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SYMPOSIUM ON 'NUTRITION AND IMMUNOLOGY'

The immunological system in health and malnutrition

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Introduction

Host defenses rely upon non-specific factors of resistance as well as upon specific immunological responses. The non-specific factors include a complex of seemingly unrelated phenomena such as endocrine functions, serum iron, and the ability to mount appropriate febrile and leukocytosis responses. On the other hand, the immunological system is a reasonably well-defined series of lymphoid tissues that are responsible for the production of antibodies and cell-mediated immunity (CMI). Immune responses are also amplified through the mediation of several other systems such as blood complement, and both specific and non-specific factors participate in the generation of adequate inflammatory responses.

One of the most important functions of the immune system is protection from infectious diseases. This point is demonstrated by the increased incidence of infections in patients with immunodeficiency diseases (Cooper, Faulk, Fudenberg, Good, Hitzig, Kunkel, Rosen, Seligmann, Soothill & Wedgewood, 1974). Persons suffering from malnutrition also sustain many infections, suggesting that a type of immunodeficiency may result from suboptimal nutrition. This concept has recently received considerable attention, and studies of the immune response have begun to piece together a picture of immunological function in malnutrition (Douglas & Schopfer, 1976). The normal immunological system is composed of at least two different types of lymphocytes. These cells are produced in central lymphoid tissues, and during their maturation and diversification they are seeded to peripheral lymphoid tissues where they function as the bulwark of host defense. This paper will briefly describe the immunological system, and several examples will be presented to show the effects of malnutrition on tissues of the normal immune system.

The immune system

The immune system consists of two lymphoid components, both apparently arising from the same stem-cell series. One of these arises in the thymus and the other in the bone marrow. Lymphocytes of thymus origin are called T-cells (T for thymus). They are responsible for many CMI reactions such as graft rejections, delayed hypersensitivity, and cytotoxicity. Lymphocytes of bone marrow origin are called B-cells (B for bone marrow) and they are responsible for immunoglobulin (Ig) and antibody production. T- and B-cells and their products, along with macrophages, complement, and certain non-specific factors of resistance, are responsible for host defenses against infections. The T- and B-cells work together in healthy persons to produce an adequate immune response to most infections, but some infections, such as pneumococcal pneumonia, are relatively more dependent on adequate B-cell function, and other infections, such as tuberculosis, are relatively more dependent on T-cell function. This division of labour within the immune system has been revealed largely as a result of extensive studies on patients suffering from primary immunodeficiency diseases (Good, Finstad & Gatti, 1970).

T-cells can be quantified according to their ability to form rosettes with sheep erythrocytes, and one aspect of their function can be measured by their proliferative response to phytohaemagglutinin (PHA), a plant glycoprotein that has a non-specific mitogenic effect on T-cells. The B-cells can be quantified according to their ability to form rosettes with either antibody-coated or antibody-and-complement-coated sheep erythrocytes. They can also be measured by membrane immunofluorescence, because they bear Ig on their cell membranes, and the type of Ig can be determined by using fluoresceinated class-specific antisera. Since B-cells produce antibodies and all antibodies are Ig, one of the most commonly used measurements of B-cell function is simply to estimate serum Ig levels, or to measure the production of antibody subsequent to antigenic stimulation.

Histopathological considerations

T- and B-lymphocytes are produced in the central lymphoid organs of the immune system, the thymus and bone marrow, and are seeded to peripheral lymphoid organs such as lymph nodes, spleen, and lymphoid aggregates in the gastrointestinal tract. Both the central and peripheral organs of the immune system demonstrate morphological and histopathological evidence of damage during malnutrition (Schonland, 1972). For example, thymus glands from healthy 4-month-old children weigh about 25 g, but thymuses from children of comparable age with malnutrition weigh less than 10 g, frequently about 1 g or even less. In addition, thymuses from healthy neonates during the first several days of life weigh about 20 g, but those from neonates who sustain intrauterine malnutrition also reveal morphological alterations and reduction in weight. Histological examination of the thymus of a normal child reveals lobules that are well demarcated by thin connective–vascular fascia, and each lobule is differentiated into medullary and cortical regions (Plate 1a). The medullary region contains large lymphocytes and

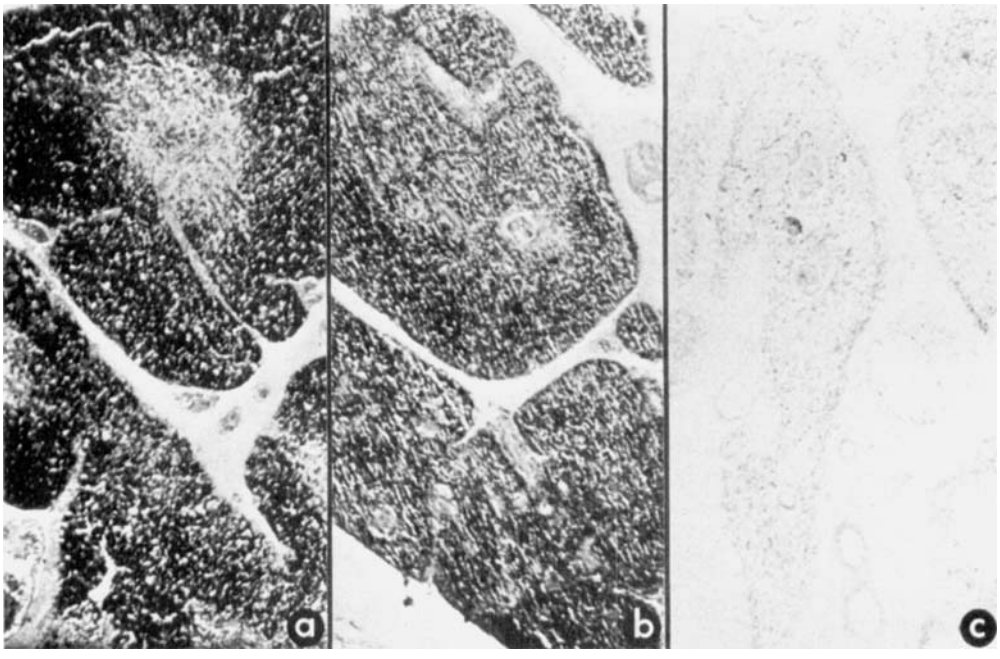

Plate 1. Human thymus in health and malnutrition. (a) Normal thymus: note demarcation between cortex (dark-staining) and medulla (light-staining). Hassall's corpuscles are found in the medulla, and the thymic cortical cells exhibit intense mitotic activity. (b) Thymus from child with intermediate malnutrition: note loss of cortico-medullary differentiation. (c) Thymus from child with severe protein-energy malnutrition: note absence of lymphoid cells and increased interlobular spaces containing connective tissue elements. (The tissues in Plates 1–4 are from children of the same age and sex.) Stained with haematoxylin–eosin (×35).

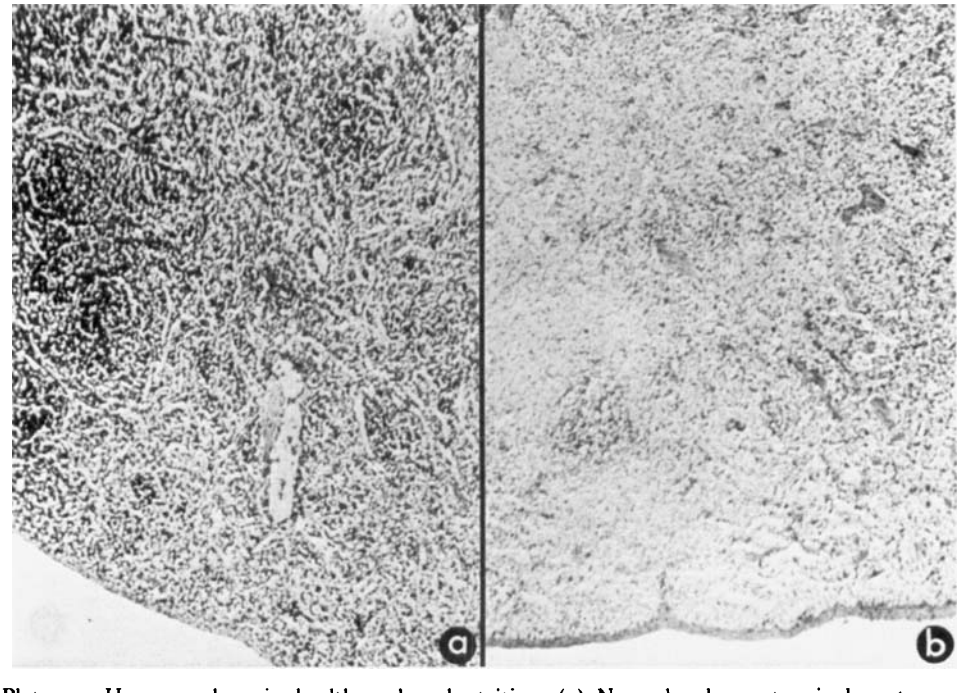

Plate 2. Human spleen in health and malnutrition. (a) Normal spleen: germinal centers are thymus-independent and periarterial lymphatic sheaths of white pulp are thymus-dependent. (b) Spleen from child with severe malnutrition: note absence of periarterial lymphatic-sheath and sparse germinal centers. Stained with haematoxylin–eosin (×35).

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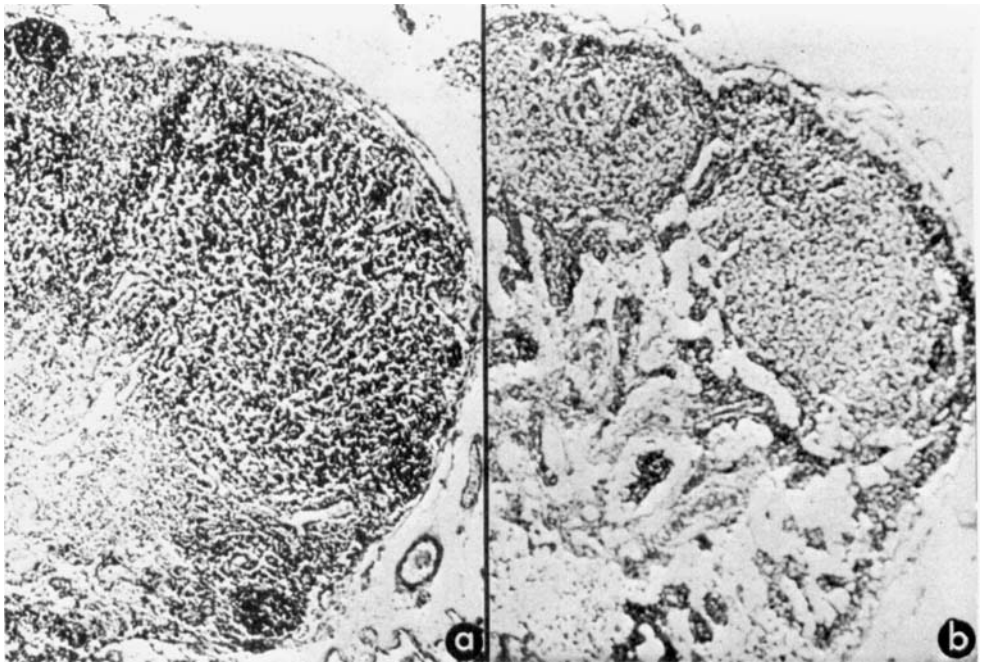

Plate 3. Human lymph node in health and malnutrition. (a) Normal lymph node: germinal centers of cortex and cells of medullary cords are thymus-independent and lymphocytes of the paracortex are thymus-dependent. (b) Lymph node from child with severe malnutrition: note that the node is virtually empty of lymphocytes and that there is an increase in connective tissue elements. Stained with haematoxylin–eosin (×35).

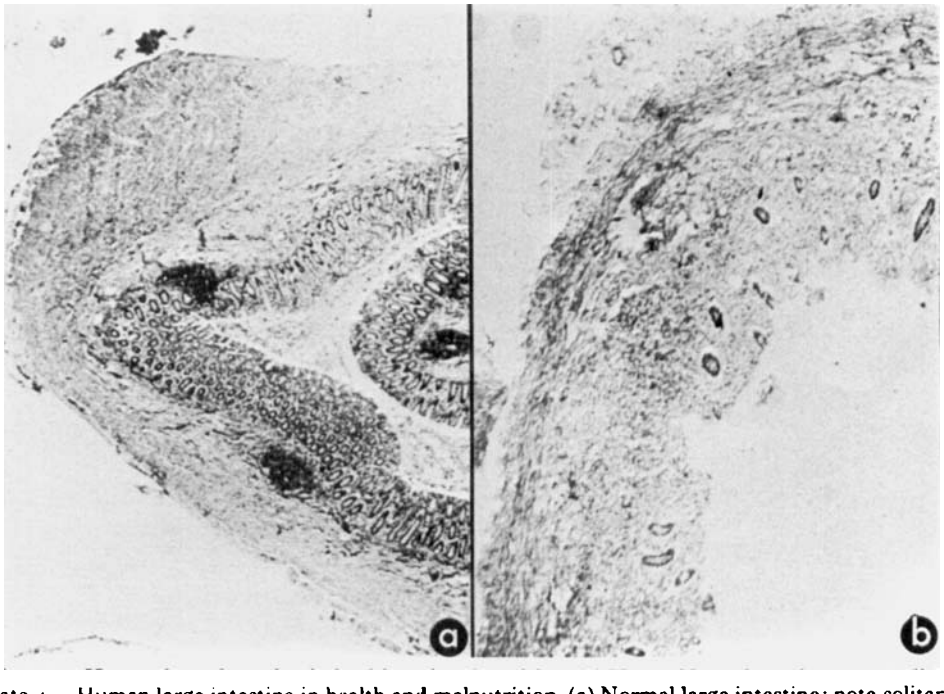

Plate 4. Human large intestine in health and malnutrition. (a) Normal large intestine: note solitary lymphoid follicles and intact mucosa. Follicles probably contain a mixed population of lymphocytes. Cells of the lamina propria are responsible for secretory IgA synthesis, and mucosa contributes to non-specific resistance. (b) Large intestine from child with severe malnutrition: note thinning of mucosa and absence of lymphoid follicles. Stained with haematoxylin–eosin (×35).

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structures of unestablished function called Hassall bodies. T-lymphocytes arise from the thymic cortex. The histology of thymuses from malnourished children varies according to the degree of malnutrition. A progressive loss of cortico-medullary differentiation and lymphocytic depletion is seen in the initial or intermediate stages of malnutrition (Plate 1b), and this can ultimately lead to the complete loss of normal thymus architecture with lymphocyte depletion, interstitial fibrosis, and degenerative changes of Hassall bodies in severe cases (Plate 1c).

The peripheral lymphoid organs of the immune system show gross and microscopical evidence of damage in malnutrition. The spleen is small and it contains fewer and smaller germinal centres (Plate 2a,b). Lymph nodes also lose their germinal centres, and the amount of fibrous connective tissue increases (Plate 3a,b). Cells from these organs taken from malnourished rats and mice incorporate much less tritiated thymidine than do comparable cells from well nourished animals. This is consistent with the observation that dividing cells are rarely seen in lymph nodes, or splenic tissues from malnourished humans. Lymphoid aggregates in the gastrointestinal tract are also hypoplastic in patients suffering from malnutrition (Plate 4a,b). It is not clear, however, whether these morphological changes in the thymus and peripheral lymphoid tissues are primarily the result of malnutrition or the secondary effects of infection or stress. Histopathological findings in thymuses from intra-uterine-malnourished neonates might suggest that the changes are primarily due to malnutrition, but the possibility of trans-placental transport of maternal stress hormones, endotoxin, or viruses as possible augumenting factors has not been excluded. Whatever the cause, it is clear that both the central and peripheral components of the immune system undergo drastic changes during malnutrition, and these histopathological alterations are clinically associated with a compromised ability of the host to deal effectively with infections.

Immunoglobulins and antibodies

Nutritional deprivation is usually not associated with a significant change in the proportion or absolute number of B-lymphocytes. Indeed, in occasional patients with prolonged infection, these cells may be slightly increased. The serum concentrations of all Ig classes are often elevated in malnutrition (Neumann, Lawlor, Stiehm, Swedseid, Newton, Herbert, Ammann & Jacob, 1975). In some subjects, IgA is increased more than other immunoglobulins, perhaps as a consequence of gastrointestinal and respiratory infections (Mata & Faulk, 1973). Serum IgE is also often elevated, particularly in patients with certain parasitic infestations. In addition, concomitant depression of CMI may contribute to the elevated IgE levels, since T-lymphocytes seem to have an inhibitory effect on IgE synthesis. In a few undernourished infants, low levels of IgG and sometimes of IgA and IgM are seen. Many such children, however, have low birth weights and are small for gestational ages (Faulk & Chandra, 1976).

The recognition that mucosal immune responses are largely independent of systemic immunity has led to the concept of locally-produced Ig. IgA present in secretions is a dimer composed of two 7S IgA monomers joined together by a joining- or J-chain and linked to another molecule called 'secretory-piece' (molecular weight 58 000) that is produced by epithelial cells (Hanson & Brandtzaeg, 1973). In malnourished children the concentration of IgA in naso-pharyngeal secretions is significantly depressed relative to the mild decrease in levels of total proteins and albumin. Consequently, IgA–antibody response to viral antigens on mucosal surfaces is impaired. Indeed, in many malnourished children no secretory-IgA antibody activity can be detected in upper respiratory tract washings. This reduction in mucosal immune response may contribute to an increased frequency and severity of infections associated with nutritional deficiency. Systemic spread may also occur more easily because of the impaired ability of mucosa to prevent pathogenic organisms from penetrating gastro-intestinal and respiratory epithelia. Other antigens seem also to take advantage of this impairment. For instance, protein antigens in the diet get across the gut wall and stimulate the formation of food antibodies (Chandra, 1975). It remains to be established if the incidence of other immunopathologic diseases known to be associated with defective mucosal immunity such as atopy, autoimmunity, and neoplasia are increased in malnourished populations.

There have been a vast number of studies of antibody responses following immunization in malnutrition (for review see Faulk, Mata & Edsall, 1975). Many of these investigations are unfortunately of little value because the antigens were not standardized, adjuvants were not specified, methods of antibody titration were inadequate, or no information was provided as to whether or not primary or secondary responses were being studied. These criticisms are entered to encourage more careful technique and documentation in future investigations. The general message that has emerged from studies thus far indicates that one might expect either a decreased or a relatively normal antibody response, and, that the response seems to be dependent on both the type of antigen and the physical state of the antigen. The fact that some vaccines fail and others succeed is compatible with current immunological concepts. Some antigens are dependent only on B-cells for antibody production, and other antigens require another lymphocyte population (T-cells, to be discussed later) to co-operate with B-cells to generate antibody production. B-cells and B-cell products are often normal in malnutrition, so one might expect that those vaccines that are primarily B-cell-dependent would produce adequate antibody responses in undernourished patients. However, the response might be inadequate if the vaccine was dependent on a depleted population of lymphocytes. Research is still required into the problem of what vaccines are dependent on what populations of lymphocytes.

The complement system

The complement system is a complex set of interacting proteins. These are present in the serum in an inactive form, and can be activated by a variety of

agents such as antibodies, microbial products, and enzymes. There are two major pathways of complement (C) activation. These are: (a) the classical pathway in which complement protein 1, C1, serves as the recognition unit, C4, C2, and C3 the activation system, and C5, C6, C7, C8 and C9 the membrane attack unit; and (b) the alternative pathway which bypasses C1, C4, and C2 and is activated by Ig aggregates and polysaccharides. This consists of the initiating factors, properdin, C3 proactivator and C3 proactivator convertase. The two pathways merge with each other at the C3 stage, sharing the membrane attack unit. The end result of the activated complement system may be membrane damage leading to cell death by lysis. In addition, several molecules involved in the reaction have contributory roles in chemotaxis, blood clotting, anaphylaxis, and other systems that amplify the immune response.

The complement system in malnutrition. Total haemolytic complement activity and levels of almost all complement components except C4 are reduced in malnourished patients (Sirisinha, Suskind, Edelman, Charupatana & Olson, 1973). The reduction is more pronounced in those with infection, which contrasts with the raised levels seen in infected, well-nourished subjects. In many samples, electrophoretically altered complement fractions are detectable, suggesting activation in vivo. There is an inverse correlation between serum C3 concentrations and immunoconglutinin titres (i.e., antibodies to activated C3 and C4) as is also reported in other conditions where complement fixation has been demonstrated. In a significant proportion of malnourished children the direct anti-globulin (Coombs') test is positive due to the presence of C4 (and occasionally other complement components) and Ig on the surface of erythrocytes. Nutritional recovery is associated with a return of complement levels and function to normal.

Cell-mediated immunity

Since T-cells are responsible for CMI it is important to know more about these cells. T-cells in mice are distinguished by characteristic membrane markers, and human T-cells are identified by several rosetting and mitogenic tests. Human T-cells form rosettes with sheep erythrocytes. These rosettes form spontaneously and do not require antibody or complement as do the B-cell rosettes mentioned earlier. This test simply provides information about the number of T-cells. It neither measures the capacity of T-cells to respond to an environmental stimulus nor does it measure T-cell function. Diminished numbers of T-cell rosettes have been reported both in chronic infections and in certain immunodeficiency states (Cooper *et al.* 1974). The capacity of T-cells to respond to an environmental stimulus can be measured either with a non-specific T-cell mitogen such as PHA or with specific antigens. In both instances a proliferative response is generated, and the number of cells responding can be measured either by counting cells in metaphase or by pulsing the culture with a labelled DNA precursor such as tritiated thymidine. Antigen of course stimulates only a small clone of committed T-cells, but the proportion of cells responding to PHA should be roughly the same as the proportion of cells forming spontaneous rosettes. Furthermore, the sum of the

percentage of T-cell rosettes in a normal blood sample plus the percentage of B-cell rosettes in the same sample should equal about 100%. Normally this is about 70% T-cells and 30% B-cells.

One aspect of T-cell function can be measured by the capacity of stimulated cells to release a soluble factor that inhibits the outward migration of macrophages from a capillary reservoir. This soluble factor, popularly known as macrophage-inhibitory-factor (MIF), is only one of a heterogeneous group of substances that are released from activated T-cells and are known as lymphokinins. It might be borne in mind that most of these assays are specific for T-cells only within the context of the test-system employed. For example, cells other than T-cells will form spontaneous rosettes with sheep cells, many cells have PHA receptors and some of these will actually incorporate tritiated thymidine when stimulated with PHA, and MIF is found in several types of cells. Nevertheless, tests for T-cell numbers, T-cell responses, and T-cell function are reliable and accurate tools if carefully used and controlled (Schopfer & Douglas, 1976), and they have produced useful information about the immunobiology of malnutrition in humans (Smythe, Brereton-Stiles, Grace, Mafoyane, Schonland, Coovadia, Loening, Parent & Vos, 1971). Some of these studies will no doubt be discussed during this symposium.

The most reproducible measure of T-cell function in human malnutrition is the skin test response to antigens, such as purified protein derivative (PPD) following BCG immunization for tuberculosis, that are characterized by delayed-hypersensitivity reactions. Many different antigens have been used in protein-energy malnutrition (PEM), and they uniformly fail to elicit normal delayed hypersensitivity reactions (Edelman, Suskind, Olson & Sirisinha, 1973). One assumes that the skin test for delayed hypersensitivity is an accurate measure of CMI, but non-immune factors, such as hormones or biochemical alterations in the skin can presumably depress the skin test. Many virus infections can also depress delayed hypersensitivity reactions in the skin, and the role of either superimposed virus infections or the activation of latent viruses in PEM has not been adequately explored.

Phagocytosis

One of the primary methods of resistance is that of phagocytosis and intracellular digestion. This mechanism is found in animals which lack a well-organized immunological system, and in mammals it is more specialized and integrated with a protease-containing system of lysosomes. Organisms are recognized and bound by phagocytic cells either by virtue of membrane receptors or by the presence of cytophilic antibodies. Bound organisms are then endocytosed into a phagosome, and the phagosome fuses with lysosomes to form a phagolysosome. Acid proteases are released from the lysosome into the phagolysosome, and the phagocytosed organism is degraded and killed. Defects can occur in each of these steps, and several diseases in man are associated with increased infections due to faulty phagocytosis and killing.

Studies done on phagocytic cells, mostly neutrophils, from malnourished children have shown that recognition and endocytosis of bacteria are normal, but

that these cells have a delayed chemotactic response, particularly in the presence of infection. There is no characteristic abnormality in the enzymes from leukocytes of malnourished individuals, but they do demonstrate increased resting activity of the hexose-monophosphate shunt, and they do not release adequate acid phosphatase from lysosomes during phagocytosis.

The most direct association of phagocytes with an increased incidence of infections in malnutrition is that of defective or delayed killing of phagocytosed organisms (Douglas & Schopfer, 1974). This has been done using several organisms including Candida, a common pathogen in malnourished children. The defective in vitro candidacidal activity of neutrophils from malnourished patients may contribute to the increased susceptibility of these children to Candida infections. However, Candida infections also occur frequently in patients with impaired CMI, which is also known to be depressed in kwashiorkor, suggesting that multiple immunological factors may be involved (for review see Douglas & Faulk, 1976).

Non-specific factors of resistance

Humoral immunity is achieved strictly by Ig, antibodies, complement and specialized cells, but there are other humoral factors that influence immunity and resistance. These are generally grouped as non-specific factors of resistance, and they include many substances such as C-reactive protein, lysozyme (*EC* 3.2.1.17), β-lysins, and hormones. Many of these factors are affected by alterations in the status of particular nutrients. There is very little information about non-specific factors in malnutrition, but some attention has focussed on the transferrins. These are Fe-binding proteins that reportedly indicate a poor prognosis if depressed. It is not altogether clear why depressed serum transferrin values should herald a poor prognosis, but it is thought to be related to their Fe-binding capacity (Bullen, Rogers & Leigh, 1972). Fe is important in the killing of endocytosed bacteria by phagocytes, and certain bacteria require Fe to manifest their pathogenicity (Lancet, 1974). Studies of the general role of Fe and transferrins in host resistance are needed, and it would seem to be particularly useful to know more about transferrins in malnutrition.

The diets of many malnourished persons also lack adequate vitamins, and several vitamins have been shown to be important in mounting an adequate immune response. This topic has been comprehensively reviewed in a WHO monograph (Scrimshaw, Taylor & Gordon, 1968). It has also been speculated that breast milk contains non-specific factors of resistance, because breast-fed infants seem to thrive better than bottle-fed babies. For instance, significant levels of resistance to enteric infection are observed among breast-fed babies, even if they are living under very deficient environmental sanitation. Mechanisms for this resistance are poorly understood. However, certian indigenous microflora which form a protective barrier against pathogenic organisms appear to be important. Breast-fed infants develop a flora predominating in Gram-positive anaerobic bacilli (Bifidobacterium), probably as a result of the combined action of bifidus factor(s), lysozyme, secretory IgA, other antibodies and macrophages and lymphocytes

present in human milk (Goldman & Smith, 1973). Bifidobacteria synthesize considerable amounts of acetic and lactic acids. Lysozyme and Ig are thought to exert a suppressing or a lytic effect, or both, on Gram-negative facultative bacilli (Enterobacteriaceae). These actions result in a milieu unfavourable for Shigella and other enteropathogenic agents. As a consequence, the incidence of infection among small infants in highly contaminated environments is low if the infants are breast-fed.

Intra-uterine malnutrition and infections significantly affect the morbidity and mortality of low-birth-weight infants. The etiological agents in these instances may be of low virulence, and systemic spread with poor localization is common. Several aspects of immunocompetence are impaired in such infants and diminished host defences may be severe and long-lasting. CMI as measured either by delayed hypersensitivity reactions in the skin or by lymphocyte responses to in vitro stimulation are usually depressed. In addition, quantitative measurements of T-lymphocytes in the blood reveal that they are reduced in number (Ferguson, Lawlor, Neumann, Oh & Stiehm, 1974). Hypoimmunoglobulinaemia, especially involving IgG, is frequent and pronounced due to a reduced materno-foetal transfer of IgG. The blood concentration of the C_3 component of complement is low, the opsonic function of plasma is reduced, and the killing of bacteria by polymorphonuclear leucocytes is slightly impaired. It is suspected that these functional defects in the host resistance of intra-uterine-malnourished infants can persist in extra-uterine life for several months. These children often sustain repeated infections, and clinically they tend to resemble children with immunodeficiency diseases (for review see Faulk & Chandra, 1976).

Future considerations

The clinical and biochemical profiles of malnourished children are extraordinarily complex, and it is unwise to make generalizations about their capacity to resist infections. However, several years ago children with immunodeficiency diseases were an equally heterogeneous and baffling lot. Research on these diseases has developed a systematic approach to diagnosis and treatment based on the pathophysiology of each deficiency, and this has led to improved prognoses in many immunodeficiency diseases. As research broadens current understanding of the immune response in malnutrition, it seems to be increasingly possible to think of the immune defects in undernutrition as being analogous to those in immunodeficiency diseases, and it has been suggested that malnourished children might be considered as having a type of secondary or acquired immunodeficiency disease (Douglas & Schopfer, 1974). This approach is useful in establishing a more intelligent understanding of patterns of host resistance in malnourished children as well as helping to build new concepts in the treatment and prevention of infections in malnourished populations. It would seem that the combined approaches of nutrition, public health, and immunology may have something of value to offer the malnourished child. Finally, WHO has co-ordinated an international collaborative study of the effects of malnutrition on the

immune response with particular emphasis on public health aspects of the nutrition–infection cycle (Faulk, Demaeyer & Davies, 1974). Considerable data have been accumulated as a result of these studies, and a profile of the immunological capabilities of malnourished children is beginning to emerge. The pathogenesis of this profile is not presently clear, but it is likely to be better defined through continuing studies and symposia in this area of human biology.

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