Environmental Health Perspectives
Vol. 18, pp. 125-131, 1976

Immunologic Response and Factors Affecting Its Assessment

by John A. Moore* and Robert E. Faith*

The potential harmful effects of toxic compounds on the developing immune system are discussed. This discussion is illustrated by results of studies on the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the developing immune system of Fischer rats. While this compound and several others have been shown to have immunosuppressive effects, the available data do not support routine evaluation of the consequence of chemical exposure to the developing immune system.

Introduction

This presentation strives to establish a rationale for investigatory research into the possible deleterious effects of chemicals or agents during immune ontogenesis, illustrate the results of such research, discuss factors which bear on interpretation of such research, and suggest basic approaches in the design of experiments that assess effects of chemicals on the developing immune system.

For the purposes of this paper, "immunologic response" is principally restricted to a response by those cells in which specific antigen recognition is inherent, e.g., production of antibody or cell mediated immune responses. We intend to discuss only depression. For clarity and brevity, cells which perform important but accessory roles are relegated to a position of benign neglect (e.g., fixed and free macrophages, mast cells, and various other leukocytic cell types). This is a calculated risk, since diminished accessory cell function may partially mask immune response given their role in the preparation of antigen for recognition by or presentation to immune competent cells or in the expression of select immune reactions.

*National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina 27709.

Need for Research on the Developing Immune System

Incorporation of tests which evaluate immune function during toxicologic evaluation of chemicals has not kept pace with the rapid advances being made in the field of immunology. Stimulation of an undesired immune response (sensitization) has been evaluated to some degree, but testing for suppressive effects has been haphazard. Failure to recognize the need for such procedures is a bit surprising given that chemicals have been identified and developed because they possess immunosuppressive properties. Corticosteroids, azathioprine, cyclophosphamide, 6-mercaptopurine, and methotrexate are chemicals utilized in the treatment of specific forms of neoplasia or in controlling host rejection processes during various grafting procedures (*1*). There is a select, but important, list of chemicals not utilized in medical applications that produce a distinct immunosuppression. These include polychlorinated biphenyls (PCBs) (*2-6*), organotin compounds (*7*), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (*8-10*), lead, and cadmium (*11-14*). While not definitive, but clearly germane to this topic, specific immunodeficiency as a possible consequence of methylmercury exposure during fetal and neonatal development has also been

reported (*15*). Exposure to corticosteroids during development also has produced immune deficiencies (*16*).

Development of immune competence is a process which requires the sequenced migration of cell types through noncontiguous tissues during an extended, but temporally ordered period of fetal/neonatal development (*17*). Development failure, delayed maturation or malfunction of an organ may arrest, delay, or otherwise compromise development of a specific population of immune competent cells (*18*). The interdependent and temporally sequenced nature of this process suggests that chemicals which affect, even in a transient way, adult immune capabilities may produce similar, more profound or permanent effects in developing systems.

Effects of TCDD on Immunity

TCDD is known to induce a pronounced atrophy of thymus, spleen, and to a lesser extent, peripheral lymph nodes of rats, mice, and guinea pigs (*8,19,20*). Although there is species variation in the degree and severity of other organ effects, the effects of TCDD on lymphoid containing tissues is consistent in all species. It is also known to be a teratogen in mice, producing cleft palate or hydronephrosis (*21,22*). It is primarily fetotoxic or lethal in rat teratology studies (*23*).

Studies of immune function in several species of animals exposed as adults found cellular immune capabilities to be impaired (*8,9*). This circumstance combined with the teratogenic data that demonstrate the ability of TCDD to gain access to the fetus prompted investigation of the possible effect(s) of TCDD on the developing immune system.

In initial experiments, 1-day-old rat pups from mothers who had received TCDD on days 11 and 18 of gestation had diminished thymus and spleen weights; the thymus on histopathologic examination showed atrophy of the cortex due to a paucity of lymphoid cells (*9*). Since a significant degree of immune maturation occurs during the nursing period in the rat, experiments were designed whereby pups were exposed to TCDD either by treatment of the mother on gestation day 18 as well as postnatal days 0, 7, and 14 or by maternal treatment on postnatal days 0, 7, and 14. The Fischer rat litters were standardized at 8 pups per litter at birth. Suppressive effects on the immune system were confirmed using a number of test procedures.

Table 1 shows the body weight and selected organ effects at four specific time periods. All 25-day old pups that had been exposed to mothers treated with TCDD either during gestation and/or during the nursing period were significantly lighter in weight than the corresponding controls. There was no significant difference between the two treated groups. The diminished body weight also was seen in rats at 39 days. At 59 days and 145 days of age, rats which had only postnatal exposure did not have body weights which varied significantly from controls. Rats exposed pre- and postnatally never attained body weights equivalent to controls. Ratios of thymus weight to body weight parallel the changes in body weight in both experimental groups at 25 and 39 days of age. This effect persisted in rats that had received pre- and postnatal exposure when evaluated at 59 and 145 days of age. Depressed ratios of spleen to body weight were also found.

Evaluations of *in vitro* immune capabilities of spleen and thymus lymphoid cells were performed. In these studies, cell suspensions from spleen and thymus were cultured in the presence of specific mitogens. The mitogens used were phytohemaglutinin (PHA) and concanavilin A (Con A); both specifically stimulate T-cells (cell-mediated response). Cells are cultured in the presence of these mitogens for 72 hr; tritiated thymidine is then added and the cells are cultured for an additional 18 hr. At the end of this time, the cold trichloroacetic acid (TCA)-precipitable material from the cultures was harvested and tritium incorporation determined by liquid scintillation spectrometry. The index illustrated in Figure 1 was derived by dividing the counts per minute (cpm) in TCA-insoluble material from cells exposed to the mitogen by the cpm in TCA-insoluble material from cells not exposed to the mitogen. The clear bar represents the controls, the stipled bar represents the rats exposed postnatally, the solid bar depicts rats that have received both pre- and postnatal TCDD exposure. Current immunologic dogma suggests that the response to PHA is primarily observed with mature T-cells (*24*). This would account for the greater PHA stimulation seen with spleen cells when compared to thymus cells. Both treatment groups showed a diminished response of spleen cells to PHA at 25 days and 59 days of age. In addition, the response at 59 days showed a significant difference between the two treated groups.

The PHA-treated thymus cells only showed a

Table 1. Selected weight effects in TCDD-exposed rats.

Age, days	Sex	No. of rats	TCDD exposure	Body wt, mean	SE	Thymus/body wt ratio	Spleen/body wt ratio
25	F	12	Controls	40.89	1.14	3.88 ± 0.09	3.97 ± 0.06
	M	6		45.14	2.35	3.13 ± 0.13	3.92 ± 0.03
	F	6	Postnatal	20.26	2.04[a]	1.86 ± 0.11[a]	5.67 ± 0.35[a]
	M	6		33.30	0.59[a]	1.76 ± 0.12[a]	3.94 ± 0.09
	F	6	Prenatal and	24.46	2.45[a]	1.91 ± 0.15[a]	7.04 ± 0.82[a]
	M	6	postnatal	30.66	1.41[a]	1.47 ± 0.13[a]	4.40 ± 0.09[a,b]
39	F	6	Controls	89.31	1.37	3.40 ± 0.14	3.15 ± 0.20
	F	6	Postnatal	69.84	3.42[a]	2.09 ± 0.12[a]	3.33 ± 0.49
	F	6	Prenatal and postnatal	57.87	4.81[a]	1.79 ± 0.19[a]	5.39 ±0.49[a,b]
59	M	6	Controls	191.98	8.91	2.01 ± 0.07	2.62 ± 0.14
	M	6	Postnatal	175.97	5.52	1.86 ± 0.06	2.68 ± 0.08
	M	6	Prenatal and postnatal	86.34	16.74[a,c]	1.25 ± 0.19[d]	3.30 ± 0.54
145	F	14	Controls	178.14	2.02	1.11 ± 0.03	2.42 ± 0.04
	F	16	Postnatal	173.81	2.01	1.00 ± 0.03	2.55 ± 0.06
	F	12	Prenatal and postnatal	74.58	13.80[a,b]	0.41 ± 0.08[a,b]	2.71 ± 0.12

[a] $p<0.01$ vs. controls.
[b] $p<0.01$, prenatal/postnatal vs. postnatal.
[c] $p<0.05$, prenatal/postnatal vs. postnatal.
[d] $p<0.05$ vs. controls.

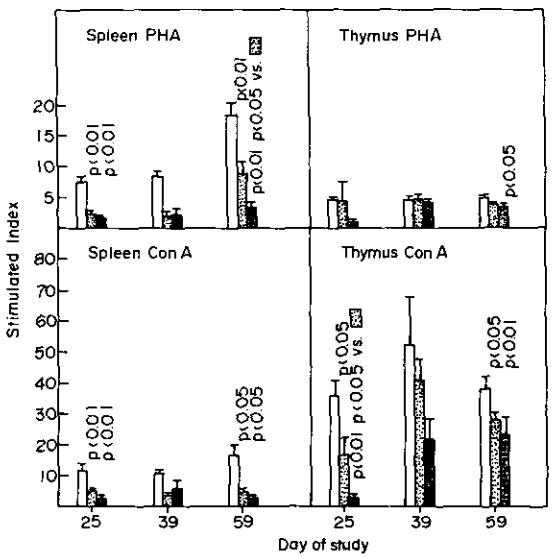

FIGURE 1. Response of isolated spleen and thymus cells from TCDD-exposed animals to mitogenic stimulation: (open bars) non-exposed animals; (stipled bars) animals exposed postnatally via maternal dosing on days 0, 7, and 14; (solid bars) animals exposed pre- and postnatally via maternal dosing on gestational day 18 and on days 0, 7, and 14.

diminished response in the pre- and postnatally treated rats at 59 days of age. The relative unresponsiveness of thymus cells to PHA was quite apparent.

Concanavilin A is more likely to stimulate the immature T-cells; therefore, a greater stimulation in cells from the thymus is expected (*24*). Although somewhat unresponsive, spleen cell stimulation by Con A was reduced at the 25 and 59 day time period. Similar results were observed in the thymus cells at both time periods.

An *in vivo* measure of cellular immune capabilities is depicted in Figure 2. The method evaluated the ability of rats to develop a delayed hypersensitivity reaction. In this experiment, the sensitizing agent was oxazolone. Rats were sensitized by painting the ears with oxazolone dissolved in ethyl alcohol; 10 days later a standardized dose of tritiated thymidine was administered intraperitoneally. At 24 hr after the thymidine injection, oxazolone in oliver oil was painted on one ear. One day later, the rat was sacrificed and a standard tissue plug taken from the ear at the site of oxazolone administration. This plug was digested and radioactivity recorded using a liquid scintillation counter. The untreated ear was handled in a similar manner and served as a control. A ratio was derived from the degree of radioactivity found in the oxazolone ear versus the untreated ear. The theory behind the test is that, in response to oxazolone application to the ear, presensitized immunocompetent cells migrate to the area; these cells also stimulate a macrophage aggregation. The macro-

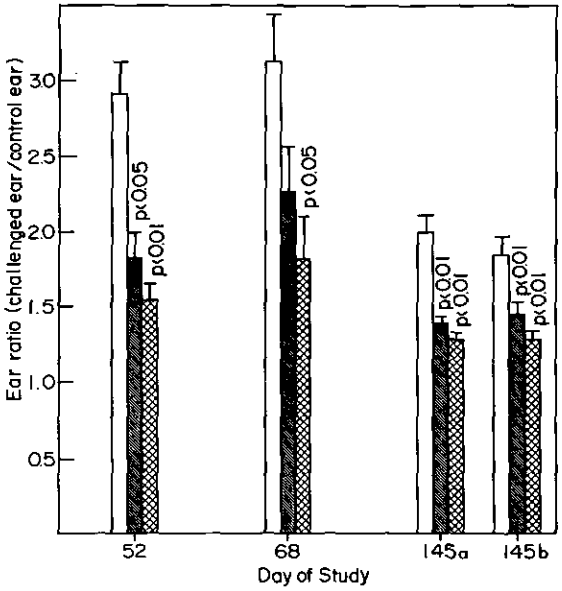

FIGURE 2. Delayed hypersensitivity responses of TCDD-exposed animals to the contact sensitizing agent oxazolone: (open) bars represent nonexposed animals; (shaded bars) animals exposed postnatally via maternal dosing on days 0, 7, and 14; (crosshatched bars) animals exposed pre- and postnatally via maternal dosing on gestational day 18 and on days 0, 7, and 14.

phages contain the thymidine label which was incorporated during their formation in bone marrow. The experiments clearly showed a diminished delayed hypersensitivity reaction in the treated rats at all the time periods evaluated. Most striking was the persistence of cellular immune depression through the 145th day.

Figure 3 illustrates serum antibody of control and TCDD-treated rats to the antigen, bovine gammaglobulin (BGG). Serum antibody is a B-cell expression, but with this antigen is dependent on competent T-cell interaction. There was no statistical difference in response of the treated versus the controls to a primary and secondary injection of BGG.

In summary, the data show suppression in cell-mediated immune response in offspring of animals which had received TCDD. The immunosuppression persisted thorugh 145 days (the last period tested). The depression of the cellular immune (T-cell) system is somewhat selective, given that a normal response to BGG was observed.

Interpretation of Immunologic Data

There are a number of environmental, physiologic, and pathologic conditions that in-

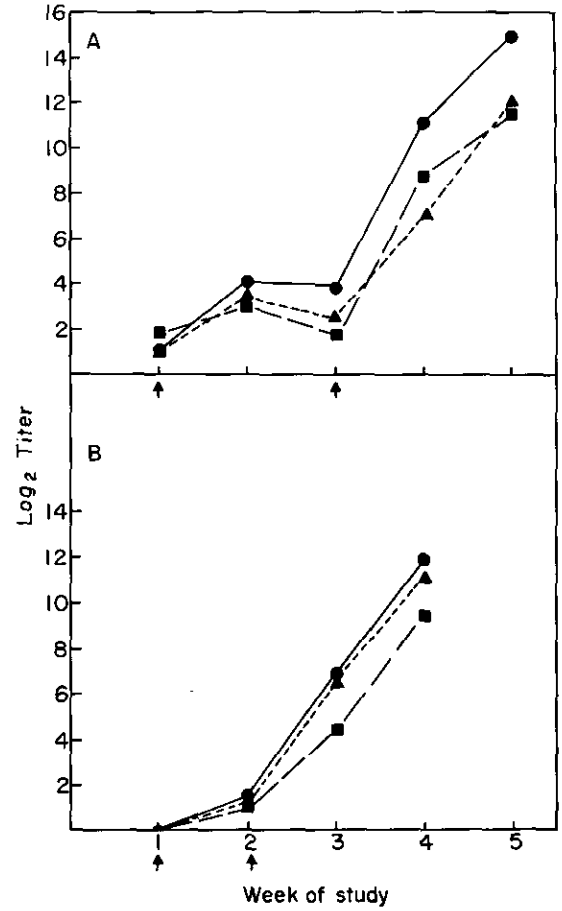

FIGURE 3. Antibody response of TCDD-exposed animals, showing (*A*) primary and secondary response to injection with bovine gamma globulin and (*B*) response of animals injected with bovine gamma globulin two consecutive weeks: (■) nonexposed animals; (●) animals exposed to TCDD postnatally via maternal dosing on days 0, 7, and 14; (▲) animals exposed to TCDD pre- and postnatally via maternal dosing on gestation day 18 and on days 0, 7, and 14.

fluence immune response. These factors need to be considered when evaluating immune response data. Such evaluation may allow a determination as to whether a suppressed state is secondary to other toxic effects. Several of these conditions merit discussion.

Adrenal glucocorticoid hormones induce lymphopenia and lymphoid depletion particularly in the thymus (*25-27*). Such an effect can be produced by endogenous or exogenous adrenal hormone. To evaluate an immune suppression mediated by corticosteroids, one should evaluate adrenal weight and morphology; the measurement of serum corticosteroid levels or the use of adrenalectomized animals would afford a more precise evaluation.

Interpretation of experiments where an immunosuppression has been produced in animals that are diminished in size is difficult. It has been clearly established that animals without a functional thymus develop a runted appearance and are deficient in cell mediated immunity (*28-30*). Similarly, general toxicity during the nursing period can produce diminished weight gain and a runted appearance. Decreased food consumption or impaired food utilization will also produce a small or runted individual. It has been shown that malnutrition, either on a net energy basis or from a selective protein deficiency, causes a diminished immune capability (*31-33*). Ascribing a runting condition to either a cause or effect is a perilous venture!

Species variation in ontogenesis of the immune response exists from the standpoint of whether it is a prepartum or largely postpartum phenomenon (*34*). In the mouse and rat, this system is being developed during the very late prenatal and neonatal period. In the guinea pig, by contrast, a well-functioning immune system is present at birth; a similar circumstance is seen in man. In species where critical maturational events occur subsequent to parturition, a number of environmental factors may have a deleterious impact. A result produced in this circumstance may not extrapolate to those species in which maturation occurs within the relatively "protected" environment of the placenta. Pharmakokinetic properties also bear on this issue. If susceptibility to chemical insult during a specific period exists, the consequence of exposure may not become manifest in an *in utero* environment due to maternal distribution; however, a high dose level could be realized during the neonatal period. TCDD shows such a dose related circumstance to some degree in that there is significant secretion of TCDD from maternal stores in milk. Thus, neonatal TCDD exposure is greater than is fetal exposure. Such factors, of course can be evaluated through appropriate cross-fostering techniques.

A final point relative to interpretation of immune function results is selectivity of immunosuppressive effects. The TCDD data clearly shows a suppression of the cellular immune capabilities. Humoral (B-cell) function seems unimpaired. Preliminary results reported with methylmercury suggest, according to Spyker (*15*), impairment of B-cell, not T-cell function. Furthermore, the TCDD studies indicate that there may be a selectivity in the suppression of cellular immunity given that response to bovine gammaglobulin, a T-dependent response, was not diminished.

Methodology

Species Selection

The rat and mouse are recommended because: (1) these are species in which teratology data may be available from which one could determine dose, and (2) many classical immunology methods have been developed in mice and, to a lesser extent, the rat. There is, however, the possible problem of extrapolation to man of data obtained from these species due to the postnatal maturation of the immune system.

Litter Manipulations

The number of pups within a litter needs to be standardized at birth to eliminate variables associated with nutritional effects. Cross-fostering techniques need to be considered in experiments which confirm initial results and determine whether the immunosuppressive effect is due to *in utero* exposure or exposure during the nursing period; i.e., milk, maternal behavior, etc.

Age of Testing

Initial assessment of the immune response shortly after the time of weaning would seem most appropriate.

Immunology Test Methods

Methods suggested are generally ones that are accepted by immunologists, and, most importantly, are well within the capabilities of many laboratories.

Production of Serum Antibodies: By selection of specific antigens, one can determine the capabilities of both B and T-lymphocytes.

Lipopolysaccharide (LPS), a commercially available product, is an antigen to which serum antibodies may be raised through a mechanism dependent only upon B-cell capabilities (*35,36*). Alternatively, sheep red blood cells (SRBC) may be used as the antigen to elicit a serum antibody at a normal level only if there are T-cells to initially interact with this antigen and B-cells to subsequently produce the serum antibody (*37-39*). Serum antibodies to these antigens can be quantified using hemagglutination techniques.

In a minimal experimental design, one should

select the sheep red blood cell test in preference ot the LPS method, since a response to SRBCs, being a T- and B-cell-dependent phenomenon, is a more inclusive method.

Delayed Hypersensitivity: This *in vivo* method is a most adequate means of evaluating cellular immune capabilities. In addition to evaluating the responsiveness of particular T-cell lymphocytes, it also gives some indication of the ability of the animal to marshall other factors associated with the hypersensitivity reaction, such as chemotaxis, macrophage infiltration, and accumulation. A specific test we recommend is the measurement of specific migration of radiolabeled cells to a site of antigenic challenge (*40*). The sensitizing agent we would currently suggest is mycobacterium through use of Complete Freunds Adjuvant. Challenge would then be carried out with purified protein derivative.

Response to Mitogen Stimulation: With this *in vitro* method, one exposes cultures cells to a substance known to stimulate mitotic activity in select populations of lymphoid cells. The degree of stimulation is evaluated through the incorporation of radiolabeled thymidine into DNA by these cells during replication. There are mitogens that selectively stimulate the cellular immune (T-cell), or humoral immune (B-cell), components. Phytohemaglutinin and concanavilin A are T-cell stimulators, while LPS seems to stimulate only B-cells (*41*). We routinely set up cell cultures from thymus as well as spleen. We then have a good likelihood of getting an indication of the nature of the immune defect; i.e., thymus has a different population of T-cells than does spleen, where mature forms of T-lymphocytes are found as are cells that will respond to a B-cell mitogen.

At the beginning of this presentation, it was stated that all chemicals should not be evaluated for possible effects on the developing immune system; it is felt there are a number of situations which would suggest that this system be assessed. Chemicals which depress immune function in adults should be evaluated in the fetus/neonate. Postnatal studies which result in runting syndrome at time of weaning or through a wasting disease later in life suggest that the immune response may be impaired. Finally, routine teratology procedures need to be revised to include an evaluation of major lymphoid organs, i.e., thymus and spleen. Such an examination should include organ weights and histologic examination. These procedures can be readily incorporated into any standard teratology screen; histologic evaluation can be done most expediently through multiple imbedding of tissues. If there is indication of diminished maturation of these systems, one should consider subsequent experiments that specifically assess immune response.

The authors are grateful to Mrs. L. D. Lawson for valuable technical assistance and to Dr. J. K. Haseman for statistical analyses.

REFERENCES

1. Penn, I. Chemical immunosuppression and human cancer. Cancer 34: 1474 (1974).
2. Friend, M., and Trainer, D. O. Polychlorinated biphenyl: interaction with duck hepatitis virus. Science 170: 1314 (1970).
3. Vos, J. G., and van Genderen, H. Toxicological aspects of immunosuppression. In: Pesticides and the Environment: A Continuing Controversy. W. B. Deichmann, Ed., Symposia Specialists, Miami, 1973.
4. Vos, J. G., and DeRoij, T. Immunosuppressive activity of a polychlorinated biphenyl preparation on the humoral immune response in guinea pigs. Toxicol. Appl. Pharmacol. 21: 549 (1972).
5. Koller, L. D., and Thigpen, J. E. Reduction of antibody to pseudorabies virus in polychlorinated biphenyl-exposed rabbits. Am. J. Vet. Res. 34: 1605 (1973).
6. Street, J. C., and Sharma, R. P. 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 (1975).
7. Verschuuren, H. G., et al. Influence of triphenyltin acetate on lymphatic tissue and immune responses in guinea pigs. Toxicol. Appl. Pharmacol. 16: 400 (1970).
8. Vos, J. G., Moore, J. A., and Zinkl, J. G.: Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the immune system of laboratory animals. Environ. Health Perspect. 5: 149 (1973).
9. Vos, J.G., and Moore, J. A. Suppression of cellular immunity in rats and mice by maternal treatment with 2,3,7,8-tetrachlorodibenz-*p*-dioxin. Int. Arch. Allergy Appl. Immunol. 47: 777 (1974).
10. Thigpen, J. E., et al. Increased susceptibility to bacterial infection as a sequela of exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Infect. Immun. 12: 1319 (1975).
11. Hemphill, F. E., Kaeberle, M. L., and Buck, W. B. Lead suppression of mouse resistance to *Salmonella typhimurium*. Science 172: 1031 (1971).
12. Jones, R. H., Williams, R. L., and Jones, A. M. Effects of heavy metals on the immune response. Preliminary findings for cadmium in rats. Proc. Soc. Exptl. Biol. Med. 137: 1231 (1971).
13. Koller, L. D. Immunosuppression produced by lead, cadmium and mercury. Am. J. Vet. Res. 34: 1457 (1073).
14. Koller, L. D. and Kovacic, S. Decreased antibody formation in mice exposed to lead. Nature 250: 148 (1974).
15. Spyker, J. M.: Assessing the impact of low level chemicals on development: behavioral and latent effects. Fed. Proc. 34: 1835 (1975).
16. Russell, A., et al.: Transplacental and neonatal effects of hypercortisonism in the rat on thymo-lymphatic system

differentiation and serum immunoglobulin levels. Adv. Exptl. Med. Biol. 27: 257 (1972).
17. Owen, J. J. T. The origins and development of lymphocyte populations. In: Ontogeny of Acquired Immunity, A Ciba Foundation Symposium. Elsevier, New York, 1972.
18. Rosen, F. S. Defects in immunological development in man. In: Ontogeny of Acquired Immunity. A Ciba Foundation Symposium. Elsevier, New York, 1972.
19. Harris, M. W., et al. General biological effects of TCDD in laboratory animals. Environ. Health Perspect. 5: 101 (1973).
20. Gupta, B. N., et al.: Pathologic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in laboratory animals. Environ. Health Perspect. 5: 125 (1973).
21. Moore, J. A., et al.: Postnatal effects of maternal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Environ. Health Perspect. 5: 81 (1973).
22. Neubert, D., et al. A survey of the embryotoxic effects of TCDD in mammalian species. Environ. Health Perspect. 5: 67 (1973).
23. Sparschu, G. L., Dunn, F. L., and Rowe, V. K.: Teratogenic study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat. Toxicol. Appl. Pharmacol. 17: 317 (1970).
24. Nielson, S. E., and Tribble, J. L. Mitogenic responses of thymus cell subpopulations. Experientia 31: 376 (1975).
25. Schlesinger, M., et al. Wasting disease induced in young mice by administration of cortisol acetate. Science 143: 965 (1964).
26. Ioachim, H. L.: The cortisone-induced wasting disease of newborn rats: Histopathological and autoradiographic studies. J. Pathol. 104: 201 (1971).
27. Balow, J. E., Hurley, D. L., and Fauci, A. S. Immunosuppressive effects of glucocorticosteroids: differential effects of acute *vs* chronic administration on cell-mediated immunity. J. Immunol. 114: 1072 (1975).
28. Billingham, R. E., and Brent, L. Quantitative studies on tissue transplantation immunity. IV. Induction of tolerance in newborn mice and studies on the phenomenon of runt disease. Phil. Trans. Royal Soc. (London) 242: 54 (1959).
29. Kaplan, H. S., and Rosston, B. H. Studies on a wasting disease induced in F_1 hybrid mice injected with parental strain lymphoid cells. Standford Med. Bull. 17: 77 (1959).
30. Parrott, D. M. V. Strain variation in mortality and runt disease in mice thymectomized at birth. Transplant. Bull. 29: 102 (1962).
31. Gebhardt, B. M. and Newberne, P. M. Nutrition and immunological responsiveness. T-cell function in the offspring of lipotrope-and protein-deficient rats. Immunol. 26: 489 (1974).
32. Chandra, R. K. Fetal malnutrition and postnatal immunocompetence. Am. J. Dis. Child. 129: 450 (1975).
33. Chandra, R. K. Antibody formation in first and second generation offspring of nutritionally depreived rats. Science 190: 289 (1975).
34. Solomon, J. B. Foetal and Neonatal Immunology. North Holland, *Co.*, Amsterdam, 1971.
35. Moller, G., and Michael, G. Frequency of antigen-sensitive cells to thymus- independent antigens. Cell. Immunol. 2: 309 (1971).
36. Andersson, B., and Blomgren, H. Evidence for thymus-independent humoral antibody production in mice against polyvinylphenolidone and *E. coli* lipopolysaccharide. Cell. Immunol. 2: 411 (1971).
37. Feldmann, M., and Basten, A. The relationship between antigenic structure and the requirement for thymus-derived cells in the immune response. J. Exptl. Med. 134: 103 (1971).
38. Takahashi, T., et al. The importance of 0 and Ig bearing cells in the immune response to various antigens. J. Immunol. 107: 1520 (1971).
39. Addison, J. E. A comparison of thymus dependent and thymus independent antibody responses made by different numbers and populations of immuno-competent cells. Brit. J. Exptl. Pathol. 55: 177 (1974).
40. Leffors, M. J. The measurement of tuberculin hypersensitivity in rats. Int. Arch. Allergy 47: 570 (1974).
41. Smith, R. T. Sepcific recognition reactions at the cellular level in mouse lymphoreticular cell subpopulations. Transplant. Rev. 11: 178 (1972).