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NUTRITION AND INFECTION

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INTRODUCTION

The critical events by which infections lead to malnutrition and malnutrition interferes with host defense have been the subject of intense study during the past 20 years (7, 8, 55, 56, 101). Some of the important mechanisms by which infection not only increases nutritional requirements but also simultaneously reduces dietary intake are now being defined. Similarly the myriad of effects of macro- and micronutrients on immune and nonimmune host defense have been recognized and are gradually being unravelled. It is clear that suboptimal nutritional status resulting from recurrent infection with associated anorexia and reduced dietary intake may lead to impaired host defense (Figure 1). Many

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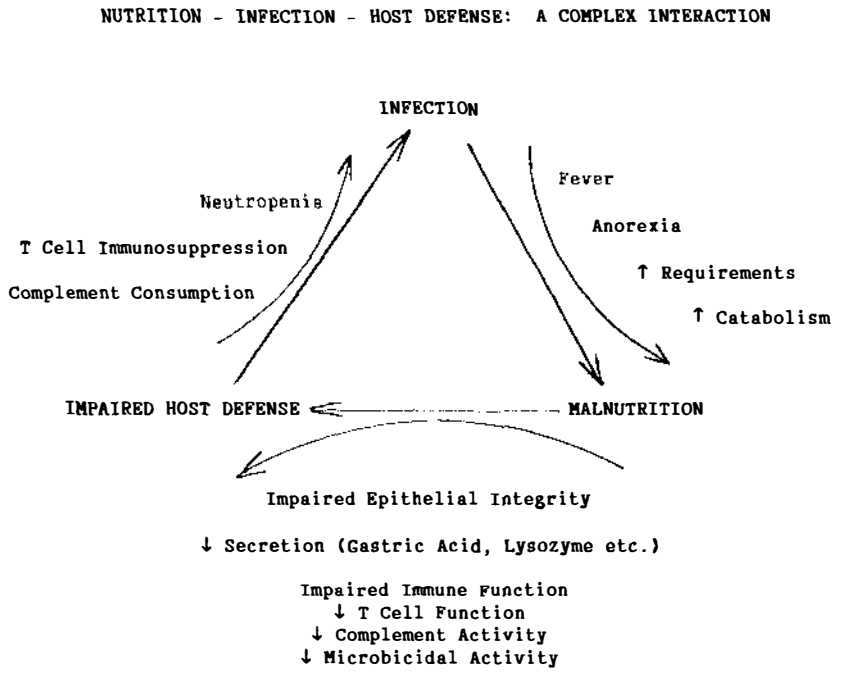

Figure 1 The triangle of interaction between malnutrition, infection, and host defense. Malnutrition may be initiated by primary or secondary dietary deficiency (e.g. malabsorptive states), or by the metabolic effects of infection. The consequence of this is impairment in host defenses, which in turn leads to an increased burden of infection and further malnutrition.

questions remain to be answered, particularly with respect to determining those key, accessible links in the triangle depicted in the figure where intervention might be effective. With this in mind, we review new information on the role of endogenous peptide mediators produced by activated leukocytes in the regulation of host metabolism during infection.

Some still argue, however, that the interaction of malnutrition and infection may not be all bad. Relative undernutrition does appear to impede the expression of certain infections under restricted conditions. However, this is a highly controversial subject and as yet has not been applied in clinical practice, that is therapeutic (?selective) starvation. Observations of the major shifts in minerals and the importance of trace minerals for both the infectious agent and the infected host have stimulated studies of the impact of altered mineral status on infectious disease. We have therefore chosen to discuss some of the controversy surrounding iron deficiency and its possible detrimental and beneficial effects on host defenses.

ENDOGENOUS MEDIATORS AND METABOLIC RESPONSE TO INFECTION

While the physiological reasons for the metabolic responses to infection have never been adequately understood, the phenomena have been clearly described by many investigators over six or more decades of work. When these events are reviewed in the context of modern physiology and clinical nutrition, however, a typical or stereotyped response to either infection or noninfectious inflammatory stress becomes obvious (8, 55). Dramatic changes in the pattern of utilization of carbohydrates, proteins, lipids, and minerals appear to anticipate inadequate food intake on the one hand, and a need to restrict the availability of some nutrients for invading microorganisms on the other hand (56). Recent studies have begun to define the mechanisms underlying these metabolic alterations, in particular the role of secreted macrophage products (endogenous mediators), which serve to link and coordinate the metabolic and the immune responses to infection.

Metabolic Sequelae of Infection

The major metabolic changes during infection are listed in Table 1. It is important to note that both anabolic and catabolic processes occur at the same time, accentuating the magnitude of the metabolic alterations. Since these processes are generally accompanied by fever, which increases metabolic rate by about 13% per °C (31), energy requirements are much greater than usual at a time when the host is often anorexic. Since carbohydrate stores are inadequate to meet these needs (15), and lipid stores are not effectively used in the infected patient (9), another source of energy is required. In most infections, this turns out to be gluconeogenesis, the production of glucose by the liver from amino acid precursors released from contractile proteins of muscle (68–71).

There is an apparent coordination of protein and energy metabolism, as the branched-chain amino acids released by proteolysis of muscle are oxidized in situ for energy, while the amino acids reaching the blood stream are taken up by the liver and utilized for new protein synthesis of acute-phase protein reactants and other anabolic repair and stress responses (70, 93). These complex adaptations are aided by increases in circulating insulin, glucagon, and growth hormone levels, loss of diurnal variation and elevation in glucocorticoid levels, and functional insulin resistance in muscle (7, 97). Gluconeogenic activity is manifested by a pseudodiabetic state, with fasting hyperglycemia, abnormal glucose disappearance curves, and exaggerated insulin secretion following a glucose load, as well as an increased glucose pool size and oxidation rate (69).

Lipid metabolism can be grossly altered as well, depending in part on the nature of the infectious stress, the duration of infection, and its severity (13, 35,

Table 1 Metabolic changes during infection that alter host nutritional status[a]

Protein metabolism	Increased nitrogen loss (negative balance)
	Catabolism of muscle protein
	Conversion of amino acids to glucose
	Decreased synthesis of albumin, transferrin
	Increased synthesis of acute-phase proteins by liver, and proliferation of phagocytes and lymphoid cells
Carbohydrate metabolism	"Pseudodiabetes"
	Increased glucose oxidation
	Peripheral (muscle) insulin resistance
	Augmented gluconeogenesis
Mineral metabolism	Removal of plasma iron to the liver
	Reticuloendothelial system uptake of zinc
	Increased plasma ceruloplasmin copper
	Urine, stool, and sweat losses of Mg, P, K, and S

[a]Adapted with permission from (56).

36). Plasma free fatty acid levels and triglycerides may be increased or decreased, depending on the activity of lipoprotein lipase and fatty acid synthetase and acetyl–coenzyme A carboxylase. Lipid changes are particularly evident during gram-negative bacillary infections, in which defective lipid clearance from serum results in extreme hypertriglyceridemia (51). Utilization of ketones is impaired, associated with decreased production of 3-hydroxybutyrate and acetoacetate, and the consequence of this failure to use lipid stores efficiently is a drain on endogenous protein stores (9).

Protein metabolism during infection is characterized by a dominance of catabolic over anabolic processes, manifested by absolute losses of nitrogen and wasting of lean body mass (55). Amino acids released from breakdown of muscle are taken up in the liver and deaminated during gluconeogenesis, with subsequent excretion of the nitrogen in the form of urea and other nitrogenous compounds in urine, sweat, and other body fluids (111). Since the carbon backbone of the amino acid is converted to glucose, which is oxidized to CO_2 and excreted by the lungs, the entire protein structure is ultimately lost from the body. This has been demonstrated in septic humans as an increased conversion rate of infused ^{14}C-alanine to glucose along with an increase in urinary 3-methyl histidine excretion, a nonreutilizable amino acid marker of muscle protein breakdown (68, 71).

Tremendous changes also occur in a number of minerals during infection (8, 55). Best documented are the decreases in serum levels of iron and zinc due to uptake in liver cells and mononuclear phagocytes, and the elevation in serum copper as a consequence of increased synthesis of ceruloplasmin, the copper

carrier protein. Iron uptake is mediated by unsaturated lactoferrin, the intracellular iron-binding protein from the specific granules of neutrophils (58). Released into the plasma from activated neutrophils, lactoferrin binds iron, and depending on the degree of saturation of the protein, the complex is subsequently cleared from the circulation, resulting in hypoferremia (108). This mechanism explains why serum iron does not decrease in infected neutropenic animals. Sequestered iron ultimately appears as hemosiderin and other nonutilizable iron storage compounds (7). Decreased serum zinc is also related to a metal-binding protein, metallothionine, which is an intracellular acute-phase protein reactant synthesized during infection or inflammation (8, 103).

The reasons for the shifts in these metals are not really known, but plausible benefits to the host can be postulated. For example, reduction in the availability of iron to microorganisms may impair their growth or production of virulence factors; uptake of zinc may prime the host to turn on cellular proliferation since many of the key enzymes involved are zinc metalloenzymes; and the presence in plasma of copper ceruloplasmin, a ferroxidase that oxidizes ferrous iron in the transfer to apotransferrin, may increase the efficiency of iron utilization for hemoglobin synthesis to compensate for the decrease in iron availability (8, 89).

Macrophage Mediators of Metabolic Responses

It is now known that the metabolic alterations during infection can be initiated by peptide mediators produced by stimulated macrophages and not directly by microbial factors. Since the metabolic response is, in fact, not identical from infection to infection, it is possible that multiple distinct mediators could be involved, each with a range of metabolic effects. Moreover, as macrophage peptide mediators are known to affect the function of the immune system as well, they could be the means to coordinate the host protective response and the metabolic needs of the infected individual.

INTERLEUKIN-1 (IL-1) One of the more important mechanisms for regulation of the immune response is the peptide monokine, IL-1 (27). Although specialized cells such as keratinocytes, epithelial cells of gingiva and cornea, astrocytes from brain, and mesangial cells of the kidney can also produce physiologically similar peptides that may function in situ, systemic effects are due to macrophage IL-1 (26). The activities of IL-1 were originally defined by assessing biological activity of leukocyte-derived peptides, for example endogenous pyrogen (EP) activity causing fever or leukocyte endogenous mediator (LEM) resulting in hypoferremia and hypozincemia (27). A multiplicity of biological effects are now known, a number of which are illustrated in Figure 2. Since the various mediators defined in this way were all small (15,000–18,000 M_r) peptides, the concept gradually arose that either one

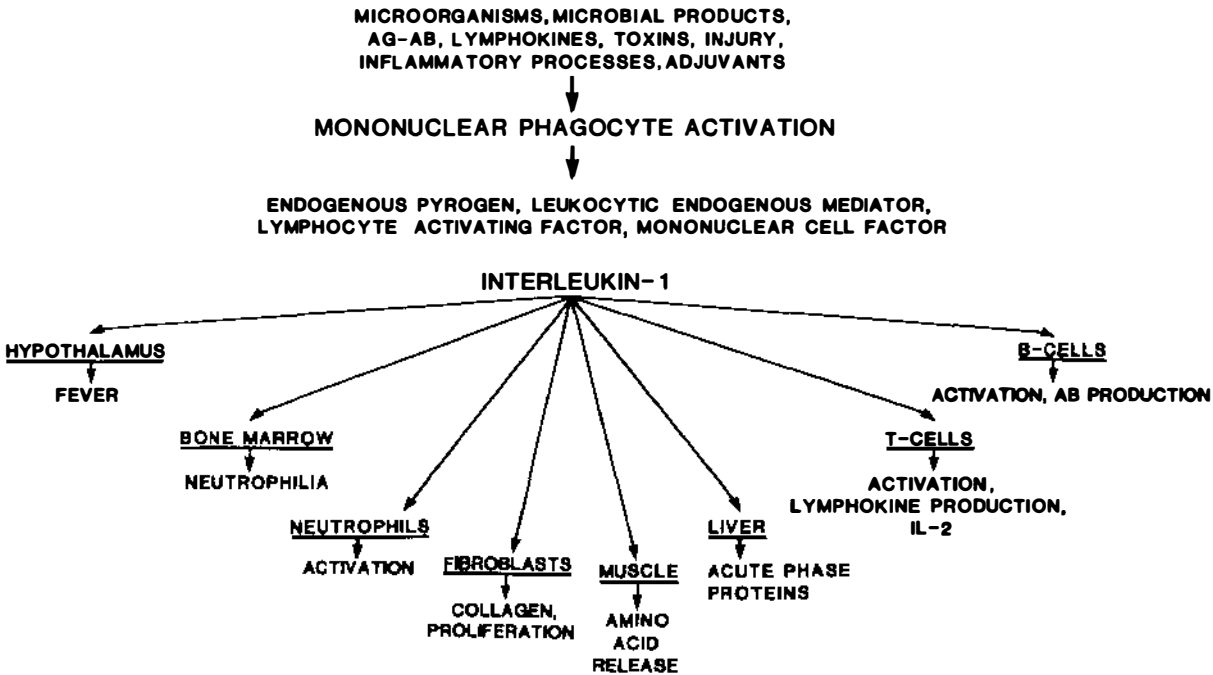

Figure 2 Initiation of the acute-phase response and the diverse effects of interleukin-1 on host nutrition and immune responses. Reprinted with permission from (26).

molecule or a family of similar mediators was produced by activated cells. In 1979 the name IL-1 was proposed for macrophage-derived mediators that prime lymphocyte immune responses, and it is increasingly the accepted term to describe the peptides affecting metabolic responses as well (27).

It is most likely that IL-1 really does represent a family of similar but distinct molecules (28). Several forms of IL-1 differing in isoelectric point are produced by human blood monocytes. Poly A-mRNA to pI 5 and pI 7 forms of human IL-1 have been used to prepare c-DNA's distinguishable by restriction enzyme analysis. These also code for peptides that are only 26% homologous in amino acid sequence.

Recently, the nucleotide sequence of human monocyte IL-1 precursor cDNA has been determined (4). This gene codes for a 269-amino-acid polypeptide of molecular weight 30,747. The molecule is quite unique in that there is no signal sequence or cleavage peptide sequence, which explains why most of the IL-1 produced remains intracellular (or possibly on the macrophage cell surface) until secretion is activated (62, 63). IL-1-specific mRNA, isolated by in vitro hybridization to this oligonucleotide, has been both translated in a cell-free reticulocyte system to produce immunoreactive IL-1, and injected into *Xenopus laevis* oocytes in which it results in production of biologically active IL-1 (4).

It is not yet clear whether there is one gene with post-translational modifications resulting in the distinct forms of IL-1 or a family of genes coding for different IL-1's (26). Although human IL-1 has never been demonstrated in plasma, IL-1 activity can be recovered in lower molecular weight fragments in plasma, urine, or peritoneal fluid (16, 17, 29, 41, 57). A 4000-M_r peptide has been found in the plasma of febrile humans that activates muscle proteolysis in vitro (21). Based on this effect, the peptide has been called "proteolysis-inducing factor," PIF, and it presumably represents a domain of IL-1. A similar biologically active peptide has been isolated as a breakdown product of IL-1 (29), further supporting the relationship of PIF and IL-1.

Metabolic effects induced by IL-1 and peptide fragments IL-1 can reproduce many of the cardinal metabolic alterations associated with infection (27). Fever was the first described effect of IL-1 and this is known to be secondary to its ability to release arachidonic acid and stimulate production of prostaglandin E_2 in the hypothalamus (30). Recent experiments in rats support the idea that fever-induced anorexia is due to the same mediator (77). In these studies, endotoxin administration resulted in both fever and reduced food intake. Antipyretics inhibited the fever response, but did not alter endotoxin effects on food intake. In contrast, animals made tolerant to endotoxin by repeated injection did not show a significant fever response nor diminish food intake. Since endotoxin-tolerant animals are also refractory to other IL-1 effects, such

as hypoferremia and production of acute-phase proteins (50, 92), it is highly likely that the anorectogenic effects of endotoxin are due to IL-1 release.

The changes in carbohydrate metabolism in infection are conditioned by the endocrine responses, and it is significant that IL-1 releases both insulin and glucagon from the endocrine pancreas (39, 86). Muscle breakdown, releasing gluconeogenic precursor amino acids into the blood stream, occurs concomitantly (20, 98). IL-1 added to muscle in vitro results in proteolysis via induction of prostaglandin E_2 synthesis (4a). At the same time, a 33-amino-acid glycopeptide probably containing sialic acid with an estimated molecular weight of 4274, was isolated from plasma of patients with sepsis and shown to cause a 221% elevation in proteolysis in the in vitro system with no reduction, and perhaps a slight stimulation, of protein synthesis rates (21). In 10 septic patients, the in vivo proteolysis rate of muscle was estimated by comparing tyrosine plus phenylalanine concentrations in femoral vein and artery blood. This correlated well ($r = 0.64$, $p < 0.05$) with the release of tyrosine from muscle in vitro. The level of this proteolysis-inducing factor (PIF) also correlated with the fever response, an indication that PIF and IL-1 are related. Direct evidence for this has now been obtained. First, low-molecular-weight fragments can be derived from highly purified monocyte-derived IL-1 and these exhibit both PIF and the well-studied in vitro biological activity of IL-1, lymphocyte-activating factor (LAF) (29). Second, PIF has been purified from plasma of febrile patients and shown to be active in the LAF assay (29). Finally, ^{125}I-labelled IL-1 also breaks down into smaller fragments that retain pyrogenicity and LAF activity (29). Thus, the evidence is consistent with the concept that PIF is a small-molecular-weight cleavage product of IL-1.

Release of amino acids into the blood of septic patients is not associated with increased plasma amino acid levels (111); rather reutilizable amino acids are taken up by the liver and used for new protein synthesis (20, 98). Both amino acid uptake by liver and synthesis of acute-phase proteins are stimulated by purified monocyte-derived IL-1 or PIF purified from septic humans (67). When IL-1 or PIF is injected into the peritoneal cavity of rats, hepatic protein synthesis is significantly elevated (70% increase in incorporation of ^{14}C-tyrosine into structural and secreted proteins) and de novo synthesis of C3 and fibrinogen increases in parallel.

These experimental results are supported by observations in patients, in whom the central plasma clearance rate for amino acids (CPCR-AA) was determined as a surrogate for actual liver uptake data (67). This measures total amino acid uptake by the central tissues, including liver, splanchnic bed, bone marrow, lymph nodes, and other tissues using amino acids for protein synthesis or energy production. CPCR-AA correlated with in vitro protein synthesis by liver tissue obtained by biopsy at surgery in the same patients, which indicates that CPCR-AA is a reasonable measure of amino acid uptake for protein

synthesis by central tissues. In addition, CPCR-AA correlated with PIF activity in patient plasma, and with the rate of incorporation of ^{14}C-tyrosine into hepatic protein by the biopsy specimens in vitro. In vitro data also show the ability of IL-1 to induce synthesis of a number of acute-phase proteins typical of infection and inflammation (38, 96, 102, 104). Thus, IL-1 and PIF not only induce muscle proteolysis, but also can direct the hepatic uptake of amino acids and accelerate the synthesis of acute-phase proteins; they are central to the catabolic and anabolic responses in infection.

Another property of IL-1 is the induction of the acute shifts of divalent cations in infected hosts (91, 103, 107). Under the influence of IL-1, lactoferrin is released from neutrophil-specific granules and binds iron and facilitates its clearance from plasma (59), while synthesis of metallothionine is triggered (103), leading to zinc uptake. Since ceruloplasmin is an acute-phase protein stimulated by IL-1, elevation of serum copper during infection is a third manifestation of IL-1 effects on minerals (94).

Finally, it is worth noting that in addition to fever and anorexia, the other generalized manifestations of infection, such as myalgias and fatigue, are probably also related to IL-1 effects. Myalgia is undoubtedly due to PIF-induced proteolysis of muscle, since this effect is blocked by antipyretics that relieve myalgias (4a). Recently IL-1 was shown to induce slow wave sleep (61), measured by EEG recording, in a manner similar to a factor (S) derived from sleep-deprived humans, except for a rapid onset rather than a delay of about one hour. Thus, factor S may act by local release of IL-1 from astrocytes in brain. The resulting sleep could benefit the host by reducing energy demands at a time when IL-1 is mobilizing host metabolism for the defense responses required for survival. This is another example of the extraordinary coordination of the host responses mediated by IL-1 to function in the most economic and efficient fashion. IL-1 is a well-conserved protein, being found in reptiles, amphibians, bony fish, and birds, as well as in mammals. This suggests that it has evolutionary significance as a protective factor (60).

Immunologic effects of IL-1 Interleukin-1 was first defined as a macrophage product (monokine) needed for the function of lymphocytes, and its ability to activate thymocytes exposed to submitogenic concentrations of PHA or ConA (LAF activity) was used as a bioassay for the peptide. The effects of IL-1 on the immune system were reviewed recently (27, 81), and only a brief summary need be presented here.

It is fair to state that IL-1 plays a critical role in immunoregulation, for it contributes to the initiation of both humoral and cell-mediated immune responses (32, 64, 81). Although the specific locus of action of IL-1 in B-cell activation remains uncertain, the molecule is one of several mediators apparently necessary for regulation of antibody production. It has become clear that the

human monocyte-derived monokine called B-cell-activating factor (BAF), which increases the anti–sheep cell response of splenocytes from antigen-stimulated nude mice when directly added in vitro, is in fact IL-1. The effect of IL-1 actually may be twofold: first a direct effect in B-cell activation and proliferation, and second an indirect effect through activation of T helper and suppressor cells. It is certainly not a chance phenomenon that most adjuvants are IL-1 inducers and pyrogens as well (27). The effect on CMI is more clearly at the early stage of T-cell activation, as IL-1 signals the production of lymphokines, the most important of which is IL-2. IL-2 and the induction of IL-2 receptors on responsive T cells, leads to clonal expansion of functional subsets of helper, suppressor, and cytotoxic T cells (73). IL-1 also augments natural killer activity and synergizes with IL-2 or interferon in this effect (24).

IL-1 also exhibits the neutrophilia-inducing activity associated with the acute-phase response (48). Partially purified pyrogen induces peak neutrophilia in 60–90 minutes after i. v. injection, and repetitive injections result in sustained elevation in neutrophil counts with no evidence of tolerance or tachyphylaxis to daily injection, as well as an increase in plasma levels of neutrophil colony-stimulating factor (49). Although the preparations of IL-1 used in these studies were not pure, Dinarello (27) has concluded that the possible level of contamination with C3a could not account for the findings and that IL-1 causes direct release of neutrophils from bone marrow.

One of the dramatic effects of IL-1 is the induction of acute-phase protein synthesis in liver. The role of the acute-phase proteins in the host inflammatory response is not fully defined but may serve, at least in part, to dampen the destructive effects of unrestrained inflammation by anti-enzyme effects, for example the antitrypsin, elastase, collagenase, plasmin, thrombin, and kallikrein activity of α_1-antitrypsin and/or α_2-macroglobulin (95). In addition, some of the acute-phase proteins, such as serum amyloid A (SAA) and C-reactive protein (CRP), are also immunoregulatory in vitro, particularly affecting T-cell responses and complement activation, respectively (10, 109). The full significance of the acute-phase protein response for host defense and immunoregulation remains to be determined.

CACHECTIN Based on observations of debilitating cachexia in *Trypanosoma brucei* infection in cattle, Rouzer & Cerami (99) experimentally infected rabbits and found that they became moribund and extremely cachectic, even when there was minimal parasitemia, and they exhibited extreme elevation of very low density lipoprotein (VLDL). The hypertriglyceridemia was shown to be the consequence of a clearing defect due to depressed lipoprotein lipase (LPL) activity. Using another model known to result in hypertriglyceridemia, endotoxin administration, Kawakami & Cerami (53) observed that endotoxin-sensitive C3H/HeN mice demonstrated the same loss of LPL in adipose tissue

and elevation of VLDL, while endotoxin-resistant C3H/HeJ mice were unaffected. However, resistance of C3H/HeJ mice was overcome by injecting serum from C3H/HeN mice treated two hours previously with endotoxin or by conditioned medium from elicited peritoneal exudate cells incubated in the presence of endotoxin (53, 90). This suggested that the endotoxin effect was due to activation of macrophages to produce a soluble mediator. Cerami and colleagues went on to prove this by demonstrating such a monokine in stimulated macrophages and macrophage cell lines (RAW 264.7) that suppressed the LPL activity of the adipocyte cell line, 3T3-L1 (11, 44, 75). This monokine was called cachectin.

Cachectin was then obtained from endotoxin-activated cultured RAW 264.7 macrophages, concentrated and desalted over an Amicon PM-10 filter, and then purified by successive isoelectric focusing in a glycerol gradient, Con A sepharose affinity chromatography, and preparative polyacrylamide gel electrophoresis (PAGE). The specific activity of the purified cachectin was increased by 80-fold, but with only a 2% yield, as assayed by ability to suppress heparin-releasable LPL activity of 3T3-L1 cells. One peak of bioactivity was obtained by isoelectric focusing at a pH of 4.7 and a single species was found by preparative PAGE under nondenaturing conditions. SDS-PAGE also demonstrated a single peptide of M_r 17,000, which contained cachectin bioactivity when eluted from sliced unfixed gels.

When the purified cachectin was labeled by the iodogen method, 70% of the bioactivity was recovered in the M_r 17,000 region on SDS-PAGE. This preparation bound specifically to 3T3-L1 cells, and $\sim 10^4$ high-affinity receptor sites were present by Scatchard analysis, with an association constant (K_a) of 3×10^9 M^{-1}. A similar number of high-affinity binding sites could also be detected on C_2 muscle cells, and specific binding to mouse liver cell membranes but not to erythrocytes or lymphocytes was also detected.

Cachectin thus resembles IL-1 in several ways: it has a similar cell of origin (monocyte/macrophage), is endotoxin inducible, and has similar pI and molecular size. Other data indicate these are distinct molecules. RAW 264.7 cells produce little LAF activity under conditions resulting in cachectin release while purified cachectin does not exhibit LAF activity and highly purified recombinant mouse IL-1 fails to compete for binding of ^{125}I-cachectin to 3T3-L1 cells (11).

The mechanism by which cachectin inhibits triglyceride clearance has been investigated using 3T3-L1 cells. This fibroblast line differentiates to adipocytes, which produce considerable quantities of three key lipid biosynthetic enzymes; lipoprotein lipase, needed for transport of exogenous lipid into the cell; and acetyl-CoA carboxylase and fatty acid synthetase, both essential for endogenous fatty acid synthesis. As already noted, adding cachectin to these cells results in a rapid decrease in LPL activity, serving as a bioassay for the

monokine. LPL activity decreases in the medium, in the cell itself, and in the heparin-releasable membrane-associated enzyme. This is not due to enzyme inhibition, since addition of cachectin to LPL does not alter the decay curve of bioactivity (53). Instead, biosynthesis of LPL is selectively decreased. When ^{35}S-methionine incorporation was measured in the presence of cachectin, there was no reduction in total protein synthesis. However, a 220,000-dalton peptide present in cultures without cachectin was missing in the cachectin-containing cultures. In addition, using antibodies to fatty acid synthetase or acetyl-CoA carboxylase for an immunoprecipitation assay, researchers observed that immunoreactive enzyme progressively decreased in the cachectin-treated cultures (90a).

Using a stable adipogenic line, TA1, derived from 3T3-L1 adipocytes, Torti et al showed that lipogenic enzymes are suppressed by a selective alteration in gene expression (105a). Under normal conditions, TA1 cells activate several genes within three days after reaching confluent growth. These genes are detectable by dot-blot hybridization of mRNA with nick-translated cDNA clones. Using clones specific for genes expressed in differentiated adipocytes but not in preadipocytes, Torti et al observed that cachectin prevented the transcriptional activation of adipose-inducible mRNA but had no effect on the noninducible actin gene (105a). No effect was found when endotoxin was added to conditioned medium from RAW 264.7 cells, which demonstrates that the role of endotoxin is to induce monokine production. Cachectin also resulted in an inhibition of oil-red–O-positive lipid accumulation in TA1 cells. All effects were reversed during incubation in cachectin-free medium.

Cachectin did not alter cell growth or viability (105a). However, because adipocytes do not proliferate in vivo, in vitro experiments were repeated with mature confluent nonmultiplying cultures. By 4–6 days in the presence of cachectin, the percentage of oil-red–O-positive cells decreased from 80% to 10%. The effect of cachectin on adipocyte specific genes was consistent with these results and even more impressive. Within 24 hours, cachectin resulted in a 90% decrease in RNA hybridizing with the cDNA's. The level of mRNA for glycerophosphate dehydrogenase also paralleled the bioactivity of the enzyme in the cell.

These results can explain the effects of certain acute infections, for example gram-negative sepsis, on lipid metabolism (51, 52). The cachectin-induced depression in LPL activity, which leads to hypertriglyceridemia in vivo, is not reversed in vitro by insulin in high concentration, although there is no alteration in either insulin receptor expression or the function of the receptor-coupled glucose transport unit in cachectin-treated adipocytes. In the septic patient, hyperglycemia due to gluconeogenesis and direct IL-1 effects on pancreatic islet β cells both lead to elevated insulin levels. Although this can result in glucose uptake by adipocytes, cachectin-affected cells cannot utilize this for

fatty acid synthesis and the glucose diffuses out of the cell into plasma, contributing in circular fashion to hyperglycemia and hyperinsulinemia. Thus, the combination of IL-1 and cachectin can explain most of the alterations in energy metabolism in the septic patient. Chronic inhibition of lipid metabolism can also explain the cachexia associated with chronic infection or malignancy.

Cachectin has a high degree of sequence homology to another previously described macrophage secretory product called tumor necrosis factor (TNF) (11a). Indeed, recombinant human TNF appears to cause lipid mobilization from adipocytes and to inhibit synthesis of lipogenic enzymes (105a), and purified cachectin has TNF activity. Antibody to cachectin that has no demonstrable reactivity with the endotoxin used to elicit the monokine has been employed to passively immunize mice prior to administration of a lethal dose of endotoxin (11a). The significant protective effect observed suggested that lethal events in endotoxic shock may be due to cachectin-induced events. This antibody did not abrogate the pyrogenic effect of endotoxin, consistent with the evidence that IL-1 and cachectin are distinct monokines. The beneficial and potentially harmful effects of cachectin remain uncertain at this writing; however, we may anticipate considerable progress in the next few years.

IRON AND INFECTION

Nutritional deficiency is usually considered to be deleterious and in particular to affect adversely the defense mechanisms against infectious diseases (55). This view has been challenged recently, especially with respect to micronutrient deficiency, notably that due to iron. Thus two extremes of opinions have now been established: (*a*) iron deficiency increases host susceptibility to infection, a situation that can be reversed by appropriate iron replacement therapy; and (*b*) since microbial pathogens require iron for survival, iron deficiency can actually reduce the likelihood of infection and correcting this deficiency may harm the host by promoting replication of the invading pathogens (45). These views present an apparently irreconcilable controversy between the possible benefit and detriment of iron for the infected human host. However, some of the data on which such views are based have not always been well controlled, and in some instances the apparent association of iron status with infection rates remains only an association without firm evidence of causality.

It is clear, however, that free iron is required by microorganisms and in vitro studies have shown that indeed the iron is necessary for microbial growth (113). In addition, iron deficiency has been shown to protect both birds and mammals from some experimental bacterial infections, while infection can be enhanced by iron administration (112). What relevance does this have for the human host and what is the evidence for and against a significant role of iron in the pathogenesis of human infectious disease?

Evidence That Iron Deficiency Promotes Infection

In 1928, Mackay (74) reported the results of a survey of 541 nonhospitalized infants in London and observed that anemia was common in both breast-fed and artificially fed subjects. Oral supplementation with iron not only increased hemoglobin but reduced attack rates of respiratory and diarrheal disease by approximately 50% compared to untreated controls. However, the groups were not selected on a random basis and it is uncertain whether or not they were well matched for other important health-related variables. Yet Mackay concluded that iron treatment resulted in "a striking improvement in general health and resistance to infection." Therefore, "artificially-fed babies should be given iron before (they are) two months old" and "many breast-fed babies also require iron treatment."

Andelman & Sered (2) examined the effect of feeding an iron-containing milk formula for a period of 6–9 months to 603 infants of a low socioeconomic status and compared the results with a group of 445 control children. While growth was similar for children in both groups, anemia was prevented in the treated infants and there was a striking reduction in the incidence of respiratory infection. More recently Lovric (72) found that children with anemia, the majority of whom had iron-deficiency anemia, had a significantly higher prevalence of gastroenteritis than nonanemic controls. Another community-based study in Maori infants showed that administration of parenteral iron during the first few days of life reduced hospital admission rates during the subsequent two years, particularly for some respiratory infections and gastroenteritis, compared to untreated controls (18).

Other interventional community-based studies also suggest that iron deficiency predisposes to infection and its correction leads to an apparent reduction in infection rate. A study of children with malnutrition in Colombia showed a reduction in infections, notably gastroenteritis, after iron deficiency was corrected with iron supplements (3). Similarly, in an urban population in Chile, the diarrheal disease rate diminished after introduction of an iron-fortified milk formula, although in this instance comparisons were made with disease morbidity during the two-month period immediately prior to introduction of the new formula (89a). Additionally, a placebo-controlled trial of prophylactic parenteral iron in early infancy significantly reduced the death rate from infectious disease in Eskimo infants (89b). However, a more recent survey of normal infants in England showed first that hemoglobin concentration was generally higher than reported previously, and second that placebo-controlled iron administration, while increasing hemoglobin in some infants, failed to make any impact whatsoever on the incidence of infection (14).

An association between the prevalence of urinary infection and anemia in pregnancy was reported by Giles & Brown in 1962 (40); they found that urinary

infection was more than twice as common in 463 anemic pregnant women compared to 447 nonanemic pregnant controls. Unfortunately, it cannot be determined from this study whether urinary infection and anemia are dependent or independent variables, and cause and effect are difficult to separate. Other studies have shown that urinary infection is associated with a marginal reduction in hemoglobin concentration (1, 100) although an extensive survey of 5000 pregnant women failed to confirm an association between urinary infection and iron deficiency (65).

Increased susceptibility to mucocutaneous candidiasis has also been reported in severe iron-deficiency anemia, which was reversed when iron stores were repleted (37, 43). At the same time, other studies have failed to show that iron status or iron repletion for iron deficiency had a significant impact on either oral or genital candidiasis (23, 110). More recently a retrospective analysis of anemic, iron-deficient, hospitalized infants in Papua New Guinea showed that meningitis and pneumonia were more common in the presence of iron deficiency (87).

Although many of these studies suggest that iron deficiency in infants and children predisposes to infection, particularly of the respiratory and gastrointestinal tracts, and that administration of iron in some circumstances can reduce infection rates, the majority of these studies have serious shortcomings. Many are uncontrolled or at best poorly controlled. Historical or retrospective controls are now unacceptable because epidemics and other seasonal factors in illness can distort data analysis and produce fallacious conclusions. Similarly, one can never be certain that an intervention such as the introduction of iron-fortified formula is the only change that occurred during that period. Few of these studies match patients for overall nutritional status, which may be a more important determinant of host susceptibility to infection for which iron deficiency is merely a surrogate measure. Finally, many of the children studied have had multiple health problems, making data analysis even more complex.

Thus none of these studies permit reliable conclusions about whether iron deficiency alone predisposes to infection and if iron supplementation and correction of iron deficiency as the sole intervention can reduce prevalence and morbidity from infection. We look forward to the results of prospective, randomized, double-blind, placebo-controlled trials of iron prophylaxis in infants (such as the study underway in Papua New Guinea), which should overcome many of the deficiencies of previous studies (88), finally ending a controversy that has remained unanswered for more than half a century.

Evidence That Iron Deficiency Protects Against Infection

During the past decade an apparently heretical concept has evolved; it proposes that nutritional deficiency, both generalized and specific, may in some situations be protective against infectious diseases (101). There is evidence to

suggest that relative or absolute iron deficiency may reduce susceptibility to certain infections and that treatment with iron exacerbates these processes. As previously discussed, it has been known for many years that serum iron falls during infection, largely because circulating iron is removed by the liver and to a lesser extent because iron absorption by the intestine is reduced (19, 42, 66). It has been proposed that this "iron shift" reduces the availability of free iron to infectious agents and therefore induces a degree of "nutritional immunity" (112, 113).

Over 100 years ago, Trousseau (106) observed that in patients with quiescent tuberculosis, iron supplementation often led to clinical recrudescence of the disease. McFarlane et al (78) suggested that in their study the rapid demise of children with kwashiorkor was related to refeeding, especially micronutrient supplementation with iron. They suggested that because of the low transferrin in these children, iron administration resulted in high levels of the free circulating iron, which was instrumental in promoting bacterial infection. Subsequently they showed that sera from children with kwashiorkor readily supported growth of *Staphylococcus aureus,* particularly when serum transferrin was low, whereas addition of purified transferrin to cultures inhibited bacterial growth (79).

Several studies followed supporting the view that repletion of iron deficiency actually enhances the prevalence of infection. Masawe et al (76) investigated infection rates in 110 anemic patients in Tanzania. Of 67 patients with iron-deficiency anemia, only 7% had bacterial infection compared with 65% of 43 patients with anemia of other etiologies. Malaria was more common in the patients with iron deficiency, but clinical malarial attacks frequently occurred after initiation of iron replacement therepy. Often cited data for the apparent protective effects of iron deficiency comes from the observational and interventional studies of Murray et al (82–84). Somali nomads are commonly iron deficient because of the limited iron available from their almost exlusively milk diet. In a survey of 90 Somali nomads, 26 were shown to be iron deficient, based on hemoglobin < 11 g/dl, serum iron < 25 μl/100 ml, transferrin saturation $< 15\%$, and a hypochromic anemia (83). None of them, however, had clinical evidence of infection. Of the remaining 64 with normal iron status, 19 (30%) had laboratory evidence of infection, particularly malaria (positive blood smear), brucellosis (agglutination titer $> 1:320$), tuberculosis (positive acid fast stain), and urinary schistosomiasis (nonquantitative examination of urine for ova). Clinical status was not reported.

Following these pilot observations, a placebo-controlled study of iron supplementation was performed in 137 iron-deficient individuals (84). Treatment was continued for 30 days, during which active surveillance for infection was maintained. In the 71 subjects receiving iron replacement therapy, there were 36 "infectious" processes, including 29 febrile episodes in 27 (38%)

subjects, compared with only 7 episodes and 6 fevers occurring in 5 of 66 (7.6%) placebo-treated controls. The most common specific infection observed was malaria, accounting for 13 episodes in untreated subjects, but only 1 in control subjects. This excess of fevers in the treated group occurred during days 20–30 of observation. Brucellosis and tuberculosis were also diagnosed more frequently in the iron-treated patients on the basis of antibody titer and acid fast stain of drainage from infected tissue (lymph node in two patients and breast in one).

In an earlier study in Central Africa, Murray et al also observed that malaria attacks were most common during refeeding after famine, and were associated with hyperferremia (82). However, an alternative interpretation can be offered to explain some results of this study. It is possible that iron repletion improved T-cell function, thus leading to an improved *Brucella* antibody response in previously infected patients and an enhanced inflammatory response to pre-existing tuberculous infections, as well as increasing phagocytic cell function, manifested as purulent lesions with fever due to IL-1 release. It is somewhat strange that schistosome ova were found in 11 of 71 untreated individuals compared to only 2 of 66 placebo-treated subjects. Since it is biologically impossible for this to reflect new infection during the 30-day observation period, the findings raise the question of some hidden bias in sample selection. At the very least, these data are in need of independent confirmation and careful reinterpretation.

Iron deficiency in infancy has been recognized in many populations, particularly in infants from low socioeconomic groups. Iron deficiency in these infants has historically been regarded as damaging to health, which persuaded some clinicians to advise routine prophylaxis with parenteral iron during the first weeks of life (105). Several studies have shown, however, this may have detrimental effects. In 1970 the National Women's Hospital in Auckland, New Zealand, began routinely to administer iron-dextran complex during the first week of life as prophylaxis against iron-deficiency anemia. In the period 1971–1972, 21 cases of *E. coli* meningitis were reported, 18 of which followed within 5 days of administration of iron dextran (6, 33, 34). In the years preceding 1970 only one or two cases of *E. coli* meningitis were observed each year. During the same period in New Zealand at Hawke's Bay, Barry & Reeve (5) observed a similar phenomenon in Polynesian infants. During a period of iron prophylaxis, the neonatal sepsis rate was 17 per 1000 births, but this fell dramatically to 2.7 per 1000 on cessation of the iron supplementation program.

A major deficit in both of these studies is that concurrent controls were not available. However, Barry & Reeve reported that neonatal sepsis rates in European infants during the same periods remained steady throughout and were comparable with sepsis rates observed in Polynesian infants when iron supplementation was discontinued. Although the dangers of giving iron have

been considered and debated for more than a century, there is as yet no satisfactory randomized, placebo-controlled, double-blind trial of iron supplementation for iron deficiency that assesses its impact on the prevalence of infection. The trial of Oppenheimer et al in Papua New Guinea should go a long way to answer these questions, certainly with respect to the effects of iron supplementation in infancy and early childhood (88).

The proposed detrimental effect of iron is also inferred from reports relating iron overload and other causes of increased serum iron concentration to the prevalence of infection. For example, hemochromatosis, an inherited disorder of iron metabolism resulting in elevated serum iron concentration and massive accumulation of iron in many organs of the body, has been associated with an increased risk of infection from *Yersinia enterocolitica* and *Entamoeba histolytica* (25, 80). Iron overload is also seen in South African native men as a result of excessive ingestion of iron in home-made beer brewed in iron vessels. Severe hepatic amebiasis is more common in these men compared with native women whose exposure to the same parasite would appear to be identical (25). In contrast the nomadic Masai who inhabit the Rift Valley are curiously free of amebiasis, which has been attributed to their custom of drinking milk, resulting in mild iron-deficiency anemia and low transferrin saturation. Iron supplementation of the Masai during a one-year period not only increased hemoglobin and transferrin saturation, but also resulted in a marked increase in prevalence of amebas in stool- and smear-positive malaria (85).

The dangers of the hyperferremic state are often considered to be exemplified by the enhanced risk of bacterial infection in conditions associated with decreased red cell survival, notably thalassemia and sickle cell anemia (113). Serum iron concentration is elevated during acute malaria attacks as a result of red cell destruction, and the increase in bacterial infection, particularly salmonellosis, has been attributed to this change in circulating iron levels (12). A similar increase in *Salmonella* and other bacterial infections has been observed during the severe hemolytic phase of bartonellosis (22); however, the mechanism is only indirectly related to iron, which is taken up by the mononuclear phagocyte system where it results in impaired clearance of circulating microorganisms (RES blockade) (54).

Although these data are used to support the hypothesis that high levels of free circulating iron are detrimental to the host and increase susceptibility to infection (112, 113), none of the above-cited studies clearly show causality. One must remain skeptical of such an association because the variables might easily be dependent.

The Current Status of the Paradox

From the data discussed above, it is difficult to develop a unifying hypothesis that explains such apparently disparate views regarding the influence of iron status on susceptibility to infection. Accepting our reservations about the

design and interpretation of data in many of the studies cited, we currently and cautiously entertain the possibility that indeed both points of view are partially true.

Iron deficiency in an otherwise well-nourished individual with normal serum transferrin may well be a risk factor for infection. Some studies supporting this point of view were performed in children in industrialized nations who did not have severe PEM and in whom serum transferrin levels would be normal or nearly normal. Therefore, iron supplementation, particularly when given orally, is unlikely to produce the very high levels of circulating free iron that have been observed in infants receiving parenteral iron-dextran. Thus, to the extent that iron deficiency impairs immune function it may result in enhanced infection, and yet iron administration is not harmful. In contrast, in children with overt PEM and very low serum transferrin levels, iron supplementation (whether oral or parenteral) can result in rapid increments in serum free iron, which may indeed be advantageous to invading pathogens. The "physiological" mild iron deficiency observed in early infancy can be considered to be an intermediate state in which the deficiency is not harmful at the same time that iron is less available to pathogens. It may be part of nature's protective design and need not and probably should not be aggressively treated. Whether or not this applies to oral iron supplementation to prevent or mitigate iron deficiency is not certain but seems unlikely.

Nonetheless, it would seem prudent to direct future studies to define iron-deficiency anemia clearly and to determine both its association with and the binding capacity of iron transport proteins to the prevalence of infection. In addition, the presence of other potentially confounding nutrient deficiencies must be ruled out and the effect of iron supplementation determined. The results of iron supplementation must also be related to changes in bound and free serum iron as well as the hematopoietic response. Only then will we be able to make reasonable judgments about the specific effects of iron nutriture on infection.

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