susceptibility to infection, allergy);
b. data obtained from experimental models suggest an immunotoxic effect;
c. the medicine or industrial chemical has structural similarities to known immunotoxic chemicals.

Different strategies are needed to assess the health effects of immunosuppressive and immunopotentiating compounds. With regard to the latter discussion will be limited to hypersensitivity reactions.

### 2. Strategy to Assess Immunosuppressive Effects in Humans

Health effects may be observed in individuals and in groups of people. Whenever possible, evidence from individuals should be supported by studies of other individuals or groups who have been similarly exposed. Furthermore these effects should be verified in suitable experimental models.

When an immunotoxic effect is identified from experimental studies, the estimation of risk to exposed humans will be complicated, as for all other toxicological studies, by differences in i) the experimental dose and the actual level of human exposure, ii) the metabolism and toxico-kinetics of the chemical in the model and in man, iii) the responsiveness of different species, iv) the route of administration and v) the age of the animal.

The observation of immunosuppression in either humans or animals may require an investigation of the relationship between exposure and occurrence of immunotoxic effects by epidemiological or other surveillance studies. In such studies particular attention should be paid to confounding factors such as age, sex, stress, use of alcohol and therapeutic medicines. Exposure should be measured and objective tests (e.g. standard haematology, serum protein

determination) should be used for the detection of immunosuppression. Initial studies may show a need for further medical examinations which could include specialised immune function tests. However interpretation of immunological tests in terms of human health risk is complicated, since little quantitative data are available on the degree to which an immunological parameter needs to be modified before an overt clinical event is produced.

## 3. Strategy for Measurement of Hypersensitivity to Chemicals in Man

### 3.1. Introduction

Since an animal model is available only for type IV hypersensitivity responses, type I, II or III hypersensitivity reactions must be assessed by studies in man. Type II hypersensitivity has been confined mainly to medicines and manifests itself in haematological disturbances. Since these parameters are easily measured by standard methods, they will not be considered in this chapter.

### 3.2. Respiratory tract allergy

When a patient has a work-related respiratory complaint which may be due to allergen exposure diagnostic tests can be performed to substantiate the relationship between allergen exposure and symptoms. These tests may include spirometry and/or peak flow measurements (Sherwood Burge, 1982), leucocyte counts (Hendrick et al.,1980), eosinophil counts and the measurement of body temperature before, during and after work exposure. If an allergen is suspected of causing the respiratory complaint, specific IgE antibody may be determined in the serum by the RAST or IgG antibody may be determined by using the ELISA technique. In addition a skin prick test with the suspected allergen can be performed. An inhalation provocation test with the suspected allergen may be carried out under carefully controlled clinical conditions, in addition to more extensive lung function measurements (diffusion capacity, compliance) and broncho-alveolar lavage.

Where allergen exposure is likely to occur in the workplace it has proven beneficial to conduct pre-employment and follow-up medical examinations. Thereafter epidemiological investigations may be carried out to study the relation between exposure and the incidence of allergic reactions. Medical examinations would include work history, respiratory symptoms, spirometry,

skin prick testing, bronchial hyper-reactivity testing and estimation of specific serum antibodies. In these examinations it is important to pay attention to the influence of confounding factors such as smoking, use of therapeutic medicines, age, sex and diurnal rhythm.

3.3. Skin allergy

In cases of work-related eczema, patch testing with the suspected compounds should be performed to identify the allergen and to differentiate between allergic and irritant contact dermatitis. It should be noted that one disadvantage of the patch test is that it can sensitise hitherto unsensitised workers.

To establish whether urticaria is work-related, skin prick testing with the suspected allergens can be performed, together with specific IgE measurements by RAST. The atopic state of such patients may be assessed from the skin prick test reactivity to common environmental allergens.

Although not common, predictive testing for the assessment of the potential of chemicals to cause allergic contact dermatitis can be carried out in humans (Stotts, 1980; Marzulli and Maibach, 1986). Ethical and practical considerations limit the scope and hence the value of such studies in man.

It may sometimes be valuable to perform epidemiological studies in workers exposed to skin sensitisers.

## I. RECOMMENDATIONS

Several aspects of immunotoxicology require further investigation to improve our understanding of the ways in which chemicals interact with the immune system. The following aspects were considered by ECETOC to be of the highest priority :

1. Improvement of the Existing Sub-Acute Toxicity Test Guidelines to Include more Detailed Assessment of the Immune System.

In contrast to longer term studies, current OECD guidelines for the examination

of chemicals in 14 - or 28-day toxicity tests pay little attention to the lymphoid organs, the only requirement being for histopathological assessment of the spleen. Studies including more detailed assessment (weight and histopathology) of the spleen, thymus, draining and distant lymph nodes, together with histopathological assessment of the bone marrow are more likely to identify adverse effects on the immune system.

It is recommended that the ability to detect immunotoxic chemicals using this type of enhanced testing should be validated by means of an interlaboratory trial (IPCS, 1986).

2. Investigation of the Functional Reserve of the Immune System

Chapters D and E have illustrated that animal and human data have implicated chemicals as immunotoxicants on the basis of a change in one or more immunological parameters. Many of the tests which measure these parameters (cf. Appendix 3) are extremely sensitive but the relevance of the changes brought about by exposure to medicines and industrial chemicals to human health is at present unclear. A possible exception is the in vivo model which measures the host response to infection. Significant perturbations of the immune system, as seen for example in patients with advanced stages of cancer or AIDS, can be correlated with major changes in certain immunological test results (e.g. reductions in T-cell numbers and lowered responses to mitogens). The significance of less profound changes in immune function test results on the health of an animal or man is not known.

It is likely that the immune system has considerable reserve capacity. It is recommended that the extent of this functional reserve is assessed in a number of animal models. In addition an assessment should be made of normal inter- and intra-individual variations in humans. This may help to provide a more meaningful interpretation of the results of the various tests. Validated ex vivo tests are particularly important for use in epidemiological studies of exposed human populations (see 5 below).

3. The Development of Experimental Models to Predict the Potential of a Chemical to Induce Respiratory Allergy.

It is desirable that an experimental model for predicting Type I hypersensitivity responses to medicines or industrial chemicals should be developed and validated.

4. Research on Mechanisms of Chemically Induced Autoimmunity

Further research is needed to clarify the mechanisms of chemically induced autoimmunity. It is desirable to develop methodology to aid in the identification of those medicines and industrial chemicals likely to cause autoimmune disease.

5. The Assessment of Immunotoxicity in Exposed Human Populations

Clinical and epidemiological studies of human populations exposed to suspected immunotoxic chemicals to correlate any health effects observed with immunological assessments and exposure levels would be desirable.

## J. BIBLIOGRAPHY


- Alanko, K., Keskinen, H., Bjorksten, F. and Ojanen, S.(1978). Immediate-type hypersensitivity to reactive dyes. Clinical Allergy, 8, 25.
- Andersen, K.E. and Maibach, H.I.(1985). Contact allergy. Predictive tests in guinea pig. In: Current problems in dermatology. Eds.: K.E. Andersen, H.I. Maibach. Publ. Karger, Basel.
- Anon (1985). Smoking, occupational allergic lung disease. Lancet, April, 965.
- Assem, E.S.K.(1981). Drug allergy. In: D.M. Davies (ed.). Textbook of Adverse Drug Reactions, second edition. Oxford University Press, New York, Toronto, p.541.
- Bach, J.F.(1975). Corticosteroids. In: The mode of action of immunosuppressive agents. Eds.Neuberger, A and Tatum, E. L..North-Holland Publ. Co.. Amsterdam.
- Bariety, J., Druet, P., Laliberte, F. and Sapin, C.(1971). Glomerulonephritis with and lc globulin deposits induced in the rat with mercuric chloride. Am. J. Pathol., 65, 293.
- Baur, X.(1983). Immunologic cross-reactivity between different albumin-bound isocyanates. J. Allergy Clin. Immunol., 71, 197.
- Bekesi, J.G.(1984). Clinical immunological studies in individuals exposed to environmental chemicals. Proceedings of International Seminar on the Immunological System as a Target for Toxic Damage. Present Status. Open Problems and Future Perspectives. In : Immunotoxicology, Eds. Berlin, A. Dean, J. Draper, M.H., Smith, E.M.B., Spreafico, F., 1987. Martinus Nyhoff Publ.-Luxembourg, Nov. 1984.
- Bekesi, J.G., Holland, J.F., Anderson, H.A., Fishbein, A.S., Rom, W., Wolf, M.S., Selekoff, I.J.-(1978). Lymphocyte function of Michigan dairy farmers exposed to polybrominated biphenyls. Science, 199, 1207.
- Bennett, W.M. and Norman, D.J. (1986). Action and toxicity of Ciclosporin. Ann. Rev. Med., 37, 215.
- Bernis, P., Hamels, J., Quoibach, A., Mahieu, Ph., Bouvry P.(1985). Remission of Goodpasture's syndrome after withdrawal of an unusual toxic. Clin. Nephrol., 23(6),312.
- Bernstein, I. L.(1981). Occupational asthma. Clinics in Chest Med., 2(1), 255.
- Bernstein, D.I., Roach, D.E., McGrath, K.G., Larsen, R.S., Zeiss, C.R. and Patterson, R.(1983). Relationship of airborne trimellitic anhydride concentrations to trimellitic anhydride-induced symptoms and immune responses. J. Allergy Clin. Immunol., 72, 709.
- Biagini, R.E., Bernstein, I.L., Gallagher, J.S., Moormann, W.J., Brooks, S. and Gann, P.H.(1985). The diversity of reaginic immune responses to platinum and platinum metallic salts. J. Allergy Clin. Immunol., 76, 794.
- Bigazzi, P.E.(1985). Mechanisms of chemical-induced autoimmunity. In: Immunotoxicology and Immunopharmacology. Eds. J.H. Dean, M.I. Luster, A.E. Munson and H. Amos. Publ. Raven Press, New York.
- Bonnyns, M. and Bastomsky, C.H. (1976). Polychlorinated biphenyl induced modification of lymphocyte response to plant mitogen in rats. Experientia, 32, 522.
- Boyd, J.E., Robertson, M.D., Davis, J.H.G.(1982). Autoantibodies in coalminers : their relationship to the development of progressive massive fibrosis. Am. J. Ind. Med., 3, 201.
- Bradley, S.G. (1985). Immunologic mechanisms of host resistance to bacteria and parasites. In: Immunotoxicology and Immunopharmacology. Eds. J.H. Dean, M.I. Luster, A.E. Munson and H. Amos. Publ. Raven Press, New York.
- Bradley, S.G. and Morahan, P.S. (1982). Approaches to assessing host resistance. Environ. Health Persp., 43, 61.
- Brewis, R.A.L.(1981). Respiratory disorders. In: Textbook of Adverse Drug Reactions, second edition. Ed. Davies, D.M.. Oxford University Press, New York, Toronto, 155.
- BTS (1983). Proceedings of British Toxicology Society. Oxford Sept. 1983. Human Toxicology (1984), 3, 145.

- Burns, C.A., Zarkower, A. and Ferguson, F.G.(1980). Murine immunological and histological changes in response to chronic silica exposure. Environ. Res., 21, 298.
- Butcher, B.T., O'Neil, C.E., Reed, M.A. and Salvaggio, J.E.(1980). Radioallergosorbent testing of toluene diisocyanate-reactive individuals using p-tolyl isocyanate antigen. J. Allergy Clin. Immunol., 68, 213.
- Butcher, B.T., O'Neil, C.E., Reed, M.A., Salvaggio, J.E., Weill, H.D.(1982). Development and loss of toluene diisocyanate reactivity : immunologic, pharmacologic and provocative inhalation challenge studies. J. Allergy Clin. Immunol., 70, 231.
- Chan-Yeung, M. and Lam, S.(1986). Occupational asthma. Am. Rev. Resp. Dis., 133, 686.
- Chang, K.J., Hsiah, K.H., Tang, S.Y. and Tung T. (1982-a). Immunologic evaluation of patients with polychlorinated biphenyl poisoning. Evaluation of delayed type skin hypersensitive response and its relation to clinical studies. J. Toxicol. Environm. Health, 9, 217.
- Chang, K.J., Hsiah, K.H., Lee T.P., Tung, T.(1982-b). Immunologic evaluation of patients with polychlorinated biphenyl poisoning : determination of phagocyte Fc and complement receptors. Environm. Res., 28, 329.
- Cockcroft, D.W., Ruffin, R.E., Dolovich, J., Hargreave, F.E.(1977). Allergen-induced increase in non-allergic bronchial reactivity. Clinical Allergy, 7, 503.
- Colley, J.R.T., Holland, W.W. and Corkhill, R.T. (1974). Influence of passive smoking and parental phlegm on pneumonia and bronchitis in early childhood. Lancet, ii, 1031.
- Cupps , T.R. and Fauci, A.S. (1982). Corticosteroid-mediated immunoregulation in man. Immunol. Rev.,65, 133.
- Davies, D.M.(1981). Disorders of muscle, bone and connective tissue. In: Textbook of Adverse Drug Reactions, second edition. Oxford University Press, New York, Toronto, 412.
- Dean, J.H., Padarathsingh, M.L., Jerrells, T.R.(1979-a). Application of immunocompetence assays for defining immunosuppression. Annals New York Academy of Sciences, 320, 579.
- Dean, J.H., Padarathsingh, M.L., Jerrels, T.R., Keys, L., Northing, J.W.(1979-b). Assessment of immunobiological effects induced by chemicals, drugs or Food additives (1). Tier Testing and Screening Approach, Drug and Chem. Tox., 2, 5.
- Dean, J.H., Padarathsing, M.L., Jerrells, T.R., Keys, L. and Northing, J.W.(1979-c). Assessment of immunobiological effects induced by chemicals, drugs or food additives (2). Studies with cyclophosphamide. Drug and Chem. Tox., 2, 133.
- Dean, J.H., Luster, M.I. and Boorman, G.A.(1982). Immunotoxicology. In: Immunopharmacology. Eds..Sirois, P. and Rola-Pleszczinski, M.. Elsevier Biomedical Press, New-York, p.349.
- Dempsey, W.L. and Morahan, P.S. (1985). Immune mechanisms of host resistance to viruses and assessment of immunotoxicologic effects. In: Immunotoxicology and Immunopharmacology. Eds.: J.H. Dean, M.I. Luster, A.E. Munson and H. Amos. Publ. Raven Press, New York.
- Denkhaus, W., v. Steldern, D., Botzenhardt, U., Konietzko, H. (1986). Lymphocytic subpopulations in solvent exposed workers. Int. Arch. Occup. Environ. Health, 57, 109.
- Descotes, J. (1986). Immunotoxicology of drugs and chemicals. Elsevier, Amsterdam, New York, Oxford.
- de Shazo, R.D. (1982). Current concepts about the pathogenesis of silicosis and asbestosis. J. Allergy Clin. Immunol., 70, 41.
- Dewair, M. Baur, X and Fruhmann, J. (1983). Inhibition of acetylcholinesterase from human erythrocytes by isocyanates. J. Occup. Med., 25, 279.
- Docker, A., Wattie, J.M., Topping, M.D., Luczynska,C.M., Newman Taylor, A.J., Pickering C.A.C., Thomas, P. and Gompertz, D.(1986). Clinical and immunological investigations of respiratory disease in workers using reactive dyes. Health and Safety Executive, U.K., Occasional Report.
- Doe, J.E.(1983). Animal models of sensitisation via the respiratory tract. In: Immunotoxicology, Eds. Gibson C.G., Hubbard R. and Parke D.V., Publ., Acad. Press, New York, London.
- Doll, N.J., Stankers, R.P., Barkman, H.W.(1983). Immunopathogenesis of asbestosis, silicosis and coal workers pneumoconiosis. Clinics in Chest Med., 4(1), 3.

- Dooms-Goossens, A., Gevers, D., Mertens, A., van der Heyden, D.(1983). Allergic contact urticaria due to chloramine-T. Contact dermatitis, 9, 319.
- Druet, P., Druet, E., Potdevin, F. and Sapin, C. (1978). Immune type glomerulonephritis induced by $HgCl_2$ in the Brown-Norway rat. Ann. Immunol. (Inst. Pasteur), 129 C, 77).
- Druet, P., Bernard, A., Hirsch, F., Weening, J.J., Gengoux, P., Mahieu, P. and Birkeland, S. (1982). Immunologically mediated glomerulonephritis by heavy metals, Arch. Toxicol., 50, 187.
- ECETOC (1980). Skin sensitisation. ECETOC Doc-10, October 1980.
- ECETOC (1985). Recommendations for the harmonisation of international guidelines for toxicity studies. Monograph No.7.
- Edwards, R.G.(1983). Structural determinants of drug allergenicity : implications and drug design. In: : Immunotoxicology. Eds. : G.G. Gibson, R. Hubbard, D.V. Parke. Academic Press, London.
- Elves, M.W.(1983). Drugs to modify the immune response. In : "Immunotoxicology". Eds. C.G. Gibson, R. Hubbard and D.V. Parke, Academic Press, London.
- Faith, R.E., Luster, M.I. and Vos, J.G.(1978). Effects on immunocompetence by chemicals of environmental concern. In: Reviews in Biochemical Toxicology, Vol.2. Eds. Hodgson E. and Bend J.R.
- Felix, R.H.(1979). Unwanted dermatological responses during drug therapy. In: Drug Toxicity, ed. Gorred, J.W.. Taylor & Francis, London, 189.
- Felix, R.H. and Stevenson, C.J.(1981). Skin disorders. In: Textbook of Adverse Drug Reactions, 2nd edition. Ed. D.M. Davies, Oxford University Press, New York, Toronto, p.429.
- Ferson, S., Edwards, A., Lind, A., Milton, G.W. and Hersey, P.(1979). Low natural killer-cell activity and immunoglobulin levels associated with smoking in human subjects. Int. J. Cancer, 23, 603.
- Fink, J.B.(1984-a). Occupational asthma and inorganic metals. In : Clinics in immunology and allergy. Vol. 4 no.1. Occupational Resp. Allergy, W.G. Saunders Company, London.
- Fink, J.B.(1984-b). Hypersensitivity pneumonitis. J. Allergy Clin. Immunol., 74(1), 1.
- Fisher, A.B.(1986). Contact dermatitis. 3rd edition. Lea and Febiger, Philadelphia.
- Foster, J.R., Cottrell, R.C., Herod, I.A., Atkinson, H.A.C and Miller, K.(1985). A comparative study of the pulmonary effects of $NO_2$ in the rat and the hamster. Br. J. Exp. Pathol., 66, 193.
- Foussereau, J., Benezra, C. and Maibach, H.(1982). Occupational contact dermatitis. Munksgaard.
- French J.G., Lowrimore, G., Nelson, W.C. , Finklea, J.F., English, Th. and Hertz, M. (1973). The effect of sulfur dioxide and suspended sulfates on acute respiratory disease. Arch. Environm. Health, 27, 129.
- Frenkel, J.K. and Havenhill, M.A. (1963). The corticoid sensitivity of golden hamsters, rats and mice : effects of dose, time and route of administration. Lab. Invest., 12, 1204.
- Fries, G.F. (1986). The PBB-Episode in Michigan : an overall appraisal. CRC Critical Reviews in Toxicology, 16, 105.
- Gardner , D.C. and Graham, J.A.(1984). Altered immunefunctions and host resistance following exposure to common airborne pollutants. Proceedings of International Seminar on the Immunological System as a Target for Toxic Damage. Present Status. Open Problems and Future Perspectives. In : Immunotoxicology. Eds. : Berlin, A., Dean, J., Draper, M.H., Smith, E.M.B., Spreafico, F., 1987. Martinus Nyhoff Publ., Luxembourg, Nov. 1984, cf.IPCS (1984).
- Gheysens, B., Auwerz, J., van de Eeckhout, A. and Demedts, M.(1985). Cobalt in induced bronchial asthma in diamond polishers. Chest, 88, 740.
- Gleichmann, E., Pals, S. T., Rolink, A.G., Radaszkiecwicz and Gleichmann, H.(1984). Graft versus host reactions : clues to etiopathology of a spectrum of immunological diseases. Immunology Today, 5, 324.
- Girdwood, R.H.(1979). The effects of drugs and their metabolites on blood and blood-forming organs. In: Drug Toxicity. Ed. : J.W. Gorred, Taylor & Francis, London, p.219.
- Goodwin, B.F.J. and Roberts, D.W. (1986). Structure activity relationship in allergic contact dermatitis. Food Chem. Toxicol., 24, 795.
- Gozodilovic, V.V. and Mandrik, E.V.(1978). The use of some immunological reactions for studying the immune response in persons presenting a high oncologic risk. Siv. Med., 9, 50.

- Hahon, N., Booth, J.A., Green, F. and Lewis, T.R.(1985). Influenza virus infection in mice after exposure to coal dust and diesel engine emission. Environ. Res., 37, 44.
- Hahon, N and Booth, J.A. (1986). Co-inhibition of viral interferon induction by benzo(a)pyrene and chrysotile asbestos. Environ. Res., 40, 103.
- Heise, E.R. (1982). Diseases associated with immunosuppression. Environm. Health Perspect., 43,9.
- Hemphill, F., Kaeberle, L.M. and Buck, W.B.(1971). Lead suppression of mouse resistance to Salmonella thyphimurium. Science (Washington D.C.), 172, 1031.
- Hendrick, D.J., Marchall, R., Faux, J.A. and Krall, J.M.(1980). Positive alveolar responses to antigen inhalation provocation tests : their validity and recognition. Thorax, 36, 415.
- Henry, J.C., Tschen, E.H. and Beaker, L.E.(1979). Contact urticaria to parabens. Arch. Dermatol., 115, 1231.
- Hersh, E.M.(1974). Immunosuppressive agents. In : Handbook of experimental pharmacology. Publ. Springer Verlag, Berlin.
- Hillam, R.P. and Ozkan, A.N.(1986). Comparison of local and systemic immunity after intratracheal, intraperitoneal and intravenous immunisation of mice exposed to either aerosolized or ingested lead. Environ. Res., 39, 265.
- Hoffmann, R.E., Stehr-Green, P.A., Webb, K.B., Evans, R.G., Knutsen, A.P., Schramm, W.F., Staak, J.L., Gibson, B.B. and Steinberg, K.K.(1986). Health effects of long term exposure to 2,3,7,8--tetrachlorodibenzo-p-dioxin. JAMA, 255(15), 2031.
- Howe, W., Venables, K.M., Topping, M.D., Dally, M.B., Hawkins, R., Low, J.S. and Newman-Taylor, A.J.(1983). Tetrachlorophthalic anhydride asthma : evidence for specific IgE antibody. J. Allergy Clin. Immunol., 71, 5.
- Hudson, R.J., Saben, H.S., Emslie, D.(1974). Physiological and environmental influences in immunity. The Veterinary Bulletin, 44, 199.
- Hughes, G.R.V., Rynes, R.I., Gharavi, A., Ryan, P.F.J., Sewell, J. and Mansilla, R.(1981). The heterogeneity of serologic findings and predisposing host factors in drug-induced lupus erythematosus. Arthritis Rheum., 24, 1070.
- IPCS (1984). Proceedings of International Seminar on the immunological system as a target for toxic damage. Present status. Open problems and future perspectives. published in : "Immunotoxicology, Eds. Berlin, A. Dean, J. Draper, M.H., Smith, E.M.B., Spreafico, F., 1987. Martinus Nyloff Publ.- Luxembourg, Nov. 1984.
- IPCS (1986). Immunotoxicology. Report of a technical review and Working group jointly supported by the International Programme on chemical Safety (UNEP; ILO ; WHO; the Commission of the European Communities; and the UK Department of Health and Social Security. London, 3-5 November 1986.
- Jaffe, I.A.(1981). Induction of auto-immune syndromes by penicillamine therapy in rheumatoid arthritis and other diseases. Springer Semin. Immunopathol., 4, 193.
- Jakab, G.J. (1987). Modulation of pulmonary defence mechanisms by acute exposure to nitrogen dioxide. Environ. Res., 42, 215.
- Juniper, C.P., How, M.J., Goodwin, B.F.J. and Kinshott, A.K.(1977). Bacillus subtilis enzymes : a 7 year clinical, epidemiological and immunological study of an industrial allergen. J. Soc. Occup. Med., 27, 3.
- Karol, M.H.(1983). Concentration dependent immunologic response to toluene diisocyanate (TDI) following inhalation exposure. Toxicol. Appl. Pharmacol., 68, 229.
- Karol, M.H.(1986). Respiratory effects of inhaled isocyanate. CRC. Crit. Rev. Toxicol., 16, 349.
- Kerkvliet, N.I. and Baccher-Steppan, L. (1982). Immunotoxicology studies on lead : effects of exposure on tumour growth and cell mediated tumour immunity after syngeneic or allogeneic stimulation. Immunopharmacol., 4, 213.

- Karol, M.H., Stadler, J. and Magreni, C.(1985). Immunotoxicologic evaluation of the respiratory system: animal models for immediate and delayed onset pulmonary hypersensitivity. Fundam. Appl. Toxicol., 5, 459.
- Kilbourne, E.M., Rigan-Perez, J.G., Health, C.W., Zack, M.M., Falk, H., Martin-Marcos, M. and de Carlos, A.(1983). Clinical epidemiology in toxic oil syndrome. New Engl. J. Med., 309, 1408.
- Kimber, I., Stonard, M.D., Gidlow, D.A. and Niewola, Z.(1986). Influence of chronic low-level exposure to lead on plasma immunoglobulin concentration and cellular immune function in man. Int. Arch. Occup. Environ. Health, 57, 117.
- Kleinhaus, D.(1985). Kontakturticaria. Dermatosen, 33(6), 198.
- Koller, L.D. and Kovacic, S.(1974). Decreased antibody formation in mice exposed to lead. Nature, 250, 148.
- Koller, L.D., Exon, J.H. and Koan, J.G.(1976). Humoral antibody response in mice after single dose exposure to lead or cadmium. Proc. Soc. Exp. Biol. Med., 151, 339.
- Koller, L.D.(1979). Effects of environmental contaminants on the immune system. Adv. Vet. Sci. Comp. Med., 23, 267.
- Koller, L.D.(1980). Immunotoxicology of heavy metals. Int. J. Immunopharmacol., 2, 269.
- Lange, A., Smolik, R., Zatonski, W. and Szymanbska, J.(1973). Serum immunoglobuline levels in workers exposed to benzene and xylene. Int. Arch. Arbeitsmad., 31, 37.
- Lange, A., Garncarck, D., Tomeczko, J., Ciechanowski, G. and Bisikiervieg, R. (1986). Outcome of asbestos exposure (lung fibrosis and antinuclear antibodies) with respect to skin reactivity. An 8 years longitudinal study. Environ. Res., 41, 1.
- Lauwerijs, R., Bernard, A., Viam, Cl. and Buchet, J.P.(1985). Kidney disorders and haematotoxicity from organic solvent exposure. Scan. J. Work Environ. Health, 11 suppl.1, 83.
- Lawrence, D.A.(1981). Heavy metal modulative of lymphocyte activities. In vitro effects of heavy metals on primary humoral immune response. Tox. and Appl. Pharm., 57, 439.
- Lawrence, D., Mudzinski, S., Rudulfski, U. and Warner, G.(1984). Mechanisms of metal induced immunotoxiciy. Proceedings of International Seminar on the Immunological System as a Target for Toxic Damage. Present Status. Open Problems and Future Perspectives. In : Immunotoxicology. Eds. : Berlin, A. Dean, J. Draper, M.H., Smith, E.M.B., Spreafico, F., 1987. Martinus Nyhoff Publ.,Luxembourg, Nov. 1984.
- Litwin, A., Adams, L.E., Zimmer, H. and Hess, E.V.(1981). Immunologic effects of hydraziline in hypertensive patients. Arthritis Rheum., 24, 1074.
- Lunn, J.E., Knowelden, J. and Handyside, A.J. (1967) Patterns of respiratory illness in Sheffield infant school-children. Brit. J. Prev. Sec. Med., 21, 7.
- Luster, M.I., Faith, R.E., Moore, J.A. (1978). Effects of polybrominated biphenyls (PBB) on immune response in rodents. Environ. Health Perspect., 23, 227.
- Luster, M.I., Dean, J.H. and Moore, J.A.(1982). Evaluation of immune functions in toxicology. In: Principles and Methods of Toxicology. Ed. W. Hayes, Raven Press, 1982.
- Malo, J.L., Ouimet, G., Cartier, A., Levitz, D., Zeiss, C.R.(1983). Combined alveolitis and asthma due to hexamethylene diisocyanate (HDI) with demonstration of crossed respiratory and immunologic reactivities to diphenylmethane diisocyanate (MDI). J. Allergy Clin. Immunology., 72, 413.
- Marzulli, F.N. and Maibach, H.I.(1986). Predictive testing in humans for contact allergy. In: Contact Dermatitis Ed. Fisher, Lea and Febiger.
- Maurer, T.H. (1983). Predictive contact allergenicity testing of drugs. Trends Pharm. Sci., 4, 104.
- Miller, K.(1983). Various mechanisms in chemically-induced thymic injury. In: "Immunotoxicology". Eds. Gibson, C.G., Hubbard, R. and Parke, D.V.. Academic Press, New York, London, 193.
- Miller, K., Maisey, J. and Nicklin S.(1986). Effect of orally administered dioctyltin dichloride on murine immunocompetence. Environ. Res. 39, 434.

- Morahan, P.S., Bradley, S.G., Munson, A.E., Duke, S., Fromtling, R.A., Marciano-Cabral, F.(1984). Immunotoxic effects of diethylstilbestrol on host resistance : comparison with cyclophosphamide. J. Leukocyte Biol., 35, 329.
- Morley, J. Wolstencroft, R.A. and Dumonde, D.C. (1978). In : Handbook of Experimental Immunology. 3rd Ed. : Weir, D.M.. Blackwell Scientific publ.
- Mostardi, R.A., Woebkenberg, N.R., Ely, D.L., Conlon, M. and Atwood, G.L. (1981). The university of Akron study on air pollution and human health effects II. Effects on acute respiratory illness. Arch. Environm. Health, 36, 250.
- Moszcsynsky, P.(1981). Organic solvents and T-lymphocytes. Lancet, i, 438.
- Murdoch, R.D. and Pepys, J.(1984). Immunological responses to complex salts of platinum I specific IgE antibody production in the rat. Clin. Exp. Immunol., 57, 107.
- Murdoch, R.D., Pepys, J. and Hughes, E.G.(1986). IgE antibody response to platinum group metals : a large scale refinery survey. Brit. Med. J., 43, 37.
- Murray, M.L., Kerkvliet, N.I., Ward, E.C. and Dean, J.H.(1985). Models for the evaluation of tumour resistance following chemical or drug exposure. In : Immunotoxicology and Immunopharmacology. Eds. : Dean, J.H., Luster, M.I., Munson A.E. and Amos, H.. Raven Press, New York, 113.
- Neilan, B.A., O'Neil, K. and Handwerger, BS.(1983). Effect of low level lead exposure on antibody - dependent and natural-killer cell mediated cytotoxicity. Appl. Pharmacol., 69, 272.
- NIEHS (1980). Target organ toxicity : immune system. Environ. Health Persp.(1982), 43. National Institute of Environmental Health Sciences.
- Nielsen, J., Sangö, C., Winroth, G., Hallberg, T., Skerfving, S.(1985). Systemic reactions associated with polyisocyanate exposure. Scand. Journ. Work Environm. Health, 11, 51.
- Norbury, K.C.(1985). Immunotoxicological evaluation : an overview. J. Am. Coll. Tox., 4, 279.
- Parkes, W.R.(1982). Occupational lung disorders. Butterworths, London.
- Paul, W.E.(1984). Fundamental immunology. Raven Press, New York.
- Penn, I.(1985). Neoplastic consequences of immunosuppression. In: J. Dean : Immunotoxicology and Immunopharmacology. Raven Press, New York, 381.
- Penninks, A., Kupper, F., Spit, B.J. and Seinen, W.(1985). On the mechanism of dialkyltin-induced thymus involution. Immunopharmacol., 10, 1.
- Polla, B., Pirson, Y., Cosijns, J.P., van Ypersele de Strihou (1983). Toxic anti-GBM glomerulonephritis. Clin. Nephrol., 19(1), 45.
- Ratner, B., Jackson, H.C. and Gruhel, H.L.(1927). Respiratory anaphylaxis : sensitisation, shock, bronchial asthma and death induced in the guinea pig by the nasal inhalation of dry horse dander. Amer. J. Dis. Child, 34, 23.
- Ravnskov, U., Lundström, S. and Norden, A.(1983). Hydrocarbon exposure and glomerulonephritis : evidence from patients occupations. Lancet, ii, 1214.
- Rees, A.J., Peters, D.K., Compston, D.A.S, Batchelor, J.R.(1978). Strong association between HLA-$DRW_2$ and antibody-mediated Goodpasture's syndrome. Lancet, i, 966.
- Reggiani, G.(1978). Medical problems raised by TCDD contamination in Seveso. Ital. Arch. Toxicol., 4, 161.
- Reidenberg, M.M. (1981). The chemical induction of systemic lupus erythematosus and lupuslike illnesses. Arthritis Reum., 24, 1004.
- Reidenberg, M.M.(1983). Aromatic amines and the pathogenesis of lupus erythematosus. Am. Journ. Med., 75, 1037.
- Reitz, B.A. and Bieber, C.P.(1982). Cancer after use of cyclosporin A in animals. Cancer Surveys, 1, 613.
- Roboz, J., Suzuki, R.K., Bekesi, G., Holland, J.F., Rosenmann, K. and Selikoff, T.J.(1980). Mass spectrometric identification and quantification of polybrominated biphenyls in blood compartments of exposed Michigan chemical workers. J. of Environ. Path. Toxicol., 3, 363.
- Roitt, I.M.(1984). Essential immunology. 5th Edition, Blackwell Scientific Publications, Oxford.

- Roitt, I.M., Brostoff, J. and Male, D.(1985). Immunology. Gower Medical Publishing/Churchill Livingstone, London and New York.
- Rothfield, N.F., Bierer, W.F. and Garfield, J.W.(1978). Isoniazid induction of antinuclear antibodies. Ann. Int. Med., 88, 650.
- Russel, A.S.(1981). Drug-induced autoimmune disease. Clin. Immunol. Allergy, 1, 57.
- Ryffel, B., Donatsch, P., Madorin, M., Matter, B.E., Ruttiman, G., Schon, H., Stoll, R. and Wilson, J.(1983). Toxicological evaluation of Cyclosporin A. Arch. Toxicol., 53, 107.
- Scheuchenzurber, W.J., Zarkower, A. and Eskew, M.L. (1982). Immunological alteration in the mouse following injection or inhalation of silica and olivin dusts. Environ. Res., 27, 398.
- Scheuchenzurber, W.J., Eskew, M.L. and Zarkower, A.(1985). Effects of prolonged inhalation of silica and olivine dusts on immune functions in the mouse. Environ. Res., 38, 389.
- Seinen, W. and Williams, M.I. (1976). Toxicity of organotin compounds. A. Atrophy of thymus and thymus dependent lymphoid tissue in rats fed n-octyltin dichloride. Toxicol. Appl. Pharmacol., 35, 63.
- Selye, H., Tuckweber, B. and Bertok, L.(1966). Effect of lead acetate on the susceptibility of rats to bacterial endotoxins. J. Bacteriol., 91, 884.
- Sharma, R.P., Kociba, R.J. and Gehring, P.J.(1984). Immunological effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals. Toxicol. Appl. Pharmacol., 45, 333.
- Sherwood-Burge, P.(1981). Occupational asthma and dermatitis : points of contact. Clin. Exp. Dermatology, 6, 235.
- Sherwood-Burge, P.(1982). Single and serial measurements of lung function in the diagnosis of occupational asthma. Eur. J. Resp. Dis., 63(123), 47.
- Shigematsu, N., Ishimaru, S., Sarto, R., Ikeda, T., Matsuba, K., Sugiyama, K. and Matsuda, Y.(1978). Respiratory involvement in PCB poisoning. Environ. Res., 16, 92.
- Silkworth, J.B. and Brabstein, E.M.(1982). Polychlorinated biphenyl immunotoxicity : dependence on isomer planarity and the Ah gene complex. Toxicol. Appl. Pharmacol., 65, 109.
- Silkworth, J.B. and Loose, L.D.(1981). Assessment of environmental contaminant-induced lymphocyte dysfunction. Environ. Health Persp., 39, 105.
- Silkworth, J.B. , Antrim, M. and Kaminsky, L.S.(1984). Correlation between polychlorinated biphenyl immunotoxicity, the aromatic hydrocarbon locus, and liver microsomal enzyme induction in C57BL/6 and BA/2 mice. Toxicol. Appl. Pharmacol., 75, 156.
- Sjögren, I., Hillendal, G., Andersson, A., Zetterström, O.(1980). Hard metal lung disease : importance of cobalt in coolants. Thorax, 35, 653.
- Sluis-Kremer, J.K., Hessel, P.A., Nizdo, E.H., Churchill, H.R. and Zeiss, E.A.(1985). Silica, silicosis and progressive systemic sclerosis. Brit. J. Ind. Med., 42, 838.
- Staines, N., Brostoff, J. and James, K.(1985). Introducing immunology. Gower Medical Immunology, London and New York.
- Starzl, T.E., Porter, K.A., Iwatsuki, S., Rosenthal, J.T., Shaw, B.W. (1984). Reversibility of lymphomas and lymphoproliferative lesions developing under Ciclosporin steroid therapy. Lancet, i, 583.
- Steinberg, A.D., Tauroy, J.D., Cowdery, J.S.Jr., Huston, D.P., Smathers, P.A., Patton-Reeves, J., Hansen, C.T. and Rayeche, E.S.(1980). In: Immunoregulation and autoimmunity. Eds.: Krahauer, R.S. and Cathcart, M.K., Elsevier North Holand, Inc., New York, p.193.
- Sternberger, L.A. (1986). Immunocytochemistry, 3rd edn.. John Wiley & Sons.
- Stotts, J.(1980). Planning conduct and interpretation human predictive sensitisation patch tests. In: Current concepts in cutaneous toxicity. Eds. Drill and Lazar, Academic Press.
- Strass, J.K., Smokler, I.A., Isbister, J. and Wilcox, K.R.(1981). The human health effects of exposure to polybrominated biphenyls. Toxicol. and Appl. Pharmacol., 58, 145.

- Street, J.C. and Sharma, R.P. (1975). Alteration of induced cellular and humoral immune responses by pesticides and chemicals of environmental concern : Quantitative studies of immunosuppression by DDT, Aroclor 1254, Carbaryl, Carbofuran, and Methylparathion. Toxicol. Appl. Pharmacol., 32, 587.
- Stuart,A.E., Habeshaw, J.A. and Davidson, A.E. (1973). Phagocytes in vitro. In : Handbook of Experimental Immunology. 3rd. ed. Ed. : Weis, D.M.. Blackwell Scientific.
- Theofilopoulos, A.N. and Dixon, F.J.(1982). Autoimmune diseases : immunopathology and ethiopathogenesis. Am. J. Pathol., 108, 321.
- Thomas, W., Holt, P.G. and Keast, D.(1973). Effect of cigarette smoking on primary and secondary humoral responses in mice. Nature, 243, 240.
- Tomilets, V.A., Donstov, V.L. and Zakharoya, I.A.(1980). Immediate and delayed allergic reactions to group VIII metals in an experiment. Fiziol. Z., 25, 653.
- Van Sittert, N.J., de Jong, G., Claren, M.G., Davies, R., Dean, J., Wren, L.J., Wright, A.S.(1985). Cytogenetic immunological and haematological effects in workers in an ethylene oxide manufacturing plant. Brit. J. Ind. Med., 42, 838.
- Vos, J.G.(1977). Immune suppression as related to toxicology. CRC Crit. Rev. Toxicol.,5(1), 67.
- Wahlberg, J.E. and Boman, A.(1985). Guinea pig maximisation tests. In: Current problems in Dermatology. Eds. Andersen, K.E. and Maibach, H.I.. Publ. Karger, Basel.
- Wedeen, R.P.(1984). Occupational renal disease. Am. J. Kidney Dis. III-4, 241.
- Wernfors, M., Nielson, J., Schutz, A. and Skerfving, S.(1986). Phthalic anhydride-induced occupational asthma. Int. Archs Allergy Appl. Immunol., 79, 77.
- Wooley, P.H., Griffen, J., Panayi, G.S., Batchelor, J.R., Welsh, K.I. and Gibson, T.J.(1980). HLA-DR antigens and toxic reactions to sodium aurothiomalate and D-penicillamine in patients with rheumatoid arthritis. N. Engl. J. Med., 303, 300.
- Young, D.A., Voris, B.P. and Nicholson, M.L.(1981). Cellular and biochemical actions of adrenal glucocorticoid hormones on rat thymic lymphocytes. Environ. Health Persp., 38, 89.
- Zeiss, C.R., Wolkonsky, P., Chacon, R., Tuntland, Ph.A., Levitz, D., Pruzansky, J.J. and Patterson, R. (1983). Syndromes in workers exposed to trimellitic anhydride. Ann. Int. Med., 98, 8.

# H. APPENDICES

## APPENDIX 1: ABBREVIATIONS AND GLOSSARY OF TERMS

### A. Abbreviations

AIDS : Acquired Immunodeficiency Syndrome.

BTS : British Toxicology Society.

EAA : Extrinsic Allergic Alveolitis.

ECETOC : European Chemical Industry Ecology and Toxicology Centre.

EEC : European Economic Community.

ELISA : Enzyme Linked Immunosorbent Assay.

HLA : Human Leucocyte Antigen.

ILO : International Labour Organisation.

NIEHS : National Institute of Environmental Health Sciences.

RAST : Radio Allergosorbent Test.

UNEP : United Nations Environment Programme.

WHO/IPCS : World Health Organisation/International Programme on Chemical Safety.

### B. Glossary of Terms

Adaptive Immune Response : A response to foreign material characterised by specificity and memory which can be mediated by antibody and/or antigen-committed lymphocytes.

Allergy : A clinical manifestation of a state of hypersensitivity. Allergy is

classically defined as an antigen-specific, altered reactivity of the host to antigen.

Anergy : Absence of immune reaction to a specific antigen.

Antibody : A protein molecule belonging to a class of proteins called immunoglobulins which are found in serum and secretions. They are produced by B-lymphocytes (plasma cells), in response to an antigen and they have the capacity to combine specifically with the antigen.

Antigen : Foreign material which can induce an immune response mediated by antibodies or committed lymphocytes (see also Hapten).

Antigen-Presenting Cells : A variety of cell types which concentrate, process and present antigens to lymphocytes in order to induce an immune response.

Atopy : A genetic predisposition towards the development of immediate hypersensitivity.

Autoimmunity : An adaptive immune response against "self" which can result in the development of clinical disease.

Cell-mediated immunity : An immune response mediated by antigen-specific lymphocytes.

Complement System : A system of proteins occurring in normal plasma and in secretions, activated characteristically by antibody-antigen interactions or by microbial cell walls and which subsequently mediate a number of biologically significant consequences e.g. cell lysis, enhancement of phagocytosis and inflammation.

Hapten : A small molecule, which although foreign, cannot be recognised by the immune system unless combined with a suitable carrier.

Humoral Immunity: An immune response mediated by factors which are 'free' in the body fluids e.g. antibodies.

Hypersensitivity : An adaptative immune response against an antigen which occurs in an exaggerated or inappropriate form and which can lead to tissue damage (see allergy). Four types of hypersensitivity are recognised (cf. Appendix 2).

Immunoglobulin : cf. antibody.

Immunopotentiation : An increase in the functional capacity of the immune response.

Immunosuppression : A reduction in the functional capacity of the immune response as observed with certain drugs which are prepared with the express purpose of suppressing the immune system.

Immunosurveillance : The mechanisms by which the immune system is able to recognise and destroy malignant cells before the formation of an overt tumour.

B-Lymphocytes : cells of bone marrow origin which migrate into blood, lymph and lymphoid tissue. They express specific surface antibody which may bind antigen causing differentiation into plasma cells.

T-Lymphocytes : cells of the bone marrow origin which mature in the thymus and then migrate into blood, lymph and lymphoid tissue. They express non immunoglobulin antigen receptors and are derived functionally into helper-, suppressor - and cytotoxic subpopulations.

Lymphokine : Generic term for a number of molecules (excluding antibody) produced by lymphocytes and involved in mediating cellular interactions during an immune response.

Macrophage : Bone marrow derived mononuclear cell found in the blood (where it is known as the monocyte), lymph and in many organs. Two main functions are recognised, phagocytosis and antigen presentation (cf. antigen-presenting cell).

Natural Killer Cell (NK Cell) : A lymphocyte-like cell which has the capacity to destroy non-specifically certain virally-infected and tumour cells.

Phagocytosis : The ingestion of foreign material into a cell, for example a macrophage.

Plasma Cell : A terminally differentiated B-lymphocyte which has the capacity to synthesise and secrete antibody.

Polymorph : A phagocytic polymorphonuclear leucocyte.

Recall Antigen : Material recognised by "memory" cells which stimulates rapid (secondary) immune responses.

Self Antigen : Antigenic component of an individual's own tissues. Normally "self" surface markers are recognised by the immune system neonatally, and immunological tolerance develops.

## APPENDIX 2 : IMMUNE HYPERSENSITIVITY REACTIONS

A Type I (immediate hypersensitivity) reaction results from the interaction between antigen and antibody (normally IgE) bound through specific receptors to the surface of mast cells or basophils. The interaction between antigen and antibody on the cell surface results in an immediate release of pharmacological mediators (e.g. histamine) which may induce clinical symptoms (e.g. hay-fever, urticaria, etc.).

A Type II reaction results from the interaction of antibody (normally IgG) and antigens (e.g. self-antigen or drug) on the surface of cells (e.g. erythrocytes). Interaction between antigen and antibody may activate the complement system leading to lysis of the cell (e.g. haemolytic anaemia) or phagocytosis of the cell through opsonic or immune adherence.

A Type III (Arthus type) reaction results from the formation of antigen-antibody complexes (in slight to moderate antigen excess) which are deposited in blood vessel walls. Complement activation results in the accumulation of polymorphonuclear leucocytes at the site of complex deposition, which release tissue-degrading enzymes damaging the basement membrane leading to haemorrhage.

A Type IV (Delayed hypersensitivity) reaction results from interaction between antigen-committed T-lymphocytes and antigen. The release of lymphokines and accumulation of effector cells at the site of antigen contact leads to a reaction characteristically-delayed by 24-48 hours after the antigen contact.

## APPENDIX 3

## Examples of Tests Available for Use in the Investigation of Possible Alterations in Immune Function

| Test | Type | Animals/ Test Systems | Endpoint | Comments | Reference(s) |
|---|---|---|---|---|---|
| elayed ypersensitivity esponse CMI*, complete) | in-vivo | mouse, (guinea pig) | Suppression of immunological reaction after repeated exposure to antigen | Antigens: Oxazolone, Sheep red blood cells, keyhole limpet haemocyanin | Vos, 1977<br>Luster et al., 1982 |
| kin graft rejection CMI, complete) | in-vivo | mouse, (rat) | Suppression of graft rejection | Allogenic skin grafts | Vos, 1977 |
| raft vs. host reaction CMI, complete) | in-vivo | mouse, (rat) | Suppression of GVH-reaction | Lymph node assay | Norbury, 1985 |
| ost responses to nfection CMI, HMI**, complete) | in-vivo | mouse, (rat) | Inhibition of antibody body production leads to expression of infection | T-cell dependent: L. monocytogenes T-cell independent: S. pneumoniae | Bradley and Morahan, 1982 |
| itogen response CMI, HMI, partial) | ex-vivo in-vitro | isolated lymphocytes | Inhibition of proli-feration | T-cell mitogens: phytohaemaglutinin/ concanavalin. A B-cell mitogen : E. coli lipopolysaccharide | Luster et al.,1982<br>Norbury, 1985 |
| erum antibody titre CMI, HMI, complete) | in-vivo | mouse, (rat) | Inhibition of antibody production (absolute amount of antibody determined) | T-cell dependent: Sheep red blood cells, T-cell, in-dependent: E. coli lipopolysaccharide | Vos, 1977<br>Luster et al.,1982 |
| laque assay CMI, HMI, partial) | ex-vivo in-vitro | isolated lymphocytes | Reduction of absolute number of antibody producing cells | -- | Luster et al., 1982 |
| ediator production CMI, partial) | in-vitro ex-vivo | isolated lymphocytes | Release of mediators from isolated sensitised T-cells | | Morley et al., 1978 |
| or T cell counting CMI or HMI partial) | ex-vivo | isolated lymphocytes or histological tissue | Differential lymphocyte count | Obtained only with specialised histochemistry methods | Sternberger, 1986 |
| acrophage hagocytosis (N)*** | in-vivo | mouse, (rat) | Inhibition of phagocytosis by macrophages | Carbon particles L. monocytogenes | Stuart et al., 1973 |

MI - Cell mediated Immunity

** HMI - Humoral Immunity

*** N - Non Specific Immunity

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