Immunological Parameters: What Do They Mean?[1,2]

Philip C. Calder*

Institute of Human Nutrition, School of Medicine, University of Southampton, Southampton SO16 7PX, United Kingdom

Abstract

The immune system acts to protect the host from infectious agents that exist in the environment and from other noxious insults. It is constantly active, acting to discriminate ''nonself'' from ''self.'' The immune system has 2 functional divisions: the innate and the acquired. Both involve various blood-borne factors and cells. A number of methodologies exist to assess aspects of immune function; many of these rely on studying cells in culture ex vivo. There are large interindividual variations in many immune functions even among the healthy. Many factors, including genetics, gender, age, nutrient status, and gut flora, contribute to the observed variation. Individuals with immune responses significantly below ''normal'' are more susceptible to infectious agents and exhibit increased infectious morbidity and mortality. However, it is not clear how the variation in immune function among healthy individuals relates to variation in susceptibility to infection. J. Nutr. 137: 773S–780S, 2007.

The immune system

Components of the immune system. The immune system acts to protect the host from infectious agents that exist in the environment (bacteria, viruses, fungi, parasites) and from other noxious insults. It serves to distinguish "nonself" from "self." In addition, the immune system plays an important role in the identification and elimination of tumor cells and in the response to injury and trauma. Thus, an effective and efficient immune system is central to host defense against infectious diseases and cancer. The immune system responds to challenge (e.g., a bacterial infection) with an increase in the activity of certain components that act in a coordinated fashion to eliminate the source of that challenge. The human immune system has the capacity to respond to millions of antigens (the precise peptide components that are recognized as "nonself" by the immune system and that trigger an immune response). The immune system has 2 functional divisions: the innate (or natural) and the acquired (also termed specific or adaptive). Both components of immunity involve various blood-borne factors and cells (**Table 1**). All cells of the immune system originate in bone marrow. They are found circulating in the bloodstream, organized into lymphoid organs such as the thymus, spleen, lymph nodes, and gut-associated lymphoid tissue, or dispersed in other locations around the body. The immune response to infection will include both innate and acquired actions that will involve a variety of cell types, mediators, and chemical agents. The exact nature of the response will depend on the origin and nature of the antigen (and so the origin and nature of the challenge). It is known that individuals with defective immune responses are more susceptible to infections and are more likely to suffer from infectious morbidity and mortality. Furthermore, it is known that improvements in the immune responses of such individuals can decrease susceptibility to infections and decrease infectious morbidity and mortality.

Innate immunity. Innate immunity is the first line of defense against infectious agents. It is present before exposure to pathogens and is concerned with preventing the entry of infectious agents into the body and, if they do enter, with their rapid elimination. The innate immune system includes physical barriers, soluble factors, and phagocytic cells (Table 1). Innate immunity has no memory and is therefore not influenced by prior exposure to an organism. Phagocytic cells express surface receptors specific for bacteria or their components (e.g., lipopolysaccharide), such as toll-like receptors. Binding of ligand to the receptors triggers phagocytosis and subsequent destruction of the pathogenic microorganism by complement or by toxic chemicals, such as superoxide radicals and hydrogen peroxide. Natural killer cells also possess surface receptors and destroy pathogens by release of cytotoxic proteins. In this way, innate immunity provides a first line of defense against invading pathogens. However, an immune response often requires the coordinated actions of both innate immunity and the more powerful and flexible acquired immunity (**Fig. 1**).

[1] Published as a supplement to *The Journal of Nutrition*. The articles included in this supplement are derived from presentations and discussions at the World Dairy Summit 2003 of the International Dairy Federation (IDF) in a joint IDF/FAO symposium entitled ''Effects of Probiotics and Prebiotics on Health Maintenance—Critical Evaluation of the Evidence,'' held in Bruges, Belgium. The articles in this publication were revised in April 2006 to include additional relevant and timely information, including citations to recent research on the topics discussed. The guest editors for the supplement publication are Michael de Vrese and J. Schrezenmeir. *Guest Editor disclosure*: M. de Vrese and J. Schrezenmeir have no conflict of interest in terms of finances or current grants received from the IDF. J. Schrezenmeir is the IDF observer for Codex Alimentarius without financial interest. The editors have received grants or compensation for services, such as lectures, from the following companies that market pro- and prebiotics: Bauer, Danone, Danisco, Ch. Hansen, Merck, Müller Milch, Morinaga, Nestec, Nutricia, Orafti, Valio, and Yakult.

[2] Author disclosure: no relationships to disclose.

* To whom correspondence should be addressed. E-mail: pcc@soton.ac.uk.

TABLE 1 Components of the innate and acquired immune systems

	Innate immunity	Acquired immunity
Physicochemical barriers	Skin	Cutaneous and mucosal immune systems
	Mucosal membranes	Antibodies in mucosal secretions
	Lysozyme	
	Stomach acid	
	Commensal bacteria in gut	
Circulating molecules	Complement	Antibodies
Cells	Granulocytes	B lymphocytes
	Monocytes/macrophages	T lymphocytes
	Natural killer cells	
Soluble mediators	Macrophage-derived cytokines	Lymphocyte-derived cytokines

Acquired immunity. The acquired immune response involves lymphocytes. It is highly specific because each lymphocyte carries surface receptors for a single antigen. The acquired immune response becomes effective over several days after the initial activation, but it also persists for some time after the removal of the initiating antigen. This persistence gives rise to immunological memory, which is the basis for a stronger, more effective immune response on reexposure to an antigen (i.e., reinfection with the same pathogen).

B lymphocytes are characterized by their ability to produce antibodies [immunoglobulins (Ig)],[3] which are specific for an individual antigen. Antibodies work in several ways to combat invading pathogens. They can "neutralize" microorganisms by binding to them and preventing their attachment to host cells, and they can activate complement proteins in plasma, which in turn promote the destruction of bacteria by phagocytes. Immunity involving antibodies (humoral immunity) deals with extracellular pathogens. However, some pathogens, particularly viruses but some bacteria as well, infect individuals by entering cells. These pathogens will escape humoral immunity and are instead dealt with by cell-mediated immunity, which is conferred by T lymphocytes. T lymphocytes express antigen-specific T-cell receptors on their surface. However, unlike B lymphocytes, they are able to recognize only those antigens that are "presented" to them on a cell surface (by antigen-presenting cells); this is the distinguishing feature between humoral and cell-mediated immunity. Therefore, infection of a cell by an intracellular pathogen is signaled to T lymphocytes by cell surface expression of peptide fragments derived from the pathogen. These fragments are transported to the surface of the infected cell and expressed there in conjunction with proteins termed the major histocompatibility complex (MHC); in humans the MHC is termed human leukocyte antigen (HLA). It is the combination of the pathogen-derived peptide fragment bound to MHC that is recognized by T lymphocytes. Intracellular pathogens stimulate cytotoxic T lymphocytes to destroy the infected cell, whereas extracellular pathogens stimulate a Th-mediated response. In delayed-type hypersensitivity (DTH), antigen-activated $CD4^+$ T lymphocytes (Th cells) secrete cytokines, which have several effects, including recruitment of neutrophils and monocytes from the blood to the site of antigen challenge and activation of monocytes to effect elimination of the antigen.

[3] Abbreviations used: DTH, delayed-type hypersensitivity; HLA, human leukocyte antogen; IFN, interferon; Ig, immunoglobulin; IL, interleukin; MHC, major histocompatibility complex; Th, helper T lymphocyte.

Communication within the immune system. Communication within the acquired immune system and between the innate and acquired systems is brought about by direct cell-to-cell contact involving cell surface proteins (e.g., adhesion molecules) and by the production of chemical messengers, which send signals from 1 cell to another (Fig. 1). Chief among these chemical messengers are proteins called cytokines, which can act to regulate the activity of the cell that produced the cytokine or of other cells. Each cytokine can have multiple activities on different cell types. Cytokines act by binding to specific receptors on the cell surface and thereby induce changes in growth, development, or activity of the target cell. Tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6 are among the most important cytokines produced by monocytes and macrophages. These cytokines activate neutrophils, monocytes, and macrophages to initiate bacterial and tumor cell killing, increase adhesion molecule expression on the surface of neutrophils and endothelial cells, stimulate T- and B-lymphocyte proliferation, and initiate the production of other proinflammatory cytokines. Thus, TNF, IL-1, and IL-6 are mediators of both natural and acquired immunity and are an important link between them (Fig. 1). In addition, these cytokines mediate the systemic effects of inflammation such as fever, weight loss, and acute-phase protein synthesis in the liver. Inflammation is the body's immediate response to infection or injury and is an integral part of the innate immune response. Thus, production of appropriate amounts of TNF, IL-1, and IL-6 is important in response to infection. However, inappropriate production or overproduction can be dangerous, and these cytokines, particularly TNF, are implicated in causing some of the pathological responses that occur in acute and chronic inflammatory conditions.

One useful paradigm to describe the regulation of the immune response is the Th1-Th2 paradigm (1). This describes the differentiation of the Th cell, defined by the appearance of the protein CD4 on its surface, along 1 of 2 phenotypic pathways (**Fig. 2**). The pathway followed depends on the nature of the antigen being presented to the undifferentiated Th cell, and the cytokines present. An intracellular antigen, which will result from the phagocytic uptake of a bacterium, and the presence of IL-12, produced by the antigen-presenting cell, will promote differentiation along the Th1 pathway (Fig. 2). The Th1 cells thus produced will generate IL-2 and interferon (IFN)-γ. IL-2 will promote the proliferation of antigen-specific T lymphocytes, and IFN-γ will activate the cells involved in elimination of bacteria, viruses, fungi, and tumor cells (e.g., monocytes, macrophages, cytotoxic T lymphocytes, natural killer cells) and will promote the generation of antigen-specific IgG2 by B lymphocytes. Such antibodies can coat pathogens to facilitate their recognition and uptake by phagocytes. Extracellular antigens produced in response to, for example, infection with helminthic worms, and the presence of IL-4 will promote differentiation along the Th2 pathway (Fig. 2). The Th2 cells thus produced will generate IL-4, IL-5, and IL-13 (among other cytokines). IL-4 will promote the generation of antigen-specific IgE by B lymphocytes. The IgE will bind to and cross-link IgE receptors on the surface of mast cells and induce the extracellular release of the contents of mast cell granules. IL-5 promotes the activation of eosinophils. Mast cells and eosinophils are the mediators of host defense against extracellular parasites. Thus, Th1 and Th2 cells are involved in promoting an appropriate immune response to different types of infectious organisms. The final element of the Th1, Th2 paradigm is cross-regulation: IFN-γ inhibits the differentiation and activity of Th2 cells, whereas IL-4 inhibits the differentiation and activity of Th1 cells (Fig. 2). Clearly this

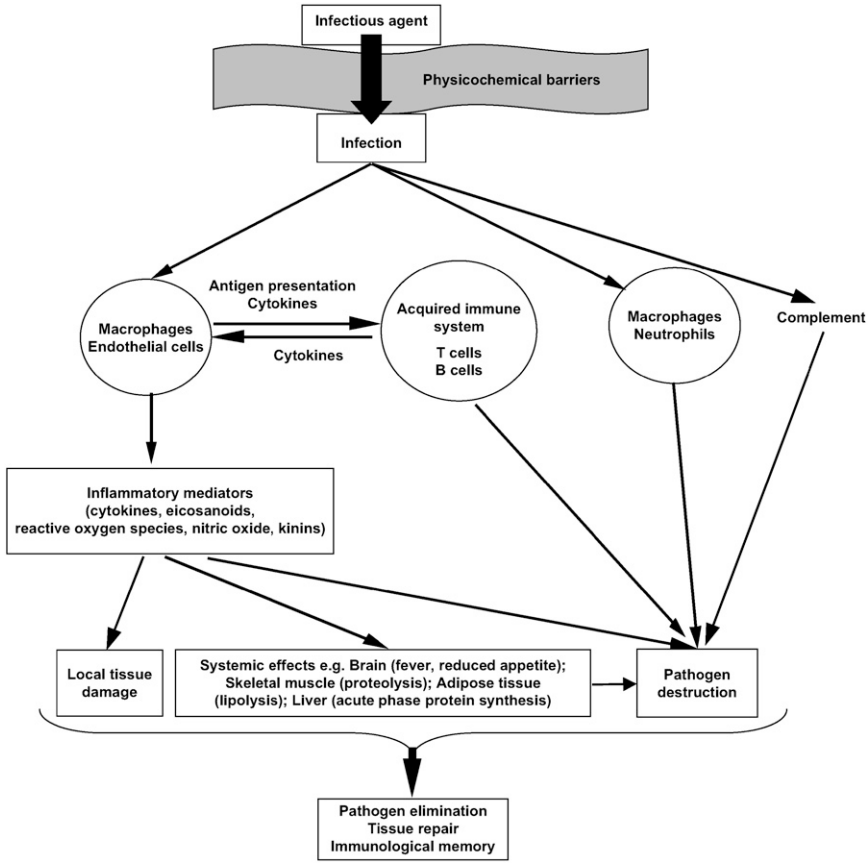

Figure 1 A general overview of the immune system.

serves to cause a transient "skewing" of the immune response while an infectious agent is present. However, once the source of the infection is eliminated, the system will return to the basal state in preparation for any subsequent infections.

The immune system in disease. Although the capacity to mount vigorous Th1- or Th2-type responses is central to effective host defense against the range of possible infectious organisms, a number of human diseases are associated with inappropriate activation or activity of the immune response. These diseases appear to be associated with an inherent, inappropriate "skewing" toward either a Th1- or Th2-type response. There is a genetic predisposition toward such skewing, with susceptibility genes frequently being associated with antigen presentation (2). It is likely that the combination of genetic predisposition and certain environmental and/or lifestyle factors allows the disease to manifest itself. In some individuals the immune system responds to presentation of a host antigen (or a normally benign

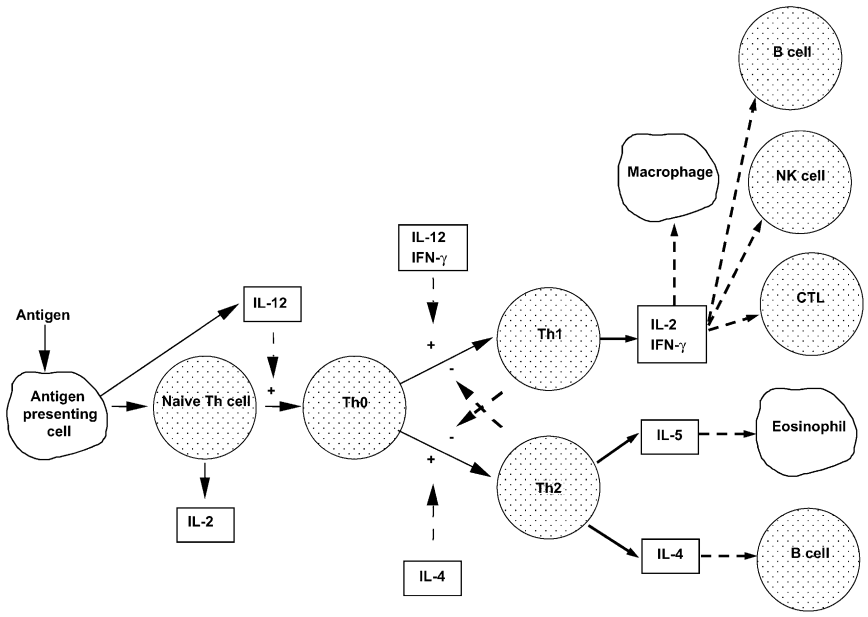

Figure 2 The Th1-Th2 paradigm. IFN, interferon; IL, interleukin; Th, helper T lymphocyte; > indicates produces;–→ indicates acts on.

foreign one) rather than a foreign antigen; this triggers a Th1-type response with the destructive actions of the immune system targeted at those body sites where the antigen (or a similar one) is expressed. An example of such a disease is rheumatoid arthritis. In rheumatoid arthritis susceptibility genes map to antigen presentation proteins, and this somehow results in host antigen being presented to T cells, which differentiate along the Th1 path. The Th1-type cytokines promote the activity of macrophages and the generation of autoantibodies by B lymphocytes (**Fig. 3**). The Th1-type cytokines also suppress the Th2-type response and so skew the system toward the Th1 phenotype (3). The host's synovial joints become infiltrated by activated T cells, macrophages, and B cells. The macrophages produce a range of cytokines including TNF-α, IL-1, and IL-6 (4). Although these cytokines have a physiological role in innate immunity, in activating cellular immune responses, and in coordinating the whole-body response to infection and injury, in excess they are involved in causing a range of local and systemic responses that are detrimental to the host. Macrophages will also produce the matrix metalloproteases that degrade the cartilage and bone of the host. Other diseases in which an inappropriate Th1-type response appears to be involved include Crohn's disease, multiple sclerosis, and atherosclerosis. Together theyes are termed chronic inflammatory diseases.

An inappropriate predisposition to skewing toward a Th2 response also occurs. Here the antigens involved are frequently normally benign foreign antigens, such as those present in cow's milk, egg, house dust mite, or tree or grass pollen and are referred to as allergens because they can trigger allergy in sensitized individuals (5). The Th2 response results in production of allergen-specific IgE, which, when and where allergen is present, will induce mast cell degranulation, and in activation of eosinophils (**Fig. 4**). This process can be termed allergic inflammation.

Biomarkers of immune function

General comments. There is a wide range of methodologies available with which to assess the status and functional capacity of the immune system, but there is no single marker of either its status or functional capacity. The activity of many of the separate components of the immune system can be measured, most frequently by studying that component under controlled ex vivo (i.e., outside of the body) conditions. It is also possible to study a coordinated immune response in vivo, usually to a controlled challenge (e.g., vaccination, intradermal application of an antigen). Animal studies can investigate the functional responses of immune cells isolated from the blood, thymus, spleen, lymph nodes, gut-associated lymphoid tissue, peritoneal cavity, and, in some cases, from the bone marrow, lungs, and liver. Human studies are often limited by the ability to sample only blood and external secretions such as saliva, although in some experimental settings it is possible to take biopsies of the gut, which may include immune tissue, or to collect bronchoalveolar lavage fluid. In most human settings, circulating cell numbers, their activation state, and their responses to ex vivo challenge can be measured. However, it must be remembered that the majority of immune cells are not in the bloodstream; for example, only 2% of total lymphocytes are circulating at any given time. Normal ranges have been established for circulating immune cell numbers and circulating Ig concentrations, but there are no normal ranges for immune cell functional responses.

Assessment of immune status. Assessments of immune status most frequently involve the measurement of various leukocyte numbers in the bloodstream (and the size and cellularity of lymphoid organs, where accessible). The total number of leukocytes and of the subclasses of white cells (e.g., neutrophils, monocytes, lymphocytes, T lymphocytes, B lymphocytes, $CD4^+$ cells, $CD8^+$ cells, natural killer cells) in the circulation can be determined using immunological staining procedures and associated analytical techniques such as flow cytometry (6–9). Because "white cell counts" are used clinically, normal ranges have been identified (10). In addition to total cell numbers, the percentage contribution of each class to the total is sometimes used (11–13). By combining antibody "stains" it is possible to obtain great detail about the subtypes of cells present (8,9,11–13). The ratio of $CD4^+$ to $CD8^+$ cells is often reported as a measure of the relative numbers of Th and T-suppressor/cytotoxic cells, but the true meaning of this ratio is unclear. The ratio of memory to naive cells (CD45RO:CD45RA) can be determined; this is an indicator of long-term activation of the immune system.

In animal studies the thymus, spleen, and lymph nodes can be removed and weighed. In human studies thymus size can be estimated by imaging techniques. This approach has been used to identify differences among infants on different feeding regimens (14) and to show an increase in malnourished children given oral zinc (15).

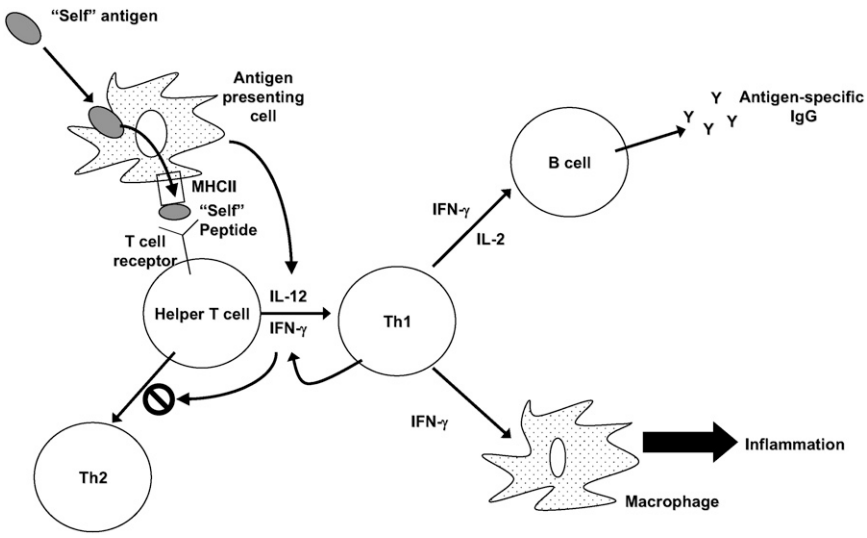

Figure 3 A generalized view of Th1-mediated disease processes. Self antigen is processed and presented to autoreactive T cells, which then differentiate toward a Th1-type phenotype. Th1-type cytokines promote production of self antigen-specific IgG and production of inflammatory cytokines by macrophages. IFN, interferon; IL, interleukin; MHCII, major histocompatibility complex II; Th, helper T lymphocyte; Y, immunoglobulins.

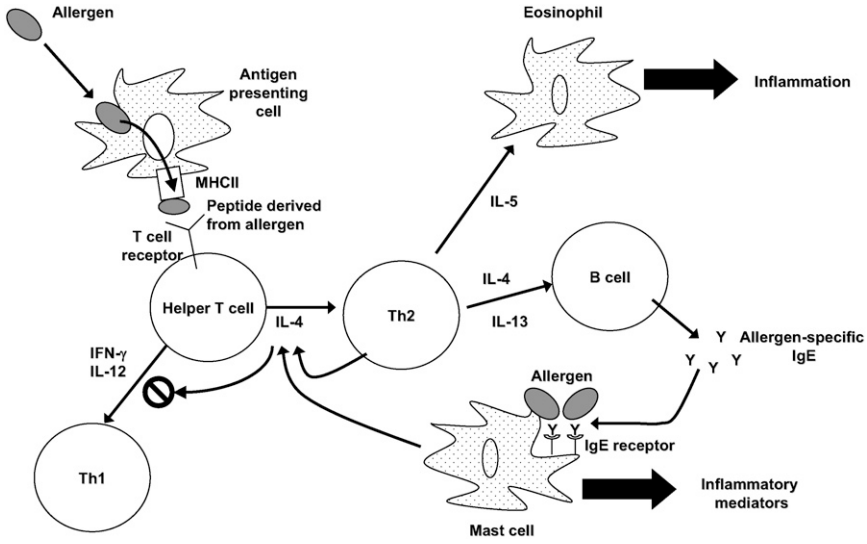

Figure 4 A generalized view of Th2-mediated disease processes. Allergen is processed and presented to T cells, which then differentiate toward a Th2-type phenotype. Th2-type cytokines (especially IL-4) promote production of allergen-specific IgE, which binds to and cross-links IgE receptors on mast cells, resulting in release of inflammatory mediators. Th2-type cytokines (especially IL-5) promote eosinophil activation. IFN, interferon; IL, interleukin; MHCII, major histocompatibility complex II; Th, helper T lymphocyte; Y, immunoglobulins.

Assessment of the functional activity and capacity of the immune response. Assessments of the functional capacity of the immune response can be made by measuring specific cell functions ex vivo (i.e., of the cells isolated and studied in short- or long-term culture), measuring in vivo responses to challenge (e.g., by measuring the changes in the concentrations of antibodies in the bloodstream or saliva or by measuring the physical response to administration of antigen), or measuring the incidence and severity of infections. In animal studies, resistance to challenge with live pathogens can be used; the outcome is usually survival, and this can be coupled with some of the above in vivo and ex vivo measures and with measures of the numbers of pathogens that are found in various organs (e.g., spleen, lymph nodes, liver). Human studies are largely restricted to naturally occurring infectious episodes, particularly in settings where these are likely to occur at a high rate (16,17).

When measures are made of immune function (either in vivo or ex vivo), it must be remembered that the responses being measured are dynamic in nature. Thus, the absolute response measured may be different at different time points. Furthermore, different responses may follow different time courses. Immune responses are related to the concentration of the stimulant used to trigger those responses in a dose-dependent fashion. Thus, the absolute response and the timing of that response will depend on the concentration of the stimulus used.

Ex vivo measures. Ex vivo measures allow the functional responses of specific immune cell types (e.g., neutrophils, monocytes, T lymphocytes, B lymphocytes, natural killer cells) to be determined. The following ex vivo measurements are possible:

Phagocytosis by neutrophils and monocytes. Substrates for phagocytosis include bacteria, sheep red blood cells, and yeast particles; these can be studied in the opsonized (i.e., complement- or antibody-coated) and unopsonized states. Some techniques (e.g., flow cytometry) allow identification of both the number of cells participating in phagocytosis and the phagocytic activity per cell (9,18–22). Measures of phagocytosis can be coupled to measures of oxidative burst (9,20–22); bacterial phagocytosis measurements can be coupled to measures of bacterial killing.

Oxidative (respiratory) burst (superoxide generation) by neutrophils and monocytes. Stimuli to induce respiratory burst include bacteria and protein kinase C activators such as phorbol esters (e.g., phorbol myristyl acetate). Some techniques (e.g., flow cytometry) allow identification of both the number of cells participating in oxidative burst and the activity per cell (9,20–22). Oxidative burst measurements can be coupled with measures of bacterial killing. Production of other reactive species such as hydrogen peroxide can also be made (23).

Chemotactic response of neutrophils or monocytes. This is the movement of these cells toward particular stimuli; stimuli used include leukotriene B_4, bacterial cell wall peptides such as formyl-methionyl-leucyl-phenylalanine, IL-8, and autologous serum (23–25).

Natural killer cell activity. This is measured as killing of tumor cells known to be specific targets for natural killer cells (e.g., K562 cells) (8,19,26–30). Killing can be expressed in various ways, such as percentage target cells killed or "lytic ratio," which is the ratio of killer to target cells required to kill a particular percentage (e.g., 25% or 50%) of target cells.

Cytotoxic T lymphocyte activity. This is measured as killing of virally infected cells known to be specific targets for cytotoxic T cells (e.g., P815 cells).

Lymphocyte proliferation. This is the increase in number of lymphocytes in response to a stimulus. Most often this is measured as the incorporation of radioactively labeled thymidine into the DNA of the dividing lymphocytes (6,7,11,12,20,21,27,28,31,32), although a number of other measures, not involving the use of radioactivity, are available. For example, it is possible to measure the incorporation of bromodeoxyuridine into DNA of proliferating cells. Agents used to stimulate lymphocyte proliferation include concanavalin A, phytohemagglutinin, and anti-CD3, which stimulate T lymphocytes; pokeweed mitogen, which stimulates a mixture of T and B lymphocytes; and bacterial lipopolysaccharide, which stimulates B lymphocytes. These agents are all known as mitogens, and the process as mitogen-stimulated lymphocyte proliferation. Most often, T-cell mitogens are used. If the individual has been sensitized to an antigen (or allergen), then the antigen (or allergen) can be used to stimulate lymphocyte proliferation (33). The proliferative response to mitogens or antibodies is much greater than that to an antigen or allergen (33). This is because mitogenic stimulation is nonspecific and will target a large proportion, perhaps all, of the T or B cells in a cell preparation. In contrast, antigenic stimulation is highly specific and targets those few cells that will recognize the antigen. Vaccines can also be used to stimulate ex vivo lymphocyte proliferation (34,35).

Production of cytokines by lymphocytes and monocytes. This usually requires the cells to be stimulated. For lymphocytes, mitogens (or antigens, if the individual has been sensitized to them) are used, whereas for monocytes, bacterial lipopolysaccharide is most often used. Cytokine protein concentrations in the cell culture medium are most frequently measured by ELISA (7,9,11,12,20–22,27,28,30,32,36,37), although flow cytometry–based techniques have now become available that allow multiple cytokines to be measured simultaneously (13). However, cellular mRNA levels can also be measured (38). Flow cytometry can also be used to measure the intracellular concentration of cytokine protein (39). This technique also allows the relative number of cytokine-producing cells to be identified and, if combined with other immunological stains, the type of cells producing the cytokine (39). The production of Th1- and Th2-type cytokines by isolated lymphocytes can be used to indicate the balance between the 2 types of response. IFN-γ is frequently used as a marker for the Th1-type response. IL-4 has sometimes been used as a marker for the Th2-type response, but IL-4 is often produced in low amounts and only after prolonged periods in culture. IL-5 is an alternative to IL-4.

Production of Ig by lymphocytes. This involves measurement of total or antigen-specific immunoglobulins by ELISA following stimulation with antigens and reflects B-cell activity.

Cell surface expression of molecules involved in bacterial recognition (e.g., toll-like recptors), antigen presentation (e.g., HLA subtypes) (40), and in cellular activation (e.g., cytokine receptors, CD69) (13) after stimulation. Stimulants used can include mitogens or antigens. Cell surface expression is most frequently determined by flow cytometry after immunological staining (13,40). The percentage of cells expressing the molecule and the average level of expression per cell can both be determined (40). If combined with other immunological stains, the type of cell expressing the molecule can be identified (13).

By definition, ex vivo measures require that cell functions be studied outside of the environment in which they normally occur, i.e., within the body. Ex vivo cell responses may not be the same as those observed in the more complex in vivo situation. This effect may be exaggerated by studying cells in increasingly purified states. Thus, measurements of cell function made in whole blood may be more similar to those seen in vivo than functions measured using purified cell preparations. Whole-blood systems retain all blood components (including plasma), and they are kept at the same ratios at which they exist in vivo; by definition cell purification removes many blood components. However, 1 advantage of using purified cells for measuring some ex vivo functional responses (e.g., lymphocyte proliferation, cytokine production, antibody production) is that the number of cells cultured can be carefully controlled; this may not be the case where whole blood is cultured.

In vivo measures. The following in vivo measurements are possible:

Circulating concentrations of total Ig and of the Ig subclasses. Measurements are made by ELISA or a similar method (21,41). In the absence of an "immune challenge" these measurements are probably not very useful.

Circulating concentrations of Ig specific for antigens after an antigen challenge of some sort (e.g., inoculation with a vaccine such as those to hepatitis B, influenza, or Pneumococcus*).* Vaccination responses are very useful because they represent a coordinated, integrated immune response to a relevant challenge. Polysaccharide vaccines such as *Pneumococcus* initiate T-cell-independent B-cell responses, whereas hepatitis B elicits T-cell-dependent responses. Depending on prior vaccination, primary and secondary antibody responses can be followed (32,34,35,42,43). The time course of the antibody response varies according the vaccine used.

Concentration of secretory IgA in saliva and tears. Total and antigen-specific secretory IgA can be measured (41). This can be a useful measure of mucosal immune responses.

Circulating concentrations of cytokines or of soluble cytokine receptors. Measurements are made with ELISA (often requiring highly sensitive assays) (44,45). The source of the cytokines measured is not known, and this limits the usefulness of these measurements.

DTH response to intradermal application of an antigen to which the individual has already been exposed; this measures the cell-mediated immune response and is often, inappropriately, referred to as a "skin test." The response is measured as the size of the reaction (termed induration) around the area of application at a period (usually 48 h) after the application (20,21,42). This measurement is useful because it represents a coordinated, integrated cell-mediated immune response to a relevant challenge. However, the test cannot be repeated on the same area of skin, and recent vaccination may interfere with the outcome.

There is wide variation in immune responses among individuals. Even when highly standardized experimental conditions are used, there are wide variations among individuals in all in vivo and ex vivo measurements of immune responses (46–48). Some of this variation is likely caused by factors such as age, gender, smoking status, obesity, dietary habits, acute and chronic effects of exercise, acute and chronic consumption of alcohol, pregnancy, etc. (47,49,50). Nevertheless, even when as many of these factors as possible are standardized, significant variation remains (46–50). Genetic polymorphisms, early life events, hormone status, and gut flora may be additional factors contributing to such variation (2,47,49,50).

What is the biological significance of differences in immune function?

It is known that individuals with defective immune responses are more susceptible to infections and are more likely to suffer from infectious morbidity and mortality. Furthermore, it is known that improvements in the immune responses of such individuals can decrease susceptibility to infections and decrease infectious morbidity and mortality. However, individuals with truly defective immune responses represent an extreme, and it is not clear to what extent variations in immune responses among apparently healthy individuals contribute to variations in susceptibility to infectious agents. Indeed, relatively small differences, decreases or increases, in indicators of immune function may not be relevant to host defense. There are 2 main reasons for this. First, there is significant redundancy in the immune system, such that a change in the functional capacity of 1 component of the immune response may be compensated for by a change in the functional capacity of another component. Second, there may be "excess" capacity in some immune functional responses, particularly those that are measured ex vivo by challenging the cells with a high concentration of stimulant. Thus, individuals with immune functions in the "normal" range may not benefit from increased immune function. In other words, increasing the activity of 1 or more components of the immune system may not necessarily be of any benefit to the individual, just as decreasing

the activity of 1 or more components of the immune system may not necessarily be detrimental to the individual. However, a sufficiently large variation or change in some immune functions has been related to improved host defense. For example, individuals with low natural killer cell activity have increased risk of cancer (51) and of mortality (52). Likewise, a poor DTH response is associated with increased mortality in surgery patients (53–55) and in cancer patients (56), with progression to AIDS in HIV-infected individuals (57), with increased risk of upper respiratory tract infections (58,59), and with progression of diarrhea in Bangladeshi children (60). These observations suggest that improvements in some immune responses might be associated with improved clinical outcome and better health.

Literature Cited

1. Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today. 1996;17:138–46.
2. Howell WM, Calder PC, Grimble RF. Gene polymorphisms, inflammatory disease and cancer. Proc Nutr Soc. 2002;61:447–56.
3. Panayi GS. Targetting of cells involved in the pathogenesis of rheumatoid arthritis. Rheumatology. 1999;38: Suppl 2:8–10.
4. Feldman M, Maini RN. The role of cytokines in the progression of rheumatoid arthritis. Rheumatology. 1999;38: Suppl 2:3–7.
5. Romagnani S. The role of lymphocytes in allergic disease. J Allergy Clin Immunol. 2000;105:399–408.
6. Meydani SN, Ribaya-Mercado JD, Russell RM, Sahyoun N, Morrow FD, Gershoff SN. Vitamin B6 deficiency impairs interleukin-2 production and lymphocyte proliferation in elderly adults. Am J Clin Nutr. 1991;53:1275–80.
7. Meydani SN, Endres S, Woods MM, Goldin BR, Soo C, Morrill-Labrode A, Dinarello C, Gorbach SL. Oral (n-3) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation:comparison between young and older women. J Nutr. 1991;121:547–55.
8. Thies F, Nebe-von-Caron G, Powell JR, Yaqoob P, Newsholme EA, Calder PC. Dietary supplementation with eicosapentaenoic acid, but not with other long chain n-3 or n-6 polyunsaturated fatty acids, decreases natural killer cell activity in healthy subjects aged > 55 years. Am J Clin Nutr. 2001;73:539–48.
9. Thies F, Miles EA, Nebe-von-Caron G, Powell JR, Hurst TL, Newsholme EA, Calder PC. Influence of dietary supplementation with long chain n-3 or n-6 polyunsaturated fatty acids on blood inflammatory cell populations and functions and on plasma soluble adhesion molecules in healthy adults. Lipids. 2001;36:1183–93.
10. MacLennon ICM, Drayson MT. Normal lymphocytes and non-neoplastic lymphocyte disorders. In: Hoffbrand AV, Lewis SM, Tuddenham EGC, editors. Postgraduate haematology. 4th ed. Oxford: Butterworth Heinemann; 1999. p. 111–21.
11. Thies F, Nebe-von-Caron G, Powell JR, Yaqoob P, Newsholme EA, Calder PC. Dietary supplementation with γ-linolenic acid or fish oil decreases T lymphocyte proliferation in healthy older humans. J Nutr. 2001;131:1918–27.
12. Wallace FA, Miles EA, Calder PC. Comparison of the effects of linseed oil and different doses of fish oil on mononuclear cell function in healthy human subjects. Br J Nutr. 2003;89:679–89.
13. Tricon S, Burdge GC, Kew S, Banerjee T, Russell JJ, Grimble RF, Williams CM, Calder PC, Yaqoob P. Effects of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 conjugated linoleic acid on immune cell function in healthy humans. Am J Clin Nutr. 2004;80:1626–33.
14. Hasselbalch H, Jeppesen DL, Engelmann MDM, Michaelson KF, Nielsen MB. Decreased thymus size in formula-fed infants compared with breastfed infants. Acta Paediatr. 1996;85:1029–32.
15. Golden MHN, Jackson AA, Golden BE. Effect of zinc on thymus of recently malnourished children. Lancet. 1977;2:1057–9.
16. Graat JM, Schouten EG, Kok FJ. Effect of daily vitamin E and multivitamin-mineral supplementation on acute respiratory tract infections in elderly persons: a randomized controlled trial. JAMA. 2002;288:715–21.
17. Meydani SN, Leka LS, Fine BC, Dallal GE, Keusch GT, Singh MF, Hamer DH. Vitamin E and respiratory tract infections in elderly nursing home residents: a randomized controlled trial. JAMA. 2004;292:828–36.
18. Arunachalam K, Gill HS, Chandra RK. Enhancement of natural immune function by dietary consumption of Bifidobacterium lactis (HN019). Eur J Clin Nutr. 2000;54:263–7.
19. Gill HS, Rutherfurd KJ, Cross ML, Gopal PK. Enhancement of immunity in the elderly by dietary supplementation with the probiotic *Bifidobacterium lactis* HN019. Am J Clin Nutr. 2001;74:833–9.
20. Kew S, Banerjee T, Minihane AM, Finnegan YE, Muggli R, Albers R, Williams CM, Calder PC. Lack of effect of foods enriched with plant- or marine-derived n-3 fatty acids on human immune function. Am J Clin Nutr. 2003;77:1287–95.
21. Miles EA, Banerjee T, Dooper MWBW, M'Rabet L, Graus YMF, Calder PC. The influence of different combinations of γ-linolenic acid, stearidonic acid and EPA on immune function in healthy young male subjects. Br J Nutr. 2004;91:893–903.
22. Rees D, Miles EA, Banerjee T, Wells SJ, Roynette CE, Wahle KWJW, Calder PC. Dose-related effects of eicosapoentaenoic acid on innate immune function in healthy humans: a comparison of young and older men. Am J Clin Nutr. 2006;83:331–42.
23. Walrand S, Moreau K, Caldefie F, Tridon A, Chassagne J, Portefaix G, Cynober L, Beaufrere B, Vasson MP, Boirie Y. Specific and nonspecific immune responses to fasting and refeeding differ in healthy young adult and elderly persons. Am J Clin Nutr. 2001;74:670–8.
24. Schmidt EB, Pedersen JO, Ekelund S, Grunnet N, Jersild C, Dyerberg J. Cod liver oil inhibits neutrophil and monocyte chemotaxis in healthy males. Atherosclerosis. 1989;77:53–7.
25. Schmidt EB, Varming K, Pedersen JO, Lervang HH, Grunnet N, Jersild C, Dyerberg J. Long term supplementation with n-3 fatty acids. ii. effect on neutrophil and monocyte chemotaxis. Scand J Clin Lab Invest. 1992;52:229–36.
26. Santos MS, Meydani SN, Leka L, Wu D, Fotouhi N, Meydani M, Hennekens CH, Gaziano JM. Natural killer cell activity in elderly men is enhanced by beta-carotene supplementation. Am J Clin Nutr. 1996;64:772–7.
27. Watzl B, Bub A, Blockhaus M, Herbert BM, Luhrmann PM, Neuhauser-Berthold M, Rechkemmer G. Prolonged tomato juice consumption has no effect on cell-mediated immunity of well-nourished elderly men and women. J Nutr. 2000;130:1719–23.
28. Yaqoob P, Pala HS, Cortina-Borja M, Newsholme EA, Calder PC. Encapsulated fish oil enriched in α-tocopherol alters plasma phospholipid and mononuclear cell fatty acid compositions but not mononuclear cell functions. Eur J Clin Invest. 2000;30:260–74.
29. Gill HS, Rutherfurd KJ, Cross ML. Dietary probiotic supplementation enhances natural killer cell activity in the elderly: an investigation of age-related immunological changes. J Clin Immunol. 2001;21:264–71.
30. Miles EA, Banerjee T, Wells SJ, Calder PC. Limited effect of eicosapentaenoic acid on T-lymphocyte and natural killer cell numbers and functions in healthy young males. Nutrition. 2006;22:512–9.
31. Kelley DS, Dougherty RM, Branch LB, Taylor PC, Iacono JM. Concentration of dietary n-6 polyunsaturated fatty acids and the human immune status. Clin Immunol Immunopathol. 1992;62:240–4.
32. Albers R, van der Wielen RPJ, Brink EJ, Hendriks HFJ, Dorovska-Taran VN, Mohede ICM. Effects of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 conjugated linoleic acid (CLA) isomers on immune function in healthy men. Eur J Clin Nutr. 2003;57:595–603.
33. Miles EA, Warner JA, Jones AC, Colwell BM, Bryant TN, Warner JO. Peripheral blood mononuclear cell proliferative responses in the first year of life in babies born to allergic parents. Clin Exp Allergy. 1996;26:780–8.
34. Kelley DS, Taylor PC, Rudolph IL, Benito P, Nelson GJ, Mackey BE, Erickson KL. Dietary conjugated linoleic acid did not alter immune status in young healthy women. Lipids. 2000;35:1065–71.
35. Broome CS, McArdle F, Kyle JA, Andrews F, Lowe NM, Hart CA, Arthur JR, Jackson MJ. An increase in selenium intake improves immune function and poliovirus handling in adults with marginal selenium status. Am J Clin Nutr. 2004;80:154–62.
36. Beck FWJ, Prasad AS, Kaplan J, Fitzgeral JT, Brewer GJ. Changes in cytokine production and T cell subpopulations in experimentally induced zinc deficient humans. Am J Physiol. 1997;272:E1002–7.
37. Miles EA, Bakewell L, Calder PC. Production of lymphocyte-derived cytokines by whole umbilical cord blood cultures stimulated with mitogens and allergens. Cytokine. 2003;21:74–83.
38. Baumann KH, Hessel F, Larass I, Muller T, Angerer P, Kiefl R, von Schacky C. Dietary ω-3, ω-6, and ω-9 unsaturated fatty acids and growth factor and cytokine gene expression in unstimulated and stimulated monocytes. Arterioscler Thromb Vasc Biol. 1999;19:59–66.

39. Kelley DS, Simon VA, Taylor PC, Rudolph IL, Benito P, Nelson GJ, Mackey BE, Erickson KL. Dietary supplementation with conjugated linoleic acid increased its concentration in human peripheral blood mononuclear cells but did not affect their function. Lipids. 2001;36: 669–74.
40. Hughes DA, Pinder AC, Piper Z, Johnson IT, Lund EK. Fish oil supplementation inhibits the expression of major histocompatibility complex class II molecules and adhesion molecules on human monocytes. Am J Clin Nutr. 1996;63:267–72.
41. Kelley DS, Branch LB, Love JE, Taylor PC, Rivera Y, Iacono JM. Dietary alpha-linolenic acid and immunocompetance in humans. Am J Clin Nutr. 1991;53:40–6.
42. Meydani SN, Meydani M, Blumberg JB, Leka LS, Siber G, Loszewski R, Thompson C, Pedrosa MC, Diamond RD, Stollar BD. Vitamin E supplementation and in vivo immune response in healthy subjects. JAMA. 1997;277:1380–6.
43. McDade TW, Beck MA, Kuzawa C, Adair LS. Prenatal undernutrition, postnatal environments, and antibody response to vaccination in adolescence. Am J Clin Nutr. 2001;74:543–8.
44. Blok WL, Deslypere J-P, Demacker PNM, van der Ven-Jongekrijg J, Hectors MPC, van der Meer JMW, Katan MB. Pro- and anti-inflammatory cytokines in healthy volunteers fed various doses of fish oil for 1 year. Eur J Clin Invest. 1997;27:1003–8.
45. Gallai V, Sarchielli P, Trequattrini A, Franceschini M, Floridi A, Firenze C, Alberti A, Di Benedetto D, Stragliotto E. Cytokine secretion and eicosanoid production in the peripheral blood mononuclear cells of MS patients undergoing dietary supplementation with n-3 polyunsaturated fatty acids. J Neuroimmunol. 1995;56:143–53.
46. Yaqoob P, Newsholme EA, Calder PC. Comparison of cytokine production in cultures of whole human blood and purified mononuclear cells. Cytokine. 1999;11:600–5.
47. Calder PC, Kew S. The immune system: a target for functional foods? Br J Nutr. 2002;88:S165–76.
48. Kew S, Banerjee T, Minihane AM, Finnegan YE, Williams CM, Calder PC. Relationship between the fatty acid composition of peripheral blood mononuclear cells and measures of immune function in healthy, free-living subjects aged 25 to 72 y. Am J Clin Nutr. 2003;77:1278–86.
49. Cummings JH, Antoine J-M, Aspiroz F, Bourdet-Sicard R, Brandtzaeg P, Calder PC, Gibson GR, Guarner F, Isolauri E, et al. PASSCLAIM—Gut health and immunity. Eur J Nutr. 2004;43: Suppl 2:II-118–II-173.
50. Albers R, Antoine J-M, Bourdet-Sicard R, Calder PC, Gleeson M, Lesourd B, Samartin S, Sanderson IR, van Loo J, et al. Markers to measure immunomodulation in human nutrition intervention studies. Br J Nutr. 2005;94:452–81.
51. Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K. Natural cytotoxic activity of peripheral blood lymphocytes and cancer incidence: an 11 year follow-up study of a general population. Lancet. 2000;356:1795–9.
52. Ogata K, An E, Shioi Y, Nakamura K, Luo S, Yokose N, Minami S, Dan K. Association between natural killer cell activity and infection in immunologically normal elderly people. Clin Exp Immunol. 2001;124: 392–7.
53. Bradley JA, Ledingham IM, Hamilton DN. Assessment of host resistance in critically ill surgical patients by the response to recall skin antigens. Intensive Care Med. 1981;7:105–8.
54. MacLean LD. Delayed type hypersensitivity testing in surgical patients. Surg Gynecol Obstet. 1988;166:285–93.
55. Christou NV, Meakins JL, Gordon J, Yee J, Hassan-Zahraee M, Nohr CW, Shizgal HM, MacLean LD. The delayed hypersensitivity response and host resistance in surgical patients. 20 years later. Ann Surg. 1995;222:534–46.
56. Aziz M, Akhtar S, Malik A. Evaluation of cell-mediated immunity and circulating immune complexes as prognostic indicators in cancer patients. Cancer Detect Prev. 1998;22:87–99.
57. Blatt SP, Hendrix CW, Butzin CA, Freeman TM, Ward WW, Hensley RE, Melcher GP, Donovan DJ, Boswell RN. Delayed-type hypersensitivity skin testing predicts progression to AIDS in HIV-infected patients. Ann Intern Med. 1993;119:177–84.
58. Gordin FM, Hartigan PM, Klimas NG, Zolla-Pazner SB, Simberkoff MS, Hamilton JD. Delayed-type hypersensitivity skin tests are an independent predictor of human immunodeficiency virus disease progression. Department of Veterans Affairs Cooperative Study Group. J Infect Dis. 1994;169:893–7.
59. Zaman K, Baqui AH, Yunus M, Sack RB, Chowdhury HR, Black RE. Malnutrition, cell-mediated immune deficiency and acute upper respiratory infections in rural Bangladeshi children. Acta Paediatr. 1997;86: 923–7.
60. Azim T, Islam LN, Sarker MS, Ahmad SM, Hamadani JD, Faruque SM, Salam MA. Immune response of Bangladeshi children with acute diarrhea who subsequently have persistent diarrhea. J Pediatr Gastroenterol Nutr. 2000;31:528–35.