ICES Journal of Marine Science, 54: 718–725. 1997

Consequences of petrochemical ingestion and stress on the immune system of seabirds

Kenneth T. Briggs, M. Eric Gershwin, and
Daniel W. Anderson

Briggs, K. T., Gershwin, M. E., and Anderson, D. W. 1997. Consequences of petrochemical ingestion and stress on the immune system of seabirds. – ICES Journal of Marine Science, 54: 718–725.

The immune system is a target of toxicants and there is increasing awareness of the role of environmental pollutants in altering immune function. Immune suppression may constitute a previously unappreciated source of both acute and chronic impacts on seabirds affected by spilled oil. Thus, it is important to determine (a) if immunosuppression occurs, (b) its importance compared to other mechanisms of impact, (c) its timing and chronicity relative to oil ingestion and post-spill cleaning efforts, and (d) if something can, and should be, done to mitigate its effects. The published evidence concerning immune suppression among oiled seabirds is incomplete and much of it is indirect. Among oiled birds, leukocyte numbers (especially lymphocytes) are depressed in the circulation and the major lymphoid organs (spleen and bursa of Fabricius). At the same time, bone marrow hypercellularity, with an emphasis on erythropoiesis, suggests an adaptive shift from white cell to red cell production in response to haemolytic anaemia. Secondary fungal and bacterial infections, common among seabirds in rehabilitation centres, emphasize the immunosuppressive qualities of petrochemicals. Furthermore, inflammation of the gastrointestinal tract following oil ingestion leads to malabsorption of nutrients (which is immunosuppressive), damage to mucosal immune defences, and impairment of responses to certain antigens, such as those of foods. Unfortunately, direct challenge by viral or bacterial pathogens has been incorporated into very few relevant, laboratory studies: compared with experimental controls, domestic birds fed petroleum distillates and/or oil-emulsifying agents suffer greater mortality, and have depressed ability to kill or phagocytize bacterial pathogens. Cell-mediated immune mechanisms are more sensitive to the toxic effects of petrochemical ingestion than are mechanisms related to antibody production. Petrochemical ingestion produces abnormal concentrations or accelerated metabolism of adrenal corticosteroids. The same is true for birds subjected to handling stress, such as occurs during experimentation with wild birds, and during cleaning of oil-soaked birds. Corticosteroid hormones affect the immune system in many ways, including changes in numbers, and depression of function among lymphocytes. Results of the few recent studies of birds released from cleaning facilities are consistent with the notion of chronic, toxic, or immune system problems. These birds suffer higher than expected mortality rates, disappear from expected breeding and dispersal areas, and generally fail to breed for one or more years. Better long-term success might be obtained with improved assessment of immune function during captivity, and with the use of non-specific potentiators of immune function.

Key words: immune system, petroleum ingestion, seabirds, stress effects.

K. T. Briggs: Agricultural Experiment Station, University of California, Davis, California 95616, USA. M. E. Gershwin: Division of Rheumatology and Clinical Immunology, University of California, Davis, California 95616, USA. D. W. Anderson: Department of Wildlife, Fish and Conservation Biology, University of California, Davis, California 95616, USA. Correspondence to K. T. Briggs: 812 Camino Ramon Road, Danville, Calfiifornia 94562, USA; tel: +5108374264; email: ktbriggs@msn.com

Introduction

Over the past three decades much scientific and public attention has been focused on overt effects of oil spills on seabirds. These include mechanical fouling, loss of buoyancy and insulation, and a large array of inflammatory and toxic effects on internal organs (Leighton, 1991). In contrast, relatively little attention has been paid to possible damage to the immune system. This is not surprising, given that the immune system is

1054–3139/97/040718+08 $25.00/0/jm970254

Table 1. General leukocyte functions and soluble products.

Cell type	Primary functions	Primary soluble products
B lymphocyte	Production of antibody, neutralize pathogens	Immunoglobulins
T_{helper} lymphocyte	Mediates leukocyte activation and differentiation	Interleukins 2, 3, 4
$T_{suppressor}$ lymphocyte	Suppresses leukocyte activities	Various suppressive products
$T_{cytotoxic}$ lymphocyte	Kills cells infected with virus, tumour cells	Cytotoxin
Natural killer	Kills tumour cells	Cytolysin
Monocyte/macrophage	Regulates other cells, degrades and presents antigens, phagocytosis and killing of pathogens, mediates inflammation	H_2O_2, hydrolases, lysozyme, tumour necrosis factor, Interleukin 1
Eosinophil	Kills multicellular parasites	Major basic protein
Basophil	Mediates inflammation	Histamine, leukotrienes
Neutrophil/ heterophil	Phagocytosis and cytolysis	Lysozyme, myeloperoxidase, hydrolases, H_2O_2
Thrombocytes	Clotting and inflammation	Histamine, serotonin

(After Yoshida *et al.*, 1989; Griffin, 1989; Lillihoj, 1993).

distributed throughout the body, depends on myriad intercellular interactions, and is not easy to study. Additionally, immune system injury will normally result in morbidity or mortality days or weeks after mortality due to direct toxicity. However, there is increasing evidence that immune system injury probably occurs as a result of oil spills, among both wild and rehabilitated birds. This may mean that the impact of spilled oil on seabird populations is greater, and perhaps longer lasting, than previously has been appreciated. Immune suppression may also present a significant barrier to survival of oiled birds that are rehabilitated, then released (Kahn and Ryan, 1991; Burger and Fry, 1993; Collins *et al.*, 1994; Sharp, 1996; Anderson *et al.*, 1996.)

In this paper we review evidence concerning immunotoxic injury due to oil contamination of seabirds, and note the important role that may be played by captivity stress in the absence of toxicity. New areas of research are identified and two areas of the seabird cleaning/ rehabilitation process are noted where progress in dealing with immunotoxic injuries may enhance the restoration of oiled birds to normal life in the wild.

Constituents of the immune system

At the most general level, the immune system can be said to be (1) disseminated throughout the body, and to feature division of labour; (2) responsive to an extremely diverse array of organic and inorganic antigens; (3) highly integrated, through the mediation of cell–cell contact and chemical messengers between cells; and (4) possessed of memory for specific antigens. The components of a seabird's immune defences can be roughly organized into three functional groupings: those that are mediated by antibody, those that operate independently of antibody, and those mechanisms that are concerned with self-recognition and tolerance (Tizzard, 1987). In this paper, we are concerned mainly with antibody-mediated and antibody-independent mechanisms for neutralization and removal of toxins and pathogens.

Antibody is secreted in response to the presence of antigens, which are molecules recognizable by antigen receptors on the surfaces of immune system cells. Antigen receptors bind antigens and transmit signals to the cells' interior (DeFranco, 1995). Examples of antibody-mediated immunity include detection and elimination of bacterial cells and virus particles. Elimination of aged or diseased red blood cells and rejection of surgical grafts are examples of antibody-independent processes. Recognition and tolerance of self-antigens are also of tremendous importance, as is illustrated by the chaos that ensues when the processes go awry. Auto-immune diseases such as Systemic Lupus Erythematosis and Autoimmune Haemolytic Anaemia involve activation of immune defences directed at healthy tissues (which are identified by self-antigens). The immune systems are integrated by direct interaction between cells and through the action of chemical messengers, including interleukins (Lillihoj, 1993).

For many purposes, the most important constituents of the immune system are the white blood cells, or leukocytes. These include T and B lymphocytes, killer and natural killer (NK) cells, phagocytes (monocytes/ macrophages and heterophils), and other granulocytes (mast cells, basophils and eosinophils). Thrombocytes or platelets also participate in the immune defences (Table 1). White blood cells utilize the blood and lymphatic circulation to survey and gain access to almost all body tissues. While they are concentrated in the peripheral lymphoid organs (spleen; lymph nodes; and gut- and bronchial-associated lymphoid tissues, GALT and BALT, respectively), the leukocytes are constantly presented with a ''parade'' of blood-borne antigens,

including those of pathogenic micro-organisms and infected cells.

All leukocytes arise from a common ancestor in the marrow (the pluripotential stem cell), but development, differentiation and acquisition of functional capabilities differ for the different kinds of cells. The phagocytes and other granulocytes develop primarily in the bone marrow, and are aided by, but are not dependent on, antibody for recognition and destruction of their target pathogens, namely bacteria, infected cells, fungi and multicellular parasites. The persistence in the circulation of monocytes and heterophils is probably on the order of 10 to 20 h, at which time they marginate (move to small vessel walls) and migrate to tissue sites, where they are drawn by a variety of chemoattractants (lymphokines) released by other inflammatory cells. Birds appear to lack fixed, tissue-resident macrophages (the activated, effector stage of the blood monocyte), so it is difficult to estimate cell lifespan; in mammals, macrophages may live for days to months (Latimer and Meyer, 1989). Heterophils probably last only a few days before they are destroyed by other cells or are lost on mucosal surfaces.

The primary phagocytic cells, macrophages and heterophils, kill pathogens by engulfing them into phagosomes, then fusing them to vacuoles (lysosomes) containing cytolytic products such as myeloperoxidase and lysozyme (Kubes, 1993; Gordon *et al*., 1995). There is also a rapid metabolic excitation – the respiratory burst – that yields such toxic oxygen metabolites as hydrogen peroxide. Cytotoxic enzymes may also be released extracellularly. The phagocytes must overcome bacterial defences against being engulfed into phagosomes. This process is aided by opsonization, or coating of bacteria with blood-borne antibodies and complement (a series of serum proteins synthesized in macrophages, fibroblasts, and other cells, and which participate in opsonization, blood clotting, breakdown or thrombi, and changes in vascular permeability; Stabler *et al*., 1994). In addition, through release of a variety of soluble mediators such as interleukins, macrophages, in particular, are very important participants in activation of, and communication between, other leukocytes (Gordon *et al*., 1995).

In contrast, the lymphocytes begin their maturation in the marrow (and perhaps also the GALT), then emigrate for differentiation and further functional development. Lymphocytes retain the ability to mitose when appropriately stimulated. This is very important in secondary (anamnestic) response to an antigen that previously has been encountered. T lymphocytes migrate to the thymus (a pharyngeal evagination of the gut); they may also undergo differentiation in the intestines. Birds differ from mammals in that B lymphocyte maturation occurs in a distinct outpocketing of the gut, the bursa of Fabricius (McCormack and Thompson, 1990; Franceschi *et al*., 1995). Considered as the interval between mitoses, T cells of mammals survive for periods up to years, while B cells survive weeks to months (Latimer and Meyer, 1989).

T lymphocytes appear in a variety of subtypes that function as both regulatory and effector elements in the immune system, and are the core of cell-mediated immunity (Anderson and Coyle, 1994). Depending on subtype, T cells may activate or may suppress other leukocytes, secrete a variety of chemical mediators, and kill all types of pathogens. T cells recognize specific antigens (e.g. cell-surface proteins, soluble toxins) through their specific surface receptors. The antigenic repertoire is thought to be enormous; it is estimated that mammalian T cells can recognize and respond to 10^9 different antigens via variations in structure of their T cell receptors. These appear to be responsive only to processed, degraded antigen that is presented to them by antigen-presenting cells, including macrophages. B cells can respond to native, undegraded antigen.

The primary function of B lymphocytes is to produce antibodies. Antigen-mediated signals, in conjunction with cytokine (chemical attractants to mobile cells) influences from antigen-stimulated T cells, combine to direct B cells to mature, proliferate, and secrete antibodies. Antigen receptors on the avian B cell surface are probably comparable to the five major isotypes seen in mammals (immunoglobulins M, G, A, D, and E); the antibody secreted by a B cell is identical in isotype to the cell's receptor class (Banchereau *et al*., 1994; Jurd, 1994). Attachment of antibodies to antigens, whether soluble macromolecules, virus particles, single cells, or multicellular parasites, leads to antigen neutralization, lysis by complement-mediated events, or uptake by phagocytic cells. Antibodies may also provide a mode for intercellular communication by binding to endogenous or host materials and cells (Coutinho, 1995).

The preferential binding of antigen to selected lymphocyte clones results in their activation and proliferation. This process is called clonal selection (Klinman, 1994). Later contact with the same antigen results in shorter cellular response times, changes in the binding affinities of antigens to their receptors, and greatly enhanced antibody response (anamnesis).

Birds and oil contamination

From the foregoing, it should be clear that seabird immune defences involve many tissue and cell types, interacting through cell–cell contact and chemical messengers to recognize, process and eliminate pathogens and toxins. At this point we consider whether extant evidence suggests that petrochemical ingestion leads to immunotoxic injury in seabirds. The two major issues here are: (1) to determine if seabirds contacting oil at sea

(but which do not immediately die or come ashore) are at risk of immunosuppression; and (2) to determine if immunosuppression contributes to poor survival of oil-contaminated birds that are cleaned, then released back into the wild (Kahn and Ryan, 1991; Burger and Fry, 1993; Sharp, 1996; Anderson *et al*., 1996).

Toxic injury can occur at several points, such that immediate or future immune responses are abnormal: (1) there may be interruption in production and development of competent, mature immune cells by toxic actions at the level of the bone marrow, during transit of immune cells through the blood or lymphatic circulation, or as immune cells reside in secondary lymphoid organs and tissues; (2) there may be accelerated loss of immune cells through direct cell death or destruction of lymphoid tissues; (3) there may be impairment of immune cell functions; and (4) there may be loss in integration between immune cells, resulting in failure to activate cells, or runaway positive or negative feedback on cellular processes. As there are several pathways of possible immunotoxicity, we should not expect such gross measures as weights of lymphoid organs or counts of circulating immune cells to be sensitive indicators of immune system status. As is emphasized by Griffin (1989), it is necessary to focus our measurements and experiments on those aspects of the immune system that are functionally important, not just those that are easily accessible to study.

A recent review shows that information concerning immunotoxic injury to seabirds is incomplete, as yet inconclusive, and mostly indirect (coming from studies of other groups of animals; Briggs *et al*., 1996). The evidence is of two types: (a) studies of seabirds contaminated in oil spills and subsequently brought to cleaning centres; (b) laboratory studies of seabirds, other birds, and mammals. As yet, there have been no published studies of immune status among seabirds in the wild, following oil spills. The most direct data, gathered at cleaning centres, show a variety of lesions in bird kidney and liver tissues, gut mucosal damage (in both the presence and absence of intestinal parasites), osmotic and electrolyte imbalances, and haemolytic anaemia (Fry and Lowenstine, 1985; Leighton, 1991; White, 1992). Most deaths occur within a few days of oil-fouling, although anaemia persists for periods up to weeks. Body weights often decline despite regular alimentation (Kahn and Ryan, 1991), and subcutaneous and abdominal fat stores are depleted (McOrist and Lenghaus, 1992).

There is evidence of depression of leukocyte numbers (especially lymphocytes), and an adaptive shift in the bone marrow toward production of red cells (erythropoiesis) to combat the effects of anaemia (Holmes *et al*., 1979; Rocke *et al*., 1984; Leighton, 1986; McOrist and Lenghaus, 1992; White, 1992). Viral, fungal, and bacterial diseases have been reported from a wide variety of captive seabirds; many of these have appeared as clinical problems among birds undergoing cleaning and rehabilitation following oil spills (Croxall, 1979; McOrist and Lenghaus, 1992; White, 1992; Burger and Fry, 1993). The (scant) available evidence shows that birds that are cleaned, then released, suffer high mortality and have very poor subsequent reproductive success (Collins *et al*., 1994; Sharp, 1996; Anderson *et al*., 1996).

The second tier of evidence comes from laboratory experiments using various petrochemicals as treatments. These experiments generally indicate that (a) after petrochemical ingestion, the phagocytic and cytotoxic capacity of monocytes and heterophils is depressed (Rocke *et al*., 1984) and (b) the immunosuppressive effects of petrochemicals are directed more against the cell-mediated constituents of the immune system (especially T cells), rather than the antibody-mediated responses (Goldberg *et al*., 1990; Arstila *et al*., 1994). However, to the degree that T cells are inhibited, so also is the expression of secondary antibody responses that depend on T cell stimulation (Vos *et al*., 1973; Blakley *et al*., 1980; Vecchi *et al*., 1980; Reddy *et al*., 1983; Arstila et al., 1994; Kaspers *et al*., 1994; Klasing, 1994).

One of the ''gold standards'' for assessing immune system competence is measurement of *in vivo* responses to direct challenge with known pathogens. This type of study has only rarely been done with petrochemical toxicants. In one relevant experiment, mallards (*Anas platyrhynchos*) dosed with petroleum distillates and/or oil emulsifiers showed reduced ability to phagocytize and kill *Pasteurella multocida*, the agent of avian cholera (Rocke *et al*., 1984). Lymphocyte functional responses to bacterial challenge have not been reported for seabirds experimentally treated with petrochemicals.

The importance of stress and gastrointestinal injury

Two noteworthy themes emerge from these studies: the pre-eminent role of damage to the liver and gut in immune suppression, and the superimposed, immunosuppressive role of physiological stress, independent of petrochemical ingestion. Hepatocellular damage can lead to disruption in production and regulation of many blood proteins, including acute phase proteins (regulators of the inflammatory responses in the hours and days after toxic injury), and mixed-function oxidases (which mediate de-toxification). Liver injury may also disrupt production of bile and other digestive products (Fry and Lowenstine, 1985; Duffy *et al*., 1997). Furthermore, the liver is prominent in regulating metabolism of adrenal corticosteroids, which in turn mediate stress responses (see below).

Damage to the gut, described by Fry and Lowenstine (1985), Kahn and Ryan (1991), and others, is especially

important for at least four reasons. First, necrosis of the intestinal villi leads to malabsorption of nutrients, resulting in protein-calorie malnutrition. This condition has been extensively studied with domestic birds and mammals. Besides weight loss and catabolism of body protein to supply amino acid and glucose needs, there is depression of T cell function (Latshaw, 1991; Lochmiller *et al.*, 1993). B cell function appears to be spared (though production of IgG in secondary responses does seem to be suppressed in protein-restricted chickens). Malnutrition can also involve vitamin and mineral imbalances, which are well known for their immunosuppressive effects in mice and domestic birds (Chandra and Newberne, 1977; Gershwin *et al.*, 1985; Lochmiller *et al.*, 1993). In the case of zinc deficiency, this can even take the form of immune depression spanning multiple generations (Beach *et al.*, 1982). The implications of trans-generational immune suppression might be important in cases like that of the ''Exxon Valdez'' spill in Alaska, where impacted seabird populations fail to completely recover, even years after the initial impact (Warheit *et al.*, 1997).

Second, with erosion of the intestinal submucosa, there is potential loss of very large numbers of lymphocytes and damage to supporting tissues (GALT). Although no data exist for seabirds, it is noteworthy that some T cell maturation is likely to take place in the subepithelial layers. The gut lining of humans is estimated to harbour 10^{10} lymphocytes per linear metre (Kiyono *et al.*, 1990). Third, loss of the mucosal barrier permits invasion of pathogens from the gut lumen and exposure of the submucosa to a large array of foreign antigens. Fourth, as a consequence of disruption of lymphocyte populations, we can expect decreased capacity to produce secretory IgA. It is noteworthy that in humans and mice, about 60% to 90% of the immense numbers of gut-associated lymphocytes are B cells committed to production of IgA (Kiyono *et al.*, 1990). This isotype is secreted into the lumen of the gut, where it coats bacterial pathogens such as *Escherichia coli*, thus preventing adherence to the gut wall and facilitating mass flushing (Lillihoj, 1991; Giannasca and Neutra, 1994). Killing of the bacteria that manage to penetrate the mucosa is mostly due to the action of phagocytes, NK cells and cytotoxic T cells (Stabler *et al.*, 1994). No controlled experiments have been carried out wherein seabird responses to gut-associated pathogens are compared for normal vs. oil-dosed birds.

These findings suggest that there will be a dose-related immune suppression among all seabirds that ingest petrochemicals at sea, during preening on land, or prior to being presented for cleaning and rehabilitation. The chronicity of these effects is unknown, but if long-lasting, this might influence morbidity and mortality in areas far removed from a spill.

Immunosuppressive effects of stress

When seabirds become contaminated with petrochemicals, the ocean environment becomes very stressful to the birds. There is the documented loss of buoyancy and insulation, birds are hampered in feeding, and there may be multiple encounters with people and spill cleaning equipment. For the birds undergoing cleaning and rehabilitation, we can add to the list of stressors an unfamiliar diet, unusual thermal and light conditions, crowding, handling, and cleaning itself (White, 1992). Much has been written about the immunosuppressive effects of physiological stress, particularly the role of increased concentrations or rapid metabolism of adrenal corticosteroids. Corticosteroids are induced by ingestion of petrochemicals (Lillihoj and Shevach, 1985; Compto *et al.*, 1987; Isobe and Lillihoj, 1992) and by the stress of handling and captivity alone (Fowler *et al.*, 1995; Holberton *et al.*, 1996). Among the effects of corticosteroid hormones are destruction and redistribution of lymphocytes, blockage of lymphocyte activation (especially T cells), changes in responses to food antigens (affecting nitrogen balance and food sensitivity), and changes in metabolism of fat and protein (Griffin, 1989; Dohms and Metz, 1991). The effects of corticosteroids are so pervasive that, when working with seabirds, it seems mandatory that we fully understand the immune functions of captive, healthy (but stressed) birds, before we can have much confidence in laboratory or clinical studies involving petrochemical toxicity.

Future prospects

Taking the available information together, we conclude that there are numerous theoretical reasons for supposing that petrochemical ingestion induces immune suppression among seabirds. However, the critical experiments using seabird models (experiments characterized by appropriate controls, analysis of dose-dependent responses, and in which there are controls for captivity stress) have yet to be done. Ideally, this should include: (1) determination of peracute (very short-term, immediate) responses to captivity/handling; (2) effects of captivity over periods up to several weeks; (3) effects of cleaning protocols in the absence of toxic injury; and (4) assessment of immune function among birds in the wild, following a spill. Until this occurs, we will have little idea whether immunosuppression does indeed occur, its magnitude and chronicity in the laboratory and in the wild, or whether stress of captivity and cleaning is more important than the supposed toxic injury itself.

Much of the debate about the efficacy of seabird rehabilitation after oil spills seems to depend on which criterion of ''success'' is adopted. If release of oiled birds from the cleaning facility is adopted as the criterion, the 50%+ releases of cleaned birds after the ''Exxon

Table 2. Non-specific immunomodulating agents of potential use in treatment of immunotoxic injury due to ingestion of petrochemicals in seabirds. (Names of commercial products are in parentheses.)

Treatment	Action	References
$Interferon_{alpha}$	Antiviral	Kruth (1995)
Complex carbohydrates (Acemannan)	Induces macrophage production of interleukins	Kruth (1995)
Bacterial slurries (Immunoregulin)	Induces macrophage production of interleukins	Kruth (1995)
Levamisol	T cell potentiation	Taki and Schwartz (1994)
Serratia marescens (extract)	Induces macrophage production of interleukins	Kruth (1995)
Recombinant granulocyte stimulating factor (e.g. Neupogen)	Stimulates granulocyte cell line production in marrow	Coe *et al.* (1993); Kruth (1995)
HMB	Protein-sparing	Nissen *et al.* (1994); Gatnau *et al.* (1995)

Valdez'' and ''American Trader'' oil spills are, indeed, impressive (Sharp, 1996). If, on the other hand, one chooses to assess survival to one or more years post-release, or evaluates successful reintegration of cleaned birds into the wild breeding population, then cleaning operations seem to be fruitless endeavours (Sharp, 1996). Clearly, the decision to engage in cleaning and rehabilitation is a societal one, but one subject to much future debate.

If, however, we assume that well-intentioned people will continue to engage in cleaning of contaminated birds after oil spills, then we should try to maximize success at all levels. We believe that two initiatives should be pursued in regard to potential immune system injury. The first of these is improvement of clinical evaluation of immune competency. The idea is that birds should not be released from cleaning facilities until appropriate measures of immune function have returned to norms characteristic of birds in the wild. Certainly, the serial measurement of circulating immune cell population levels is important, as is monitoring of blood chemistry (White, 1992). It is also important to begin routine monitoring of corticosteroid levels and to focus new attention on acute phase proteins, such as haptoglobin and fibrinogen, as measures of ongoing immune suppression and ongoing inflammation, respectively (Fowler *et al.*, 1995; Duffy *et al.*, 1997).

There are several direct measures of immune competence that might be employed as part of routine monitoring protocols. The ease of testing and potential usefulness of cutaneous hypersensitivity reactions make them well suited to this task. In this type of test, a small amount of mitogenic compound (such as the plant lectin, phytohaemagglutinin, or PHA) is injected into an easily accessible spot (e.g. the wing web), and the degree of inflammation is assessed hours or days later (often by measurement of the raised plaque and by cytological examination of infiltrated cells). Little handling of the birds is required. This is a powerful, *in vivo* test of integrated immunocyte responses: the mitogenic capacity of T cells, cellular integration via cytokine production, and mobility and ''homing'' abilities of monocytes and other cells are all involved (Latshaw, 1991; Lochmiller *et al.*, 1993).

The second aspect of treatment protocols that should be considered is the use of immunomodulators (immunopotentiators). A number of non-specific potentiators have been developed in human and veterinary practice to boost the performance of immune cells (Table 2). Among these are treatments that specifically stimulate production of interleukins by macrophages, treatments that are specifically virucidal (interferons), and drugs that stimulate the performance of T cells (Kruth, 1995). For example, one metabolite of dietary leucine, HMB, has been shown to improve survival and growth in a variety of domestic birds and mammals under the stress of intensive production husbandry (Nissen *et al.*, 1994). Note that administration of such response modulators as interleukins cloned from other species is probably not worthwhile, due to formation of anti-IL antibodies in the treated individual. In contrast, administration of interferons appear to elicit much less response, while potentiating anti-viral capabilities (Coe *et al.*, 1993; Kruth, 1995).

In our view, the laboratory study of immunosuppression in seabirds exposed to petrochemicals should be undertaken in parallel with studies involving clinical monitoring and immunopotentiation. Work in these three areas should be viewed as complementary and synergistic. Taken together, they will improve our ability to understand, predict and mitigate the effects of marine oil spills.

References

Anderson, G. P. and Coyle, A. J. 1994. TH2 and ''TH2-like'' cells in allergy and asthma pharmacological perspectives. Trends in Pharmacological Science, 15: 324–332.

Anderson, D. W., Gress F., and Fry, D. M. 1996. Behaviour and disappearance of brown pelicans after oiling, rehabilitation and release. Marine Pollution Bulletin, 32: 711–718.

Arstila, T. P., Vainio, O., and Lassila, O. 1994. Central role of CD4+T cells in avian immune responses. Poultry Science, 73: 1019–1026.

Banchereau, J., Briere, F., Liu, T. J., and Rousset, F. 1994. Molecular control of B lymphocyte growth and differentiation. Stem Cells, 12: 278–288.

Beach, R. S., Gershwin, M. E., and Hurley, L. S. 1982. Gestational zinc deprivation in mice: persistence of immunodeficiency for three generations. Science, 218: 469–471.

Blakley, B. R., Sisodia, C. S., and Mukkur, T. K. 1980. The effects of methylmercury, tetraethyl lead and sodium arenite on the humoral immune response in mice. Toxicology and Applied Pharmacology, 52: 245–254.

Briggs, K. T., Yoshida, S. H., and Gershwin, M. E. 1996. The influence of petrochemicals and stress on the immune system of seabirds. Regulatory Toxicology and Pharmacology, 23: 145–155.

Burger, A. E. and Fry, D. M. 1993. Effects of oil pollution on seabirds in the northeast Pacific. *In* The status, ecology and conservation of marine and shoreline birds of the North Pacific, pp. 254–263. Ed. by K. Vermeer, K. T. Briggs, K. H. Morgan, and D. Seigel-Causey. Special Publication of the Canadian Wildlife Service, Ottawa. 263 pp.

Chandra, R. K. and Newberne, P. M. 1977. Nutrition, immunity and infection mechanisms and interactions. Plenum Press, New York. 246 pp.

Coe, N. E., Frand, D. E., and Roth, J. A. 1993. Effect of recombinant human cytokines on porcine neutrophil function. Veterinary Immunology and Immunopathology, 37: 39–47.

Collins, R., Brazier, H., and Whelan, J. 1994. Rehabilitating a herd of oiled mute swans (*Cygnus olor*). Proceedings of the Royal Irish Academy, 1: 83–89.

Compto, M. M., Caron, L. M., and Cidlowski, J. A. 1987. Glucocorticoid action on the immune system. Journal of Steroid Biochemistry, 27: 201–208.

Coutinho, A. 1995. The network theory: 21 years later [editorial]. Scandinavian Journal of Immunology, 42: 3–8.

Croxall, J. 1979. Birds and oil pollution. *In* First aid and care of wild birds, pp. 174–191. Ed. by J. E. Cooper and J. T. Eley. David and Charles, Newton Abbot, London.

DeFranco, A. L. 1995. Transmembrane signaling by antigen receptors of B and T lymphocytes. Current Opinions in Cell Biology, 7: 163–175.

Dohms, J. E. and Metz, A. 1991. Stress – mechanisms of immunosuppression. Veterinary Immunology and Immunopathology, 30: 89–109.

Duffy, L. K., Bowyer, R. T., Testa, J. W., and Faro, J. B. 1997. Acute phase proteins and cytokines in Alaskan mammals as markers of chronic exposure to environmental pollutants. Symposium of the American Fisheries Society (in press).

Fowler, G. S., Wingfield, J. C., and Boersma, P. D. 1995. Hormonal and reproductive effects of low levels of petroleum fouling in magellanic penguins (*Speniscus magellanicus*). Auk, 112: 382–389.

Francheschi, C., Monti, D., Sansoni, P., and Cossarizza, A. 1995. The immunology of exceptional individuals: the lessons of centenarians. Immunology Today, 13: 12–16.

Fry, D. M. and Lowenstine, L. J. 1985. Pathology of common murres and Cassin's auklets exposed to oil. Bulletin of Environmental Contaminants and Toxicology, 14: 723–737.

Gatnau, R., Zimmerman, D. R., Nissen, S. L., Wannemuehler, M., and Ewan, R. C. 1995. Effects of excess dietary leucine and leucine catabolites on growth and immune responses in weanling pigs. Journal of Animal Science, 73: 159–165.

Gershwin, M. E., Beach, R. S., and Hurley, L. S. 1985. Nutrition and immunity. Academic Press, Orlando.

Giannasca, P. J. and Neutra, M. R. 1994. Interactions of microorganisms with intestinal M cells: mucosal invasion and induction of secretory immunity. Infectious Agents and Disease, 2: 242–248.

Goldberg, D. R., Yuill, T. M., and Burgess, E. C. 1990. Mortality from duck plague virus in immunosuppressed adult mallard ducks. Journal of Wildlife Disease, 26: 299–306.

Gordon, S., Clarke, S., Greaves, D., and Doyle, A. 1995. Molecular immunobiology of macrophages: recent progress. Current Opinions in Immunology, 7: 24–33.

Griffin, J. F. T. 1989. Stress and immunity: a unifying concept. Veterinary Immunology and Immunopathology, 20: 263–312.

Holberton, R. L., Parrish, J. D., and Wingfield, J. C. 1996. Modulation of the adrenocortical stress response in neotropical migrants during autumn migration. Auk, 113: 558–564.

Holmes, W. N., Gorsline, J., and Cronshaw, J. 1979. Effects of mild cold stress on the survival of seawater adapted mallard ducks (*Anas platyrhynchos*) maintained on food contaminated with petroleum. Environmental Research, 20: 425–444.

Isobe, T. and Lillehoj, H. S. 1992. Effects of corticosteroids on lymphocyte subpopulations and lymphokine secretion in chickens. Avian Diseases, 36: 590–596.

Jurd, R. D. 1994. Reptiles and birds. *In* Immunology. A comparative approach, pp. 137–172. Ed. by R. J. Turner. John Wiley and Sons, Chichester.

Kahn, R. A. and Ryan, P. 1991. Long-term effects of crude oil on common murres (*Uria aalge*) following rehabilitation. Bulletin of Environmental Contaminants and Toxicology, 46: 216–222.

Kaspers, B., Lillehoj, H. S., Jenkins, M. C., and Pharr, G. T. 1994. Chicken interferon-mediated induction of major histocompatibility complex class II antigens on peripheral blood monocytes. Veterinary Immunology and Immunopathology, 44: 71–84.

Kiyono, H., Taguchi, T., Aicher, W. K., Baegley, K. W., Fugihashi, K., Eldbridge, J. H., and McGee, J. R. 1990. Immunoregulatory confluences: T cells, Fc receptors and cytokines for IgA responses. International Reviews in Immunology, 6: 263–273.

Klasing, K. C. 1994. Avian leukocytic cytokines. Poultry Science, 73: 1035–1043.

Klinman, N. R. 1994. Selection in the expression of functionally distinct B-cell subsets. Current Opinions in Immunology, 6: 420–424.

Kruth, S. A. 1995. Cytokines and biologic response modifiers in small animal practice. *In* Kirk's current veterinary therapy XII. Small animal practice, pp. 547–554. Ed. by J. D. Bonagura. W. B. Saunders, Philadelphia. 1520 pp.

Kubes, P. 1993. Polymorphonuclear leukocyte-endothelium interactions: a role for pro-inflammatory and anti-inflammatory molecules. Canadian Journal of Physiology and Pharmacology, 71: 88–97.

Latimer, K. S. and Meyer, D. J. 1989. Leukocytes in health and disease. *In* Textbook of veterinary internal medicine, pp. 2181–2284. Ed. by S. J. Ettinger. W. B. Saunders, Philadelphia. 2399 pp.

Latshaw, J. D. 1991. Nutrition – mechanisms of immunosuppression. Veterinary Immunology and Immunopathology, 30: 111–120.

Leighton, F. A. 1986. Clinical, gross and histological findings in herring gulls and Atlantic puffins that ingest Prudhoe Bay crude oil. Veterinary Pathology, 23: 254–263.

Leighton, F. A. 1991. The toxicity of petroleum oils to birds: an overview. *In* The effects of oil on wildlife: research, rehabilitation and general concerns, pp. 43–57. Ed. by J. White and L. Frink. Sheridan Press, Hanover, PA.

Lillihoj, H. S. 1991. Avian gut associated immune system: implication in coccidial vaccine development. Poultry Science, 72: 1306–1311.

Lillihoj, H. S. 1993. Avian interleukin-2 and interferon. *In* Avian immunology in progress, pp. 105–111. Ed. by INRA, Paris.

Lillihoj, H. S. and Shevach, E. M. 1985. A comparison of the effects of cyclosporin A, dexamethasone and ouabain on the Interleukin 2 cascade. Journal of Immunopharmacology, 7: 267–284.

Lochmiller, R. L., Vestey, M. R., and Boren, J. C. 1993. Relationship between protein nutritional status and immunocompetence in northern bobwhite chicks. Auk, 110: 503–510.

McCormack, W. T. and Thompson, C. B. 1990. Somatic diversification of the chicken immunoglobulin light chain. Advances in Immunology, 48: 41–67.

McOrist, S. and Lenghaus, C. 1992. Mortalities of little penguins (*Eudyptula minor*) following exposure to crude oil. Veterinary Record, 130: 161–162.

Nissen, S., Fuller, J. C., Jr, Sell, J., Ferket, P. R., and Rives, D. V. 1994. The effect of B-hydroxy-B-methylbutarate on growth, mortality and carcass qualities of broiler chickens. Poultry Science, 73: 137–155.

Reddy, R. V., Sharma, R. P., and Taylor, M. J. 1983. Dose and time related response of immunologic functions to aflatoxin B1 in mice. *In* Developments in the science and practice of toxicology, pp. 431–434. Ed. by A. W. Hayes, R. C. Schnell, and T. S. Miya. Elsevier, Amsterdam.

Rocke, T. E., Yuill, T. M., and Hinsdill, R. D. 1984. Oil and related toxicant effects on mallard immune defenses. Environmental Research, 33: 343–352.

Sharp, B. E. 1996. Post-release survival of oiled, cleaned seabirds in North America. Ibis, 138: 222–238.

Stabler, J. G., McCormick, T. W., Powell, K. C., and Kogut, M. H. 1994. Avian heterophils and monocytes: phagocytic and bactericidal activities against *Salmonella enteritidis.* Veterinary Microbiology, 38: 293–305.

Taki, H. N. and Schwartz, S. A. 1994. Levamisole as an immunopotentiator for T cell deficiency. Immunopharmacology and Immunotoxicology, 16: 129–137.

Tizzard, I. 1987. Veterinary immunology. W. B. Saunders, New York. 401 pp.

Vecchi, E. S., Mantovani, A., Sironi, M., Luini, W., Spreafico, F., and Garattini, S. 1980. The effect of acute administration of 2.3.7.8-tetrachlorodibenzo-p-dioxin on humoral antibody production and cell-mediated activities in mice. Archives of Toxicology, S4: 163–165.

Vos, J. G., Moore, J. A., and Zinkle, J. G. 1973. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the immune system of laboratory animals. Environmental Health Perspectives, 5: 149–162.

Warheit, K. I., Harrison, C. S., and Divoky, G. J. 1997. ''Exxon Valdez'' oil spill restoration workshop. Technical Publication 1 of the Pacific Seabird Group (in press).

White, J. 1992. Protocol for the rehabilitation of oil-affected waterbirds. *In* Proceedings of the oil spill and wildlife emergency response conference, pp. 1–11. University of California, Davis.

Yoshida, S., Golub, M. S., and Gershwin, M. E. 1989. Immunological aspects of toxicology: premises not promises. Regulatory Toxicology and Pharmacology, 9: 56–80.