1981

Chapter 5

IMMUNOTOXICOLOGY AND CHEMICAL CARCINOGENESIS

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ABSTRACT

The influence of environmental chemicals on mammalian systems has been given much attention over the last several years. Bioassays of a chronic duration as well as short-term genetic assays have been developed with an eye toward the early recognition of the occurrence of a lesion in some target organ. Physiological and biochemical changes are targeted for analysis and surveillance in an effort to develop a screen that will signal the possibility of the occurrence of chronic disease.

Although the liver and kidney have been most intensively investigated, other systems have been used in this effort to establish an early warning system. The majority of the studies carried out have not taken into account the possibility that the immune system may be the ideal vehicle for such bioassays. For whatever reasons, investigators have underestimated the importance of the immune system as

The author wishes to acknowledge helpful comments and suggestions contributed to the author by Dr. M. M. Yokoyama, M.D., Ph.D., Director of Clinical Immunology, and Dr. John Bederka Ph.D., Chief of Toxicology and Pharmacometrics, of the University of Illinois Hospital and Medical School Staff.

The author also wishes to acknowledge the very helpful clerical assistance provided by Mrs. Debbie Fript, who did the typing and editing of the manuscript.

The author also accepts the blame for any errors or inconsistencies which may be found, and requests any reader to please notify the author of such mistakes as may occur, without intent.

related to disease states. This author feels that a nucleus of information has been developed by immunological investigators to indicate that the immune system organs and tissue (thymus, spleen, bone marrow, lymphocytes, monocytes) may provide a fertile soil for such investigations that attempt to provide early detection of capacity for chronic disease by environmental chemicals.

Immune deficiency or modulation of the immune response has been known for many years. Irradiation, malnutrition, thymectomy and certain anticancer drugs currently in use are proven to cause a change in the immune system. Although the immune system is complex and not completely understood, sufficient information is available to indicate that various elements in the immune system appear quite sensitive to chemicals.

It is well established that active cells are the most sensitive to chemicals. A good deal of information has been developed on the use of anticancer agents and their capability to induce secondary and tertiary cancers in patients undergoing anticancer treatment. Some of the changes associated with such treatment at the cellular level are also seen *in vivo* and *in vitro* and of course raises the question of whether this activity can be at work during exposure to environmental chemicals? This author feels that there are some relationships that make the next decade in immunotoxicological research possibly the most exciting and potentially the most important.

Introduction

During the last five years, many of us have heard the statement that "a large number of cancers may have their etiology in the environment." This statement helped to propel an already energetic national desire to seek cancer causes and cures. Although there are many different opinions as to the actual numbers of cancer cases contributed by the various environmental factors, it has been generally agreed upon, among biomedically oriented research scientists, that environmental carcinogenesis is a real entity.[1] In an effort to seek a cure or to begin a prevention pro-

gram, the environmental chemicals that present a carcinogenic risk to humans, are an ideal target for investigation.[2]

In order to establish some measure of risk, predictive toxicology has had to use the various available tools; epidemiology, statistical extrapolations of pharmacological dose response studies, the multiple specie end target organ bioassays, microbial, biochemical studies, and chemical structure relationships.[4-9] These tools, used cautiously, have served us very well. However, the fact that human data is lacking in a majority of the cases has been a source of consternation.

Recent developments in the area of immunology have provided a nucleus of data and of concepts that may help resolve some of these problems associated with extrapolations to humans. Although immune mechanisms and the system has not been defined absolutely, research data has been developed that shows that immune system cells (small lymphocytes, T and B cells, macrophages) and products such as lymphokines play a very important role in the body's immune reaction to possible pathology from microbial infection, graft rejection, and tumour production.

The fact that these cells are readily accessible and are human tissue present in extensive amounts in peripheral blood and have potential for rapid proliferation makes them an ideal vehicle for the study of potential toxicity.

Predictive Toxicology in Retrospect

Epidemiology is the discipline that integrates the impact of the various determinants involved in the disease process. In assessing environmental hazards, it has been used as a tool that helps to provide direction toward actual laboratory investigations. The statistical correlations of the distribution of the determinants of disease have been able to relate the possible chemical toxicants capable of causing pathology to the occasion of disease states among humans who have been exposed. Thus, epidemiology has been an important tool in providing the first step in the process of predictive toxicology—helping to determine in many cases whether a suspect chemical warrants investigation.[3,11,12,13]

Microbial assays have supported predictive toxicology due to the successes of Ames and McCann in establishing aberrations

seen in mutagenesis assays as being related to the process of carcinogenesis.[14,15,16] The philosophical basis for this procedure lay in the fact that a chemical capable of causing DNA related lesions could be detected in microbial systems. If a chemical had the capacity to cause mutations in microbial cells, then genetic toxicity was a reality. Using the strain of *Salmonella typhimurium*, which has a histidine negative mutation, Ames and co-workers were able to show that exposure to chemicals causing genetic lesions did cause a reversion or back mutation. The normal Salmonella cell was modified by introducing membrane defects so a chemical could enter. The excision repair system had to be neutralized so that any lesion that could occur remained, and a bacterial plasmid was introduced to insure errors in DNA replication. Finally, rat liver extract was introduced to provide a mammalian metabolic system for activation of the chemical that may not be a direct carcinogen and requires transformation.

Since this system was introduced, others[17,18] have validated the assay system using hundreds of chemicals and have reported that approximately 90 percent of the chemicals tested, which were reported in animal systems to be carcinogenic, were in fact mutagenic in the Ames assay system. Although the correlation is fairly good, caution and prudence must be exercised. Doctor U. Saffiotti of the National Cancer Institute has expressed confidence in the Ames system as having the ability to predict carcinogenic potential, but also added that animal bioassays, using mammalian species are required as conclusive evidence of a chemicals' ability to cause carcinogenesis.[19]

When an appropriate experimental design is used, the mammalian bioassay generates the biological data that has been most sensitive in determining whether a given chemical may have carcinogenic potential. Due to the fact that animals used for carcinogenesis bioassays are highly inbred, some strains may express a predisposing genetic tendency toward the occurrence of neoplastic response from chemical exposures. It was therefore recommended by the National Academy of Sciences[21] that carcinogenic studies be carried out in multiple species of mammalian laboratory animals, so that any expression of a genetic lesion causing neoplastic response be validated or supported as much as possible. Animals,

as do humans, have varying susceptibilities, so that the larger data base presents a greater possibility of providing effective extrapolation when predicting possible toxicity.

Anderson and Schein[23] have used the dog and monkey to provide a suitable overlap in combined studies on anticancer chemotherapeutics and found that they were in fact very favorable predictors in preclinical studies. However, the size and cost of such large animals for carcinogenesis assay, given the large number of chemicals in the environment, would present an extreme cost burden in dollars and in human resources.

Carcinogenic or teratological studies have been reported using many species.[24,25] Clegg[26] has indicated that the mouse has provided the most success, but he and Jensen[27] caution that this specificity alone is unsuitable for establishing carcinogenic response. Organ tissue morphology is different; rate of onset of polyploidy, biochemical-enzymic differences, and rough to smooth endoplasmic reticulum change during exposure cause a great deal of difficulty in interpretation of the actual pathology. They do suggest however, such data should be used as a warning signal for further study.

In their report on principals for evaluating environmental chemicals, the National Academy of Sciences (NAS)[21] recommends the use of randomly bred hamster, rat, and mouse as the animal species ideal for carcinogen bioassay.[20] Once again, this is no guarantee of successfully being able to predict human toxicity, but it is all that we have developed up to the present time.

Similar arguments exist for any of the animal species that are used in carcinogenic bioassay. The everpresent reality is that thus far the multiple species bioassay data have provided the best evidence for extrapolating possible chronic pathology from exposure to environmental chemicals.

The recent megamouse study reported by Littlefield et al.[28] has confirmed that the dose-response studies performed on some 26,000 plus mice has validated that such information is absolutely necessary, from a standpoint of both dose and time. He has reported that given different lengths of time of exposure to 2-acetylaminofluorene, an animal could respond with two different end points. Mice that were dosed for eighteen months and sacrificed later showed urinary bladder neoplasms; they also required continual

presence of the carcinogen although the neoplastic response was induced early in the study. However, in animals that were exposed for thirty-three months, doing serial sacrifices, a liver neoplasm became evident only late in the study, although it was induced early during exposure and did not require the continual presence of the carcinogen.

This data provides substantial evidence of the necessity of the long-term bioassay. A product of that study was a confirmation of a principle applied to regulatory function in public health programs that "there is no level of exposure greater than zero for a toxic substance, which can be assumed to be without harmful effect."[28]

In contrast to that principal, risk benefit analyses have been introduced based on the public's right of acceptability of possible adverse effect to a given chemical exposure. The calculated risk allows some level of a toxin to coexist in the realm of human existence, correlated with the fact that humanity derives some benefit from that coexistence. In order to calculate that "level of acceptability," it is necessary to extrapolate from the dose of the experiment, associating some end point with a given level and length of time of the exposure. A source of consternation has been, which part of the curve to use when calculating allowable dose to exposed humans.[29–35] Based on the data of the megamouse study, both lesions are induced, in their respective tissues, early in the exposure, suggesting a linear no threshold dose-response. Thus the lower end of the dose curve would be the ideal place to begin extrapolations.[28] Although this philosophy is not espoused by all, it is a practical approach that can provide consistency, assuming the data from bioassays provide sufficient evidence that chronic pathology is a real possibility. Wilson,[36] with some reservations, supports this philosophy, suggesting it is useful for making comparisons with benefits easier to calculate.

The point I have tried to make is that regardless of the method(s) being utilized, problems exist that prevent the unanimous acceptance of any of the given assay systems. Even when taken in concert, the major stumbling block has been that, we do not have information as to how this material will react in humans.

Cellular Basis of Toxicology

The influence of environmental chemicals on the various biochemical, morphological, and histopathological parameters has been intensively investigated in various laboratory animals. Epidemiological and statistical analyses have produced more sophisticated and complex mathematical formulas dealing with the extrapolation of dose-response levels and the prediction of toxic associations. Since our predictive ability and the skill we use in evaluating exposure are dependent upon basic biological processes, it is important and prudent that we should direct our energies to answer the basic questions describing how toxicity occurs at the cellular level. Disturbances at the membrane, cytoplasmic, or nuclear levels and at micromolecular structure-activity levels need to be very carefully analyzed to determine the sites and modes of toxicity. Whether for acute or chronic toxicity, these are the fundamental questions that must be answered in order to determine the levels of chemicals humans may tolerate without adverse effect. This is not a new or reorganized philosophy, but a reconfirmation of the original principles laid down by our toxicological forefathers.

As the numbers of chemicals entering into the environment have increased, the long-term bioassays for all chemicals becomes less and less possible due to the length of time and large costs involved. Sequentially, it becomes more important to further develop cellular bioassays as genetic defect predictors. The quicker response time and reduced costs are obvious benefits, although extrapolation is a difficult constraint. Since the correlation of animal responses to human situations are problematical, I suggest we devote more of our attention and energies toward use of human cells. Since the protection and well being of human life is our ultimate end point, data that can be derived from culture and analysis of human cells can provide us with the best evidence for making predictions and risk assessments.

Immune Cell Analyses

One area that has not been well investigated but deserves attention is the human immune system. Vos[10] as well as Silkworth and Loose,[37] have found evidence that supports the concept that envi-

ronmental chemicals can produce modifications in the activities of the immune system cells. Antibody mediated resistance, cell mediated resistance, as well as increased levels of mortality due to lowered resistance to bacterial infections have been reported in their reviews. Silkworth proposes that cellular and humoral mediated responses may be the appropriate indicators of environmental toxicity. He indicates they are quite sensitive, they can be conveniently evaluated, and he has had limited success using this test system in the evaluation of polychlorinated biphenyls (PCBs), Arochlor 1016 (ACLR), and hexachlorobenzene (HCB). Taylor[38] has analyzed various immune functions in the guinea pig during dietary studies; however, he had conflicting results due to various other factors that could not be controlled. Luster et al.,[39] as well as Luster and Faith,[40] have evaluated the rationale for using immunocompetence as a form of laboratory investigations. They recommend using multiple parameters of study to properly evaluate environmental-chemical effects, since normal immune responses are dependent upon macrophages and at least three sets of lymphocytes. These cells interact among themselves and with various cell products (lymphokines and/or complement), so it can become quite complicated. However, the studies they performed did show that exposure to various chemicals produced immune-cell modulation. Dean et al.[41] have examined cell and humoral mediated immunocompetence and also recommend multiple parameter assays, with most of them dealing with systems designed to study tumour/cancer.[10,37–41]

It is obvious that since the immune system deals with a large population of cells of human origin that are available from the peripheral blood, it is an ideal group of cells in which to study toxicity.

Since these cells are present in the circulating peripheral blood, we can correlate their reactions to chemical exposure in a realistic manner, because it is similar to an environmental exposure. Chemicals that are taken in by humans must be absorbed and transported through the circulating blood, which provides the media for close association and residence time. Generally speaking, most chemicals are not biotransformed within the absorptive area of the intestinal tract. In their contact with the blood elements during

circulation, not only do you get contact, but also a realistic mode of how and/or what may occur given a chemical and a sensitive or susceptible cell. Cellular response may be at the membrane surface, within the cytoplasmic confines, at the genetic-nuclear level or at all levels. Since immune lymphocytes can be stimulated into differentiation, they can show sensitivity at all levels, making them an ideal vehicle to study cell toxicity modes. With that type of information, immuno– and predictive-toxicology would greatly benefit.

Since immunotoxicology is at its infancy, large amounts of input in developing additional methodologies for detecting cellular determinants and/or receptors and for characterizing pathways and/or molecular constituents are needed. It is a complex task, but these areas must be explored and the quantification of such reactions at the cell level must be identified if we are to gain any further skill in prediction and extrapolating doses to humans.

Immunotoxicology: The Link to Humans?

The immune defense is a dual system composed of natural nonspecific barriers such as skin keratin and pH, of the natural mucociliary barriers as well as the normal bacterial flora that act to prevent bacteria from penetrating. A third nonspecific mechanism begins operating when that first barrier has been breached. Macrophages and polymorphonuclear leucocytes, together with specific humoral soluble factors and lymphokines, act in concert to sequester, degrade, and neutralize the material recognized as foreign.

If these natural and nonspecific barriers have been overwhelmed, the second and more specific defense mechanism comes into play. It involves the activation of lymphocytes that are preprogrammed to combat infection. These lymphocytes are the main element of this system and compose the basis of the humoral and cell mediated immune defense mechanisms. They are indistinguishable morphologically, but, because of differences in the programming sequences, the lymphocytes have different capabilities.

Totipotent (or pluripotent) stem cells derived from the bone marrow are distributed to the peripheral circulation and home in on certain tissue for education and processing. Lymphocyte clones

are processed by the thymus gland and are programmed with certain specific surface antigens and cellular memory for use in response to fungi, parasites, intracellular viral infections, tissue grafts and cancer cells. These are the T-lymphocytes, and are responsible for cellular mediated response. That is the response whereby the T-cell is mobilized or stimulated to seek out the foreign material for specific reaction and neutralization. Another group of lymphocytes are processed by gut associated lymphoid tissue (GALT), the supposed equivalent of bursa tissue in the avian models and are then called B-cells. They respond to bacterial infection by transformation into a plasma cell that secretes antibodies. The antibodies are located in the peripheral blood, circulating about until they meet an antigen and then begin the process of neutralization and digestion. Each lymphocyte (T or B) has received specific information during processing and is a clone with a response specificity. The specificity is due to chemical markers imbedded in cell surface conformations that are sensitive only to specific antigens. There are some populations of lymphocytes that although thought to be end cells, have been shown to live for many years and can recirculate from lymph node to lymph and to blood.[a]

Another lymphocyte population that does not possess either immunoglubulin (B) or T-cell surface markers is present and they are called null cells. They are considered to be involved in target cell killing termed *antibody dependent cell mediated cytotoxicity* (AD CC).[a]

Genetic Regulation

Immune responses are controlled genetically by a series of linked genes known in mice as the major histocompatibility complex (MHC) and in humans as the HLA complex. A similar series of genes has been identified in each mammalian species examined. This phenomena was discovered when guinea pigs inoculated with an antigenic complex of Dinitrophenyl conjugated poly l-lysine (PLL) responded by demonstrating antibody production and delayed hypersensitivity. Strain #2 responded well; however, strain #13 responded poorly. This was taken as evidence that the

[a]The reader is urged to consult one of the currently available texts on immunology for more detailed information.

specific response is due to the carrier, poly l-lysine, and its specific relationship to the PLL gene, which has the capacity to respond.[45]

Further studies were carried out using variations of polymers of amino acids, and seperate genes have been delineated. Strain #2 responded to a copolymer of glutamic acid and alanine (random grouping of AA's) but no response was seen in strain #13. On the other hand, strain #13 responded well to glutamic acid and tyrosine, but strain #2 did not. F1 generation hybrids (13 × 2) however responded to both immunogens, indicating that two different genes were involved, with both having the gene indicating a capacity to respond.

Further observations demonstrated that the ability to respond was linked to the MHC locus. Since that development, genetic control of immune responses and of antigens evoking allograft recognition have been localized to the H-2 gene complex of mouse chromosome #17, and the human leukocyte antigen (HLA) complex in the human on chromosome #6. In the mouse, two regions have been identified: H-2k and H-2d, which determine tissue transplantation antigens and cellular immune cytotoxicity, and the "I" region, which controls induction of immune responses. The H-2k and H-2d regions have been shown to code for the cell *surface antigens* that serve as targets for cell mediated immune reactions as well as the antibody response. This mechanism, which identifies some surface factor of the cell, is used not only to recognize allograft differences, but also plays a role in the recognition of an individual's own cells, which may become changed or altered as in viral infection or neoplastic disease. The mixed lymphocyte reaction is controlled by these loci, and they will proliferate and take up radio thymidine if cells from two different individuals are mixed together. If the cells come from individuals with identical MHC loci, there will be no reaction, and no thymidine will be incorporated. Thus, such an activity as normal response to nonself invasion could be upset should a given gene be affected by a chemical exposure. Since the cell's surface markers and determinants are expressions of genetic activity, those surface membranes may have unusual conformations that could be identified.

Immuneosurveillance

Cell Surface Recognition System

Within the immune defense system there exists a mechanism that has the capacity to recognize the foreign nature of intrusions into a host. Bacterial infections occur, and the antibody response causes neutralization. Foreign tissue graft (allograft) causes the cell mediated response to reject that tissue. Particulate material is sequestered, and phagocytosis causes the degradation of such material till it becomes an innocuous residual body or is discharged from the host. In each case, the immune system recognizes that this material is not identified as self, and sets about to remove the invasion. The immune system has developed and matured in each host, whereby it has learned to recognize the tissue it was associated with during its process of maturation and development. It can distinguish between self and nonself.

The T-lymphocytes, responsible for cell mediated immunity, are part of the internal policing system that is supposed to recognize aberrant cells and remove them from the system. It is able to recognize these cells by surface determinants that reflect the aberrant nature of the cell and would not be found on a normal cell.[43] It is this change in surface antigen markers that separates the self from nonself recognition scheme. This concept was developed for experimental studies in which "nude" (athymic) mice lack the ability to reject tissue grafts because of the defect in the processing of lymphocytes into T-cells.[44] Thus, the animal cannot respond to the foreign nature of a transplanted tumour cell and provides an acceptable medium for growth of various tumour cells, whereas, if a tumour is transplanted into a normal mouse, there is a rejection of the tumour tissue. It is now generally accepted that most transplanted tumours have tumour specific antigens that are the basis for the induction of the immune rejection reaction. They are called tumour specific transplantation antigens (TSTA's).

This concept of tumour specific antigens has had a profound impact on tumour immunology. Mice that were inoculated with polyoma virus were later capable of rejecting a polyoma tumour that was grafted onto a synegenic mouse (genetically identical). This was interpreted as an example of the immune system's rec-

ognition that the tumour tissue was indeed foreign and that preimmunization was directed at these surface markers. It was also hypothesized that the immune system was capable of limiting tumour development. A logical extension of that concept held that if there is a condition that exists in the animal that modifies or alters the immune system's capabilities to respond to a nonself entity (immunemodulation), then the variant cell or foreign entity can take hold, develop and mature into perhaps a disease state.[42–45]

Immune Modulation

Immune modulation can take variable forms of stimulation or suppression, and it can be specific or general. Stimulation (specific) of the system *directly* occurs in immunization with microbial cells or products or *indirectly* as in passive transfer of serum, cells, or cell products among histocompatible donors and recipients. There are also nonspecific or general responses that can occur as with *Bacillus calmette guerin* (BCG), *Corynebacterium parvum*, levamisole, and pokeweed. These cells or products are referred to as mitogenic since they are nonspecific and have the ability to stimulate B and T cells, as well as macrophages or the complement system.

Immunosuppression, on the other hand, is the reduction of available immune elements directly or indirectly. Lymphoid drainage, cytoxic drugs, antilymphocyte serum are direct, as is thymectomy, which will reduce T-cell populations and response. Irradiation is general suppression of the immune response. Bursectomy in fowl will lead to loss of B-cell response. Indirectly, one can also modify the response by increasing or reducing the levels of corticosteroids or using various known antiinflammatory agents.

Based on this ability to modify the immune response, medical science began the use of chemotherapeutics. In the clinical sense, the goal is to suppress or eliminate the ability to produce an immune response to specific antigen while allowing other antigens to evoke a response, as in organ transplants.[45,46] Such suppression has been used in the clinical treatment of neoplastic disease. Some cancer cells, as do bone marrow cells and intestinal mucosa, show an extremely rapid growth rate and, additionally, may have an abnormal complement of nucleic acids. Alkylating

agents as well as blocking agents are used to interfere with metabolism of such cells. Because some neoplastic cells metabolize at accelerated rates, they are more likely to pick up higher doses of antimetabolic agents. However, the immune lymphoid system cells are also more active so they can also be affected to a greater degree. Induction of an immunocompromised individual through the use of chemicals has become well known in cases of clinical chemotherapy.[46]

Immune Deficiency and Malignancy

One of the most significant advances in cancer information is the finding that immunodeficient states are associated with an increased incidence of malignancy. Kersey[41] has shown that patients who have naturally occurring states of immunodeficiency such as Wiskott-Aldrich disease, Ataxia telangiectasia, or agammaglobulinemia have an unusually high incidence of malignant disease.

Numerous experimental bioassays are cited in his review, showing that immune suppression facilitates transplants of malignant cells, increases a normally low incidence of viral or chemically induced cancers and accelerates growth of metastases.

Immunosuppressive therapy has been used for some twenty odd years and has had a significant impact in producing secondary and tertiary cancers in patients who were under such treatment. Penn[46] has been maintaining a tumour registry of patients who were on immunosuppressive therapy and classified them into five groups with the following results.

Patients with Transplanted Cancers

Sixty-one patients who had received organ transplants from donors who were neoplastic or within several months subsequent to donation developed evidence of malignancy showed that twenty-one patients or 34 percent had evidence of transmitted cancers. Cessation of immunosuppressive therapy and removal of the graft resulted in the complete disappearance of the disseminated neoplasms.

Transplant Patients with De-novo Malignancies

In a long-term follow up of the University of Colorado series of renal homografts, 32 of 564 patients developed cancer, an inci-

dence of 5.7 percent. The Denver Transplant Tumor Registry has data on 401 *de novo* cancers that have occurred in 378 patients who have received kidneys. The average age of the patients were thirty-nine years old (range eight to seventy years) and the neoplasms occurred from one month to one hundred fifty four months (1 to 154) after the transplant (average 32 months). After the transplants, the following immunosuppressive procedures were used: Prednisone, Azothioprine, anti-lymphocyte globulin (ALG), Actinomycin, cyclophosphomide. Radiation, splenectomy, thymectomy and thoracic duct fistula procedures were also in use. In conjunction with ALG treatment, 6-mercaptopurine, methotrexate, and azaserine were also used.

The patients receiving irradiation, splenectomy, thymectomy, or thoracic duct drainage treatment accounted for 217 *de novo* cancers, while the patients on pharmacologic therapy accounted for the remaining 164 cancers. The development of malignancy could not be related to the use of any one agent, but appeared to be an effect of the general immunosuppression. A significant finding was that the incidence rate of solid lymphomas among the organ transplant patients was disproportionally higher than the general population. One variety, Reticulum Cell Sarcoma, was calculated to be 350 times more common. The lymphoma patients were slightly younger than the other cancer patients (36.5 versus 40 years old), and the tumours appeared earlier than the other cancer patients (twenty-three versus thirty-five months). The solid lymphomas occurred in ninety-five patients with the following breakdown:

Reticulum cell sarcomas	68
(One patient also had a Kaposi's sarcoma.)	
Kaposi's sarcoma	11
Lymphoma	8
(Including 1 plasma cell lymphoma).	
Lymphosarcoma	5
Lymphoreticular malignancy	2
Hodgkins Disease	1
Histiocytic Reticulosis (?)	1

Non Transplant Patients Treated with Immunosuppressives (Antiinflammatory therapy)

Data has been collected that indicates that of seventy patients who have been under antiinflammatory therapy, seventy-two cancers developed during treatment with various agents.

Disease	*No. Cases*	*Therapy*	*No. Cases*	*Cancer Type*	*No. Cases*
Psoriasis	24	Methotrexate	23	Lymphoma	4
		Aminopterin	3	Leukemia	1
		Other	8	Skin	5
				Misc.	16
Renal Disease	13	Azathioprine	7	Skin	5
		Cyclophosphamide	7	Lymphoma	2
		Other	10	Misc.	6
Rheumatoid Arthritis	10	Cyclophosphamide	8	Lymphoma	5
		Other	8	Leukemia	3
				Lymphoma	2
				Misc.	4
Systemic Lupus Erythematosus	7	Cyclophosphamide	3	Kaposi's Sarcoma	1
		Azotheoprine	6		
		Other	6		
Other Inflamatory Diseases	16	Agents Used (as above, alone or as combined)	31	Cancers (Lymphomas, Leukemias, Hodgkins, and cancer of skin, bladder, colon, bronchus.)	16

Neoplastic Diseased Patients Without Transplants

In nontransplant patients with neoplasms receiving immunosuppressive cancer therapy, Penn[46] and Kersey[81] showed that second and even third tumours have arisen during treatment with chemotherapeutics. Of 185 patients with tumours treated with either Melphalan, cyclophosphamide, busulfan, 6-mercaptopurine,

chlornaphazine, chlorambucil, thiotepa, methotrexate, and prednisone, alone or in combination, 194 new malignancies developed. Various leukemias accounted for eighty-two of the new malignancies: thirty-five lymphomas, twenty bladder carcinomas, two cases of cancer of the cervix, and one Hodgkins disease occurred. The remainder of the cases were various and miscellaneous forms of malignancy and totalled fifty-five.

Acute leukemia occurred in thirty-nine cases where the patients originally had multiple myeloma. Twenty-seven solid lymphomas developed in cases where the patient's original neoplasm was chronic granulocytic leukemia. These unusual occurrences would tend to dispel any ideas that these cancers were transition forms of the existing malignancy. Thus, one can cautiously extrapolate the data and identify a significant association between the development of malignancy of lymphoid elements and the use of chemicals that have the capability of suppressing elements in the immune/host defense system. (Chlornaphazine is the one agent capable of directly causing cancer in man since it metabolizes to betanaphthylamine. It has caused bladder cancer in aniline dye workers.)

Leukemias and Chemical Immune Modulation

Of the four major types of cytological leukemia, myeloid and lymphatic constitute the greatest percentage. Myeloid cases peak out at about 60 percent of all leukemias at age thirty to forty, then declines thereafter. The lymphatic type has the highest prevalence in children peaking at about 50 percent and then declines till age thirty to forty. It then rises to 60 percent peak between ages eighty to ninety. On the way up to its peak, the lymphatic type passes myeloid type leukemia at about seventy years of age.[47]

An epidemiological study of solvent exposure and leukemia was conducted among rubber workers by McMichael et al.[48] indicating that an association between leukemia and jobs that involved using solvents existed. Prior to this time, Kessler and Lillienfeld (1969) as well as Vigliani and Saita[49] had prepared papers indicating they thought that the current evidence supported benzene, phenylbutazone, and chloramphenicol as being leukemogens; however, they had found little epidemiological evidence to support this

posture. McMichael et al.[48] had analyzed some 6600 co-workers (male rubber workers) who were working during the years 1964 to 1972, but had an employment duration of twenty-five years. They were followed for nine years with a 1 percent loss. Matched controls were also analyzed, with standard mortality ratios and proportional mortality ratios calculated. Of the various relationships that were analyzed, the association of death from lymphatic leukemia with a history of having worked in solvent exposure environment stood out. It is of interest that the lymphatic leukemia stands out as associated with solvent exposure jobs. This leukemia tends to be the myeloblasic or the stem cell type. McMichael had indicated that the leukemogenic effects may have been the result of concomitant exposure to other solvents that were used in the rubber industry.

However, Infante et al.[50] studied a population of workers who were occupationally exposed to only benzene during 1940 to 1949 and who were followed until 1975. Comparisons with two control groups show a significant excess of observed leukemia (p less than 0.002). A five fold excess risk of all leukemias and a ten fold excess risk of death from myeloid and monocytic leukemias combined were demonstrated in the comparison between populations. The environments of those workers were analyzed, and records have shown that the benzene levels were generally lower than the recommended limits at the time they were measured.

The observations are in agreement with Vigliani and Saita,[49] whereby a specific type of leukemia is associated with the exposure to benzene. The myelogenous or monocytic leukemia has been shown to correlate very well with the exposure and confirms the suspicion that benzene is a powerful bone marrow poison.[55] Goldstein[51] has written an excellent review of the toxic hemopoeitic effects of benzene exposure. These effects, although somewhat complicated by concomitant exposure in some cases, are fairly well demonstrated.

The mechanism by which this toxicity occurs is not known; however, the alteration of *stem cell* function is apparent.[51,52,53] Occupationally exposed persons as well as laboratory animal studies have shown chromosomal abnormalities, and correlates well with the known clinical picture of benzene toxicity.[54,55,56] Goldstein

has reported that in mice chronically exposed to 100 ppm of benzene, leukopenia, due primarily to lymphocytopenia, was clearly demonstrated. These manifestations of pancytopenia may represent a destruction of the stem cells, failure of the cells to mature, or prevention of differentiation at some critical stage. Other agents known to produce pancytopenia act in this fashion through modification of nuclear material. They include ionizing radiation chloromycetin, vinblastine, mitomycin, puromycin, colchicine and cytochalasin B. Many of these latter compounds have been used in clinical treatment of malignancy.

Forni et al.[52] carried out cytogenic studies and have confirmed chromosomal aberrations occurring in peripheral blood lymphocytes, due to benzene exposure.

If one examines the ontogeny of lymphocytes, it is readily apparent that bone marrow stem cells develop into two different lines of cells. The one line produces hemopoietic precursors, and the second line produces the lymphoid cells. Logically, interference at the stem cell level is bound to affect both the blood elements as well as the immune system elements. Laboratory investigations have observed pancytopenias such as monocytic, and myelogenous leukemias due to benzene exposure also show stem cells that have produced abnormal mature erythrocytes, which further supports stem cell nuclear function interference.

Wolman[54] has indicated that ample evidence is present to show that chromosomal aberrations can be induced through exposure to benzene. Gaps and junction breaks have been observed in cultured human cells (leukocytes and HeLa cells) at 1.1 or 2.2 x 10^{-3} M. benzene. Higher doses caused inhibited DNA synthesis. Peripheral lymphocytes stimulated by phytohemaglutinin (PHA) exposed to benzene for seventy-two hours revealed both numerical and structural alterations. Aneuploidy and chromosome breakage occurred seven and eight times more frequently in treated cells.

Immunochemicals and Cancer

Advances in pharmacological therapy has produced problems as well as remissions in the treatment of disease states. Patients who have been treated with antihypertensive, antiarrythmic agents as well as antitubercular or anticonvulsive agents have shown to be

susceptible to a syndrome related to the immune disease called systemic lupus erythematosus (SLE).[42,44,45] In SLE, the patient develops antibodies stimulated by the patient's own cellular material, especially the nuclear elements. It affects various tissues, and has a fatal form. One of the major findings in diagnosis has been the presence of an LE cell (polymorphonuclear leucocyte—PMN) that contains phagocytized nuclear material. This cell is formed as a consequence of reaction between an antibody present that can bind cellular nucleoproteins. Presumably the mechanism involves the lymphocytic nuclei that reacts with the antibody. Lymphocytes become saturated on their active sites, and PMN cells come to engulf the swollen lymphocytes. Digesting away (phagocytosis) the remainder of the lymphocyte, the PMN packages the nucleoprotein material as a residual body called LE. Although this disease is usually virally induced, therapeutic agents such as diphenylhydantoin, isoniazid, hydralazine, and procainamide have induced the syndrome that generally disappears upon withdrawal of the drug. These mechanisms that induce the SLE syndrome are not yet well understood; however, chemical influence on the immune system has produced antibodies that include anti-DNA, antinucleoprotein, antihistones, antinucleolar RNA, and antibodies to fibrous or particulate nucleoproteins. The importance of these data reflect that exogenous chemicals, which modify or influence biological systems, have unusual effects that are not always readily apparent. How this important defense mechanism has been modulated by interplay with chemicals is unknown, but any real capacity to effect nuclear material creates greater possibilities of biological dysfunction.

Zarrabi et al.[57] studied four groups of humans under treatment for some two and one-half years, with chlorpromazine and various other antipsychotic drugs. The main observation was the prevalence of immunologic and thromboplastic coagulative disorders. In patients on long-term chlorpromazine, the authors found that patients had increased levels of serum IgM, 405 ± 55 (in chlorpromazine treated patients), whereas controls had 157 ± 23, and normal is considered to range from 60 to 250: patients who were given combined therapy, chlorpromazine plus another drug, for the same length of time showed levels of serum IgM at 493 ± 75. Other

antipsychotics used were thioridizine, trifluoperazine, thiothixene, perphenazine, haloperidol, lithium, and amitryptilene. This group alone did not provide any significant differences when comparisons were made between the groups studied. Also noted was an increase in length of partial thromboplastin time. In both instances, significant correlation between increased levels of IgM in serum along with thromboplastin time were noted in relation to dose and duration of therapy. Both groups had a positive antinuclear antibody test (63%), both had nucleoprotein antibodies (58%), and both groups had antibodies to native DNA (40%).

It is interesting to note the authors' conclusion, "the IgM was the coagulation inhibitor," and was identified through immune neutralization and immunoglobulin isolation techniques. A product of the immunological analyses was the finding that the percentage of T-lymphocytes were below normal in thirteen out of forty-one patients treated with Chlorpromazine and twenty out of forty-two patients under single or combined treatment developed splenomegaly.

Recently, Doctor I. Fidler[58] at the Detrich Maryland Cancer Research Center, working with macrophages from mouse peritoneum, found that for some odd reason the macrophages began losing their tumoricidal activity during *in vitro* studies. Ordinarily, such macrophages in the peritoneum are not cytotoxic to tumour cells *in vitro*; however, a lymphokine released by an activated lymphocyte, referred to as macrophage activating factor (MAF) has the ability to stimulate the macrophage to become tumoricidal. Such peritoneal exudate macrophages (PEM) can also be stimulated to become cytotoxic by bacterial products (lipopolysaccharide—LPS), endotoxins, pyran copolymers, double stranded RNA, or during chronic infection with obligate bacteria. The *in vitro* studies were stopped and, in certain cases, the procedure modified because the PEMs were found to have lost the cytotoxic capability. A careful examination of the occurrence of lost activity seemed to correlate with a minor change in the drinking water used for the mouse colonies.

Water fed to the mice had a chlorine level of about twelve to sixteen parts per million (ppm). This high level is necessary to reduce the rate of early death syndrome in the colonies due to *Pseudomonas* infection since the mice had been lethally irradiated.

Due to an unusually high incidence of such early death syndromes, the chlorine level was raised to 25 to 30 ppm, and the experimental studies were carried on as usual.

At the start of the experiment and just before treatment, the mean number of PEMs per mouse was 21 ± 4 × 10^6. One week later the level of PEMs in the mice receiving hyperchlorinated water had decreased to 13 ± 2 × 10^6 per mouse. Controls were yielding 25 ± 3 × 10^6 per mouse. On consecutive weeks, PEM yield increased from the control mice, but mice on hyperchlorinated water had reduced PEMs or remained lower than controls. Mice that were receiving tap water yielded macrophages that when stimulated were tumorcidal *in vitro* to B16 melanoma cells or to UV 112 fibrosarcoma when activated by Concanavalin A-MAF, as measured by release of radioactivity.

The mice drinking hyperchlorinated water exhibited lowered levels of cytotoxic ability for the first two weeks, and, by the end of the third week of treatment, the cells, although stimulated by Con A-MAF, were not tumoricidal.

These studies show that hyperchlorinated water produces profound alterations in the numbers and tumoricidal capacity of PEMs. Lower levels (10-15 ppm) may also exert such influence although over a longer period of time.

Supporting these effects was a report by Fidler that showed that patients on long-term hemodialysis developed acute hemolytic anemia when treated with water that, although filtered by reverse osmosis, has 2 to 4 ppm chlorine. Chlorine compunds brought about a denaturation of the hemoglobin by direct oxidation and also by inhibition of the direct oxidative pathway (Hexose monophosphate shunt) of red blood cells (RBCs). The damage to RBCs was found to be cumulative over several periods of dialysis.

Although the mechanism for this depression of macrophage tumoricidal activity is unknown, Fidler suggests several possibilities. The vacuoles of the macrophage system are probably involved in the cytotoxic mechanism. This has been shown by Hibbs and Weinberg[39] whereby inhibition of the lysosomal enzymes of the macrophages occurs by addition of trypan blue, and stabilization of the lysosomal membranes occurs with the addition of hydrocortisone, and the cytotoxic activity is suppressed.

Macrophages that have been activated by lymphokines have enhanced bactericidal activity, and metabolically are shown to have a four to eight times increase in the uptake of glucose and its oxidation as compared to controls. Since the chlorine (compounds) inhibits the HMPS pathway[59] (that is the major path for glucose oxidation in the RBC's), there is a possibility that chlorine and/or its compounds may sufficiently inhibit glucose oxidation to the point whereby macrophages that have reduced tumouricidal capacity will allow aberrant cells to continue growing and relocalizing! Concurrently, indirect pathology also occurs! Chlorine levels affect erythrocytes' glucose metabolism, and large amounts of hemoglobin degradation products and/or large numbers of damaged RBCs can suppress macrophage tumoridical activity.

Regardless of the mechanism by which the chlorinated water or chlorine compounds may exert their influence, the important fact remains that macrophage activity is compromised. Since it carries a major role in host defense against neoplastic disease, the possibility exists that a host who is exposed to such compounds may in fact become immunocompromised. Silica, carrageenan, and trypan blue are substances that also have the ability to suppress macrophages in lab studies have been reported to decrease host resistance against transplantable tumours.

Dandliker et al.[60] recently reported their studies of the effects of pesticides on the immune response. Hamsters (LHC/LAK) five to eight weeks old and weighing about 100 grams were given a dose of pesticide equal to one-half the LD^{50} dissolved in 1 ml of corn oil. Arochlor 1260, Dinoseb, Parathion, pentachloronitrobenzene, piperonyl butoxide, mixed pyrethrins, and resmithrin were administered intragastrically. The animals were examined for an end point of "redness and swelling" (inflammatory response) and change in temperature of the foot pads, as well as histological exam, after an antigenic challenge. Serum antibody titer, binding affinity, and heterogeneity were determined by fluorescent polarization measurements.

The animals were first given an injection of fluorescein labeled ovalbumin, allowed only water *ad lib* for twenty-four hours, and then given a bolus of food with the pesticide by intragastric feeding tube twenty-four hours after the immunization. The most

striking feature reported by the authors after immunanalysis indicated marked humoral and cellular immunosuppression to single doses of Dinoseb and Parathion, and a marked stimulation of the cellular response by resmethrin. The other pesticides showed little or no effect under those conditions.

In another study[61] *using the pesticides* Ametryne, Carbaryl, Chlodimeform, DDT, Malathion, Mirex, and parathion, a single dose of pesticide was given orally at the LD^{50} or the 0.1 LD^{50} five days before, two days before, or two days after immunization with sheep erythrocytes. Assays were then conducted using antibody plaque forming cells four days later. (Plaque forming cells—antibody producing cells that can form a hemolytic plaque in the presence of complement and erythrocytes.) All animals receiving the higher dose exhibited significant depressions in splenic plaque forming cell numbers. Low dose animals receiving the dose for either eight or twenty-eight days prior to immunization exhibited no significant reduction in the antibody plaque forming cell numbers. The author indicates that a lack of information prevents a conclusion as to the efficacy of these compounds on modulating the immune system. Since the methodology only uses one test for the erythrocyte receptor, little can be concluded. This is why multiple parameter assays are necessary.

Faith and Luster[62] have performed extensive investigations on the pre- and postnatal effects of Tetrachlorodibenzodioxin (TCDD) on the immune system and have found that TCDD appears a relatively excellent immunosuppressive in the Fischer/Wistar rat strains. The Fischer strain is reputed to be less of a responder than its Fischer/Wistar cousin. However, dosing of nursing females has shown that the TCDD has the capability of causing immunosuppression in littermates. The effects have lasted as long as 270 days, from three doses to the mother at days zero, seven, and fourteen, applied at five micrograms per kilogram (ug/kg) body weight. At days eighteen and thirty-five, both female and male littermates showed depressed body weight as well as depressed thymic weights. These depressed values were evident at day 128 postdosing. The weight of the spleens were also found to be affected.

Effects of TCDD exposure on the homing patterns of lymphocytes were also studied in these same rat strains. Splenic cells taken from

the TCDD exposed rats were injected into nonexposed rats, and the thymus was found to significantly increase the uptake of such cells. Thymic cells taken from nonexposed rats were injected into TCDD exposed rats, and it was found that there was decreased homing ability to the thymus. The authors proposed that a change in cellular metabolism occurred altering the cell membrane, or that insertion of the TCDD into the membrane caused surface alterations, and this change in the cell modified its normal homing patterns. Various investigators have shown such alterations in immune function due to TCDD exposure.[63,64,65]

Thigpen[63] has shown that subclinical doses of TCDD had the ability to affect host response, when subsequent exposure to *Salmonella* infection resulted in reduced time to mortality. Thus far, exposure to TCDD has been shown to cause an increase in the susceptibility to bacterial infections (suppression of immune response) as well as suppression of mitogen responsiveness, suppression of the skin graft rejection, and depression of the delayed hypersensitivity response. (Suppression of the T-cell dependent immune functions appears to occur as an isolated response.)

Phenol

Lavia et al.[66] also had occasion to come upon the effects of phenol on immune function through a case of serendipity, as did Fidler. Phenol was being used to disinfect the cages of mice, and was found to be causing depression of the immune response to t-dependent antigens in the time period of four to six weeks. Further analysis of the phenol showed the disinfectant to be a mixture of o-phenylphenol (5.0%), o-benzyl-p-phenol (4.5%), and p-tert-amylphenol (1.0%). OPP, OBP, and PTA, respectively. Such compounds are used in biocides throughout the world.

Oehme[67] has studied the metabolism of one compound, o-phenylphenol (OPP) in the cat and dog and has found that although the two animals metabolize OPP according to two different routes and rates, excessive tissue levels are found in the spleen. Lavia[66] decided to study this phenomena on immune function knowing that the spleen plays a major role in the immune response. Groups of BALB/c female mice were dosed with the phenol derivatives (all three derivatives were used, but each group of mice received only

a single compound) at levels of 0.46 milligrams per kilogram (mg/kg) of OPP, 0.41 mg/kg OBP, and 0.09 mg/kg PTA in their drinking water. At weekly intervals, three mice were immunized intraperitoneally with 1.0×10^8 sheep erythrocytes (SRBC). Four days later the number of IgM plague forming cells (PFC) was determined. Before the end of the second week of exposure, 45 percent of the mice showed immunodepressive effects. The response after four weeks showed depression to be occurring in 77 percent of the dosed mice. Comparison of OPP with a mixture of phenol compounds showed the immune depression to be the same, indicating that OPP appears to have the same capacity for immune suppression as the mixture and must be exercising dominance in producing the response. Monocytes (Macrophages) were also analyzed for their capacity to phagocytize yeast cells. This capacity was significantly reduced as compared to controls. Measurement of T and B lymphocyte numbers in control and OPP exposed mice showed no significant differences.

The net effect was that the macrophages appear to have sustained some defect that reduced their ability to present antigen for phagocytosis or may have just resulted in a reduced number of circulating macrophages that could affect the cooperative cell–cell activation upon B-lymphocytes. Archer[68] has also studied the suppression of the immune system using gallic acid, a phenolic derivative, and has concluded that the mouse spleen cells that were studied showed a marked depression of the immune system from such exposure probably at the macrophage level. These data are very important, because it shows that immune suppression has occurred at low dose levels and with short periods of exposure, which situation mimics many of the environmental exposures. This is true especially with drinking water. To extrapolate this evidence without asking other pertinent questions clearly is not yet justified, but when taken in concert with the studies done by others, it can be seen that the immune system is in fact a very sensitive system that may be an ideal vehicle for indicating potential toxicity to humans. Although ultrastructural morphology or DNA damage studies have been used prior to this time for indications of toxicity, it appears that immune cell biochemistry may be more sensitive to such low level doses that are prevalent in the envi-

ronment and would complement such bioassays.

Metallo Organics

Extensive investigations have been made of the effects of organo tin compounds on the immune system and it has been shown by Seinen and Penninks[69] that Di-n-octyl-tin chloride causes a depletion of lymphocytes in the thymus and thymus dependent areas in the spleen and lymph nodes. There was a dose dependent relationship showing a decrease in the number as well as the viability of thymocytes. Spleen cell numbers and viability were slightly less pronounced, and no effect was found on bone marrow and/or peripheral lymphocytes and/or monocytes. Thymic atrophy reversed upon discontinuation of the exposure. The results of exposure to Di-n-butyl tin chloride was identical to the Di-n-octyl tin chloride. Di-n-ethyl-, and Di-n-propyl tin chloride compounds showed less pronounced effects. In contrast, Di-n-methyl-, Di-n-dodecyl-, and Di-n-octadecyl tin chlorides as well as monooctyl-, tri-n-octyl-, and tetraoctyltinchlorides did not show any thymic atrophy. In this excellent review, they examined a large body of data dealing with lead and cadmium on the immune system and established that the major effect is an increased susceptibility to infection by gram negative bacteria that contain endotoxin. Lead is also shown to decrease the resistance to viral disease, so one can conclude that the humoral response is somehow affected causing B-lymphocyte defects in antibody generation, or perhaps in the modification of circulating antibody molecule itself. Koller,[70] using rabbits dosed with lead acetate, 2200 mg/liter (equal parts per million) in water showed that after ten weeks of exposure, both the primary and secondary response to pseudorabies virus had been depressed. Koller and Kovacic[71] showed that mice dosed with lead acetate had a significantly increased number of IgM plague forming cells to SRBC. This further adds to the complexity of the situation, since the effect may be occurring in the lymphoid organ(s) associated with development of B-cells. Additional cells may be stimulated without proper maturation and/or they may have defective antibody response capability. Chronic low level dosing of lead to rats pre- and postnatally by Faith et al.[72] showed the thymus weights to be suppressed along with decreased responsiveness to

mitogen stimulation of lymphocytes and reduced delayed hypersensitivity response. Of greater importance is the fact that the offspring of females dosed with 25 or 50 ppm of lead in drinking water showed no inhibition of growth, as exhibited by body weight gain, nor overt signs of toxicity. However, the analysis of the immune system functions of offspring did show decreased mitogen responsiveness, as well as the depressed delayed hypersensitivity reaction. The inescapable fact is that some facet of the immune function has been altered. The doses of 25, 50 ppm lead used for the mice produced blood levels of 29.3 and 52.8 micrograms per 100 ml blood, which are comparable to blood levels found in human children, makes currently allowable lead levels somewhat undesirable. In light of the relationship of humans to lab animals, and to this evidence, supplied by the more sensitive immunological indicator, covert toxicity may in fact be occurring[72–74] even at low levels.

Hoffman and Niyogi[74] have studied metal carcinogens and have indicated that lead and the other metal salts were able to interfere with the fidelity of DNA synthesis. Such interference would, if occurring in the B-cells, have a profound effect on resistance since the ability to differentiate in lymphocytes is absolutely necessary for host defense. Should it interfere with the DNA at a small lymphocyte blast stage perhaps a major clonal species of lymphocyte could be permanently impaired!

Additional studies by Vos et al.[75] using hexachlorobenzene on rats has shown the immune system to be stimulated, which contrasts other data presented. Sharma[76] has exposed mice to vinyl chloride and found the immune system lymphocytes to be profoundly stimulated.

Miller[77] and Kagan and Miller[78] also have observed immunostimulation in patients who have asbestosis. Some disturbance may have occurred in the immune regulatory mechanism, since they have shown hyperactivity in the humoral immune response, increased reproduction in the serum globulins, secretory IgA, and a variety of autoantibodies. The reason for this activity is not clear; however, asbestos studies done on the lung suggest that macrophages trying to digest the mineral fibers are damaged, spilling out lysosomal enzymes, which may bring on autoimmune

pathology. Drath et al.[80] have established that smoke from tobacco causes morphological biochemical and functional alterations in the pulmonary alveolar macrophages, which are functionally impaired with respect to phagocytosis. Since it is well established that macrophages are an integral unit of the immune responses,[79] there is a significant modulation occurring that cannot be denied, but whose total impact is yet to be discovered.

Because of a known role in the immunosurveillance mechanism, the possibility of modulation by environmental chemicals of the immune cells certainly seems an exciting speculation.

Summary

The influence of environmental chemicals upon the public health is of considerable importance. Determining how such chemicals may cause adverse effects upon humans is a continuing problem that perplexes all phases of scientific inquiry. Genetic chronic toxicity and dose responses are defined from animal bioassays and microbial DNA studies for human use, and constitute a major source of controversy among predictive toxicologists.

A major step forward in resolving such problems could be attained with the use of human lymphoid cells, which are readily available from the peripheral circulating blood. The lymphoid cells of the immune system are an integral part of the defense mechanism known as immune surveillance and are very important in the recognition and removal of abberant or malignant cells. It is this relationship that may be disturbed by environmental chemicals and allows carcinogenesis to proceed in susceptible individuals.

The use of immunosuppressive therapy has shown there is an increased frequency of various types of malignant disease associated with continued use of such agents. Secondary as well as tertiary cancers have been produced by therapeutic suppression of the immune system. Concurrently, investigators have also shown that occurrence of malignant disease is also found in individuals with genetic immunodeficiencies at a higher frequency than in immune normal hosts. So it has become apparent that malignant response in a given host may in fact be very seriously dependent upon an immune system that has been somehow compromised.

In vitro and *vivo* studies using pesticides, metallo-organics and various pharmacological agents, have shown that certain activities of lymphoid cells are modulated by the presence of many of these compounds.

Lymphocytes have been either suppressed or stimulated, and either condition may be affecting immune response. The blood monocytes, which play a vital role in the B-cell/T-cell interactions, are shown to be very severely disturbed by excessive amounts of chlorine or hemoglobin degradation products. So, direct or indirect effects can modulate the immune response, showing the sensitivity of the lymphoid population to environmental influence. The cells themselves may become defective through direct action, or the tissue in which they mature and differentiate may be modified, producing an impotent cell.

The cell may be affected at the surface, within the cytoplasm where antigenic determinants are synthesized, or within the nuclear protein and/or chromosomal levels. Thus there exists a cell for all seasons; morphologists, biochemists, immunologists, pure chemists, pure biologists, all will find an abundance of suitable material to investigate. The most rewarding portion of our work may well be that we will be closer to effective extrapolation for human exposure.

Conclusion

The mechanisms whereby chemicals influence the immunological surveillance system are not understood. In fact, there are many who have asked questions that may dull the excitement over the immunosurveillance theory. However, they have not been sufficiently substantiated. What has been elucidated in this presentation is that we cannot deny the influences external chemicals have on the cells and products of tissue from the lymphoid-immune system. The role of chemically induced malignancy in the immune suppressed patients receiving therapy or the excess occurrences of malignant disease in immunodeficient individuals is significant and cannot be ignored. The complexity of the immune system, details of mechanisms, in fact, whether cells or products may be influencing this system is in most cases not known. However, it can be stated that many of the substances mentioned here today

are in the environment and they do have immunomodulating effects. How does this role of immunomodulation affect interpretation of previous studies that discerned that a mouse, rat, guinea pig, or some other lab animal has or has not responded with malignancy to a carcinogenic chemical? Have those modulating effects been taken into account in concluding that some chemical is or is not a carcinogen? Clearly such questions can establish a compromised position when making conclusions as to whether any chemical should be allowed in the human environment. Mice, rats, hamsters, and guinea pigs have variable systems. Has the chemical tested caused a depression of the system that allowed some virus to induce a cancer? Or has the animal perhaps a depressed immune system, due to repression of genetic expression due to inbreeding that now allows a neoplastic response to occur?

Immunotoxicology is the new kid on the block and is able to ask some very difficult questions. Questions for which we have not all the answers.

In the area of predictive toxicology, however, I would suggest the following. Because immunotoxicology may bring about an additional dimension in extrapolation, I would suggest that future studies be directed toward examining these immune system elements and how they respond to mutagenic or carcinogenic agents. Not only in the lab animal species, but by using the peripheral blood elements from humans. Establishing a tissue culture procedure with human lymphocytes and/or macrophages, even though *in vitro*, would allow function and surface identification studies as well as biochemical investigations to procede and perhaps promote greater confidence when possibly identifying toxic responses to humans. Other human cells have been cultured, such as fibroblasts and the HeLa cells, surely the same could be done for the immune system cells. It would go far in impacting the program of public health for which we are all responsible.

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