Nucleic Acids and/or Their Components: A Possible Role in Immune Function

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Summary Dietary sources of nucleic acids and/or their components, have not been considered essential for normal growth and development. However, growing evidence shows that the compounds regulate various steps of the immune system and demonstrate the necessity of the compounds in the response to immunological challenge. The significance of exogenously administered purine or pyrimidine bases, nucleotides, and nucleosides in the immune response is reviewed.

Key Words nucleoside, nucleotide, infection, immunity, parenteral formula, enteral formula

Nucleic acids and/or their components in the diet have not been considered essential for normal growth and development because it was generally assumed that living organisms, including humans, could synthesize adequate amounts of the compounds required for normal growth and development and that dietary sources are not utilized. This assumption was based on studies done on normal healthy individuals who were not confronted with an immunological challenge (1,2). Recent studies have documented that the gut and the immune system depend mainly on salvage of purine and pyrimidine bases and that the de novo synthesis is not adequate to meet the demand of various metabolically active cells or tissues. This report reviews current knowledge on the role of nucleic acids and/or their components.

Nucleotides are low molecular weight biological compounds that are involved in almost all biochemical processess. They consist of either purines or pyrimidines (nitrogen containing bases). The major purine bases are adenine and guanine, and the major pyrimidines are thymine, cytosine, and uracil. Nucleotides are the building blocks of DNA and RNA and play essential roles in structural, metabolic, energetic, and regulatory functions particularly as components of adenosine triphosphate and other nucleotide triphosphates and in many co-enzymes. The daily

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requirements of nucleic acids from all sources in the adult is 2 g/day (3), with the
maximum safe limit of RNA/DNA being 4 g/day (4). The daily dietary intake of
nucleic acids for Japanese adults is estimated to be 500–900 mg/day; whereas the
intake for Americans is 1,000–2,000 mg/day (3). Beef, chicken, pork, lamb, livers,
meat extracts, mackerel, anchovies, and sardines are found to contain high values
of purines (150–800 mg/100 g); whereas fish, seafoods, beans, peas, lentils, and
mushrooms contain moderate amounts (50–150 mg/100 g). Vegetables, cheese,
potatoes, eggs, fruits, cereals, and milk are found to contain very low levels of
purines (0–20 mg/100 g) (5). The data is typical of dietary programs for the
treatment and prevention of gout and therefore only the purine contents are shown.
However, most dietary nucleic acids occur in the form of RNA/DNA mixtures,
therefore the purine and pyrimidine bases must be present in comparable amounts. In
humans and mammals maintained on normal regular diets, deprivation of nucleic
acids (nucleotides) seemed unlikely to occur. However, such deprivation may
occur in patients solely on parenteral nutrition, elemental or semi-purified diets, as
nucleic acids are not supplemented in these feeding formulae. Although human
breast milk contains appreciable amounts of nucleic acids (6), most infant feeding
formulae are not fortified with nucleic acids.

Earlier reports (1, 7, 8) have shown that exogenous supplementation of nucleic
acids in diets is not necessary for normal growth and development as the body
needs are met de novo. However, Leleiko et al. (9) noted a significant decrease in
RNA in the small intestine of rats fed RNA-free diets. Uauy et al. (10) noted that
dietary nucleosides (adenosine, guanosine, cytidine, and uridine) enhanced gut
growth and maturation of the intestine in weanling rats. Van Buren et al. (11, 12)
demonstrated that dietary nucleotides enhanced in vitro and in vivo cell-mediated
immunity to alloantigens and cardiac allografts. We showed that mice fed
nucleotide-free diet containing 21% casein showed no recovery of the immune
response (13), and that the host response against bacterial and fungal pathogens
was decreased (14, 15). These findings suggest that exogenous supply of nucleic
acids and/or their component from dietary sources may be beneficial in maintaining
normal body functions.

The digestion and absorption of these compounds involve complex chains of
reactions in humans and mammals. Dietary nucleotides are ingested as nucleoproteins
derived from nuclear material. Digestion of nucleoproteins is initiated by
proteases. Nucleic acids undergo partial hydrolysis in the stomach and are then
subjected to pancreatic nucleases and phosphoesterases to yield nucleotides and
nucleosides. The presence of the charged phosphates of nucleotides greatly impede
their transport across cell membranes. However, mammalian cells possess intracellular (16)
and/or extracellular (17) nucleotidases which remove the charged phosphates, thus facilitating their transport across the membrane. The dietary
nucleotides which reach the cell cytoplasm in the form of nucleosides, are then
utilized through the salvage pathway. Some cells and tissues in the human body,
such as erythrocytes, polymorphonuclear leukocytes, intestinal mucosa, bone
marrow, hematopoietic cells, brain cells are incapable of de novo synthesis of purine and pyrimidine bases and their nucleotides (18). These therefore need exogenously supplied nucleic acids that can be utilized via the salvage pathway for optimal function. Although the liver is the principal site for the formation of nucleic acids and their components to be salvaged and utilized by cells incapable of synthesizing the components de novo, in certain clinical conditions such as immune challenge, the endogenous supply may not be adequate for optimal function. Under these circumstances, the supply of nucleic acids from dietary sources is deemed necessary and could be beneficial in maintaining normal bodily and immune functions.

EFFECT OF NUCLEIC ACIDS ON HEPATIC GROWTH AND COMPOSITION

The use of nutritional support, particularly parenteral and enteral nutrition is often a key part of overall management and care in patients (with cancer, gastrectomy, hepatectomy, etc.) who undergo major operative procedures, and particularly in patients who sometimes develop life-threatening complications. Nutrients, such as amino acids, constitute the bulk of parenteral and enteral feeding formulations employed, as they are considered to provide the precursors for the synthesis of numerous structural and functional body proteins. Nucleic acids and/or their components have not been incorporated in these formulae partly because they were not considered essential for normal growth and development, and could be synthesized de novo. Relatively little effort has been expended to determine the role of these compounds in total parenteral nutrition (TPN) and total enteral nutrition (TEN).

Ogoshi and colleagues (19) noted that supplementation of a nucleoside-nucleotide mixture to the TPN solution in rats after hepatectomy resulted in earlier restoration of the nitrogen balance in the first 3 days than in the solution without supplementation. During the first 3 days after hepatectomy, the group given the TPN supplemented with nucleoside-nucleotide (N-TPN) showed a positive nitrogen balance whereas the standard total parenteral nutrition (S-TPN) group showed a negative nitrogen balance. The improved nitrogen balance in the nucleoside-nucleotide mixture group resulted in the acceleration of RNA and DNA synthesis leading to increased protein synthesis. Throughout the experimental period, the mean nitrogen balance remained higher in the N-TPN group compared to the S-TPN group. The supplementation had no unfavorable effects on the compositions of blood and urine, and particularly the metabolism of nucleic acids such as uric acid, β-alanine, and aminoisobutyric acid, and also significantly improved glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) levels. In a related study, Novak et al. (20) observed that hepatic cholesterol and lipid phosphorus were significantly higher in weanling mice fed nucleotide-free diet as compared to nucleotide-free diet supplemented with nucleosides and adenosine 5'-monophosphate, respectively. In the same study, the liver weight expressed as a percentage of body weight was significantly lower in the animals fed nucleotide-free
diet than in the 2 supplemented diets. Ohyanagi et al. (21) and Yamaguchi et al. (22) also observed that in the liver, extracellular nucleosides and nucleotides modulate hepatocyte growth and regeneration and play an essential role in the synthesis of glycogen. Clifford and Story (23) also documented that adenine supplementation markedly increased the activities of hepatic adenine phosphoribosyl-transferase 5'-nucleotidase, and adenosine deaminase, and the concentrations of free adenine in rats. Nilson et al. (24) reported that enhanced hepatocyte respiration improved survival after infusion of nucleotides. These findings suggest that provision of nucleic acids and/or their components is vital for liver regeneration and growth. The provision resulted in an increase of the nucleotide pool in the liver, acceleration of protein synthesis in muscle and liver, and improved nitrogen balance.

**NUCLEIC ACIDS AND IMMUNITY**

The basis of the immune system resides in the ability of subpopulations of lymphocytes to recognize, react, and interact in a specific manner to a wide variety of encountered stimuli. These responses are partly nucleic acid-dependent. An adequate supply of nucleic acids seems necessary for any cell type, particularly in the growing state. Recent studies suggest that purine and pyrimidine metabolism, and regulation of cell proliferation and function may be closely linked in lymphocytes than in any other cells. For example, most cancer chemotherapeutic agents (immunosuppressant) are analogues of purines and pyrimidines which interfere with lymphocyte functions excluding other cell types. Also defects in the catabolic pathways of nucleic acids result in many immunodeficiency diseases (25–28).

The functional integrity of the immune system is known to be altered by various dietary factors. Malnutrition affects the structure and function of every tissue and physiological system in the body including the immune system. Deficiencies of selenium, iron, zinc, pyridoxine, and fatty acids cause transient changes in thymocyte-dependent cell-mediated immunity. The absence of dietary nucleotides cause a significant decrease in many specific and non-specific immunologic responses in dietary hosts. The observed effects can be reversed by the addition of nucleotides (RNA and uracil) to the diet suggesting that pyrimidines play a larger role in the maintenance of cellular immune response. In a number of studies, Van Buren et al. (11) demonstrated that splenic lymphocytes from animals on nucleotide-free diet and a significantly decreased proliferative response to phytohemagglutinin (PHA) and as compared to animals fed nucleotide-free diet supplemented with RNA and uracil. The response to lipopolysaccharide (LPS) was affected slightly, indicating that a nucleotide-free diet affects the proliferative response to T-cells and not B-cells. In a related study, we noted that animals fed nucleotide-free diet and sensitized with sheep red blood cells (SRBC) or bacteria failed to show any differences in the serum antibody activity against the antigens, thus emphasizing a down-regulation of T-cell mediated response by a nucleotide-free diet (29). Szondy
and Newsholme (30) documented that the addition of adenosine and uridine to the cultured medium supported the proliferative process of rat cervical lymph node T-lymphocytes after stimulation by concanavalin A. Similar blastogenic responses of spleen cells were observed by Yokoyama and colleagues (31). Such an effect on the immune system has been confirmed and characterized by investigating in vivo functional activity. Van Buren et al. (32) demonstrated that dietary nucleotides influence the induction and expression of immune cell surface markers and enhances their immunocompetency. Using a fluoresceinated monoclonal antibody reagents against various cell surface markers (anti-0, anti-Lyt 1, anti-Lyt 2, anti-immunoglobulin markers), it was noted that mice fed a nucleotide-free diet had significantly lower Lyt 1+ cells and a decrease in positive theta cells. There was no change in Lyt 2 or immunoglobulin markers. In the same study, it was demonstrated that dietary nucleotides significantly augmented in vivo lymphoproliferative responses and expression of interleukin-2 (IL-2) which affected the activation and function of T-helper cells. These results indicate that nucleic acids and/or their components play a specific regulatory role in the proliferative process.

The relationship of nucleic acids and/or their components to cellular immune functions are increasingly becoming evident. The delayed-type hypersensitivity (DTH) responses to various antigens has been advocated as a reliable means of assessing, predicting, and monitoring nutritional immunomodulation. We noted that the addition of RNA or uracil to nucleotide-free diet can be beneficial in restoring immune responses to foreign antigens. The DTH responses in mice fed nucleotide-free diet supplemented with RNA, or uracil were significantly higher than those fed nucleotide-free diet, when challenged with purified protein derivative, SRBC, and dinitrofluorobenzene (29). We also demonstrated that the addition of RNA and uracil to protein-free diet caused the recovery and/or improvement of the in vivo immune response in mice (13). Even though there was remarkable improvement in body weights, addition of 21% casein to the protein-free diet did not cause restoration of the immune response. The in vivo immunoproliferative response of popliteal lymph node (PLN) expressed as a stimulation index improved when nucleic acids were added to protein-free diet (Table 1). Similar results were reported by Pizzini et al. (33). Van Buren and colleagues (12) also demonstrated that in patients treated by renal allografting and who have been on total parenteral nutrition, the immune responses to allografting were lower compared to patients on total parenteral nutrition enriched with RNA. In experiments with cardiac transplants of BALB/c mice, the same workers noted that mice fed nucleotide-free diet showed a significant prolongation of heart allograft survival, but supplementation with RNA shortened the survival of the cardiac transplant (11). On the basis of these results, one can reasonably speculate that nucleic acids and/or their components have stimulating effects on cell-mediated immunity. Availability of nucleic acids is likely to be an important limiting factor in regulating the cellular immune response.

The effect of nucleic acids on the humoral immune system has also become
Table 1. Effect of various diets on in vivo PLN response.

| Dietary groups | Allo PLN (mg) | Syn PLN (mg) | SI (allogeneic/syngeneic) |
|----------------|--------------|--------------|--------------------------|
| PF-PF          | 3.1±0.6      | 1.2±0.2      | 2.9±0.7                  |
| PF-NF          | 2.9±0.2      | 1.4±0.1      | 2.2±0.2                  |
| PF-NFR         | 7.0±0.8      | 1.4±0.2      | 5.4±0.8*                 |
| PF-NFU         | 9.8±1.0      | 1.7±0.3      | 5.8±1.0*                 |
| F-F            | 8.6±1.2      | 1.5±0.3      | 6.7±1.3*                 |

BALB/c mice were fed protein-free diet (PF) for 10 days. The mice were then placed on the following diets: nucleotide-free 20% casein diet (NF), 2.5% RNA (NFR), 0.6% uracil (NFU), and normal rodent chow diet (F). At the onset of supplementation, mice were challenged with 10⁷ irradiated allogeneic (Allo) and syngeneic (Syn) splenic lymphocytes in the hind footpads, respectively. On the 7th day postchallenge, popliteal lymph nodes (PLN) were removed, weighed, and expressed as stimulation index (SI) difference between allogeneic and syngeneic lymph node weights. Data were modified from Kulkarni et al. (13). * Indicates significant difference from the PF-PF dietary group (p<0.05).

INFECTION STUDIES

Another aspect of the relative importance of dietary nucleotides is its role in both bacterial and fungal pathogens. The prevalence and incidence of sepsis in critically ill or immunocompromised hosts is an area of increasing concern to clinicians. As indicated above, the functional integrity of the immune system can be improved by dietary factors. There is enough clinical and experimental evidence
of attendant depression of host defense mechanisms together with increased infectious diseases. It is therefore reasonable to assume that dietary manipulations may be used to improve the host's immune system for therapeutic benefit. An example is the recent evidence of enhancement of the immune system by dietary nucleic acids against bacterial and fungal pathogens.

We (14) and Fanslow et al. (15) noted that the addition of RNA and uracil to nucleotide-free diet improved significantly the survival of mice and enhanced the immune response to both Staphylococcus aureus and Candida albicans, suggesting that RNA and uracil are essential nutrients required for regulation of the immune response against these organisms.

Methicillin-resistant S. aureus (MRSA) has been a problem to clinicians world-wide and management of MRSA infections is difficult (37). Although the microbiologic characteristics (38), antibiotic sensitivity (39), control measures (40), and nosocomial transmission of MRSA have been reported (41), there were no attempts through nutritional means to improve the host resistance against MRSA infections. We have recently demonstrated that intraperitoneal administration of a nucleoside-nucleotide mixture either before (42) or after (43) challenge with MRSA markedly increased survival (Fig. 1). The profound improvement of resistance to the infection by the mixture was certified by the significant reduction of MRSA organisms recovered from the spleen and kidney of the surviving mice 20 days post-challenge. These results suggest the need for nucleic acids in total TPN

Fig. 1. Survival rates of BALB/c female mice administered nucleoside-nucleotide mixture (○) or saline (■) intraperitoneally following inoculation with viable MRSA \((2.1 \times 10^8 \text{CFU/ml})\) organisms. Number of mice were 25 and 24 for both experimental and control groups, respectively. Asterisk indicates significant difference from the control group \((p<0.01)\). Data from Adjei et al. (42).
and TEN formulae following induction of sepsis.

NUCLEIC ACIDS AND GUT FUNCTION

The gut plays a very important role, not only for digestion and absorption of diets, but also as a barrier function to protect the internal milieu from pathogens. Bacterial translocation from the gut is emerging as an important pathogenic phenomenon and are potential sources of septicemia in critically ill or immunocompromised hosts (44, 45). Bacterial translocation can be stimulated by a disruption in the gastrointestinal microflora due to surgery, antibiotics, radiation, and impaired immune function (46). Although the process of translocation is complex, an intact intestinal immunity and microbial homeostasis are necessary for prevention of bacterial translocation from the gut. Nutrition appears to be an important factor for an intact intestinal immunity. Although current nutritional feeding formulae, enteral and parenteral, can achieve an appreciable and acceptable nutritional response, several workers have demonstrated that the formulae seem unable to improve or enhance intestinal immune function.

Nutrients such as nucleoside-nucleotide mixture, glutamine, and arginine, have been found to have stimulating properties on gut function. In the course of studying the influence of nucleic acids and/or their components supplementation in parenteral nutrition, Iijima and associates (47) observed that a nucleoside-nucleotide mixture averted the intestinal mucosa atrophic changes triggered by TPN. Strikingly, the effects tended to be significant in comparison to glutamine-enriched formula. Moreover, protein, DNA, and RNA contents of the small intestinal mucosa were higher in the nucleoside-nucleotide mixture-supplemented group as against the TPN; and that the addition of nucleoside-nucleotide to the standard TPN subsequently led to increased proliferative activity of the crypt cells and improved the mucosal growth and maturity of the small intestine. Yokoyama and colleagues (48) showed that standard TPN nutrition formula enriched with nucleosides and nucleotides administered to rats resulted in a significant reduction of S. aureus and facultative anaerobic organisms from the mesenteric lymph nodes (MLN), compared with rats administered the standard formula without supplementation.

Nucleoside supplementation increased the rate of maturation and growth in the young rat as determined by mass, RNA, DNA, and protein concentrations and activity of brush border enzymes (10). Nucleotide supplementation restored the biochemical atrophy of the small intestine at proximal and distal sites, and improved intestinal development after induction of chronic diarrhea (49, 50). In a separate study, we observed that intraperitoneal (51) and oral (52) administration of nucleosides and nucleotides inhibited the incidence of endotoxin-induced bacterial translocation, enhanced survival, decreased intestinal injury, and reduced the recovery of colony forming units of both Gram-negative and Gram-positive enteric and facultative microorganisms in protein-deficient mice (Fig. 2). A preliminary
Fig. 2. A: Terminal ileum from a control (20% casein) mouse killed 48 h after an IP endotoxin (50 μg) administration showing normal mucosal architecture (×200). B: Terminal ileum from a protein-free diet (PFD)-fed mouse killed 48 h after an IP endotoxin (50 μg) administration. Atrophy of the intestinal mucosal wall is evident. The villous height and crypt depth are both diminished, with a marked decrease in the thickness of the intestinal wall (×200). C: Terminal ileum from a protein-free diet supplemented with 0.5% nucleoside-nucleotide (PFD+NNM) fed mouse killed 48 h after an IP endotoxin (50 μg) administration. Trophic changes of the intestinal mucosa and wall are comparable to that of A. The villous height, crypt depth, and wall thickness are well developed and remain intact (×200). Data from Adjei et al. (52).

Study over a 3-month period in young infants suggested that a nucleotide-supplemented diet decreased the incidence of diarrhea (53).

Nucleotides also modify the type and growth of the intestinal microflora.
Tanaka and Mutai (54) observed that the addition of nucleosides to bifidobacteria in minimal culture media in vitro enhanced their growth. Gil et al. (55) also reported that young infants fed nucleotide-supplemented formula had higher percentages of fecal bifidobacteria and lactobacilli, and lower percentage of Gram-negative enterics compared to formula-fed infants. These results emphasize that the addition of nucleotides and nucleosides to the diet may have several benefits for the development, maturation, and repair of the gastrointestinal tract.

NUCLEIC ACIDS AND HEMOPOIESIS

Various nutritional components are known to affect the body’s immune response. These effects can be beneficial or detrimental. Cells of the immunologic system include those that participate in alloimmune responses, as well as cells that are dedicated to hematopoiesis. Van Buren et al. (12) and Kulkarni et al. (56) recently reported that nucleotide-free diet suppresses both allograft and graft-versus-host response. Thus, there is evidence in the literature that diet may potentiate hematopoiesis.

Growth and differentiation of hematopoietic cell precursors in vivo and in vitro is regulated by colony-stimulating factors. We demonstrated that dietary nucleotides function as modulatory nutrients for hematopoiesis in mice (57). Supernatants prepared from activated spleen cells from mice on various diets were tested for both IL-2 and interleukin-3 (IL-3) activity. These 2 lymphokines act as progression factors during the early G1 phase of the cell cycle, causing the target cells to move into the S phase and resulting in proliferation of mature T-cells (IL-2) (58) and proliferation and differentiation of T-cell precursors (IL-3) (59). When bone marrow cells from control chow-fed animals were cultured with supernatant from mitogen-activated splenocytes of animals on nucleotide-free diet and nucleotide-free diet supplemented with RNA and uracil, the nucleotide-free diet supernatants significantly decreased the bone marrow proliferative response compared with the response observed with RNA and uracil. When stimulated with purified IL-3, RNA bone marrow cells had higher levels of Thy 1.2 or Lyt surface markers. In the in vivo splenic colony formation assay, spleens from RNA and uracil fed animals had a significantly higher number of colonies than spleens from nucleotide-free diet-fed animals (57). These studies suggest that nucleotide-free diet decreases both the in vivo lymphoproliferative response to alloantigen and hematopoietic growth factor production, rendering the host splenic environment deficient for stem cell growth.

In a related study, Rudolph et al. (60) noted that spleen, thymus, bone marrow from mice fed nucleotide-free diet had a significantly higher number of cells positive for terminal deoxynucleotidyl transferase, a specific marker for immature T-cells as compared with lymphoid cells from RNA diet-fed mice, suggesting that there were increased numbers of null of immature T-cells in lymphoid organs of nucleotide-free diet-fed mice. In another development, we observed that nucleotide-free diet...
supplemented with nucleosides and nucleotides stimulated the proliferation, differentiation, maturation, and function of peripheral blood neutrophil number in mice challenged with MRSA (61) or treated with cyclophosphamide (62). The supplemented diet group subsequently led to increased incorporation of bromodeoxyuridine (an analogue of thymidine) into the S phase of the bone marrow cells as compared to the non-supplemented group (Fig. 3). These results emphasize that nucleotide-free diet affects hemapoietic growth factor production in vivo and in vitro, resulting in an immunodeficient state.

CONCLUSION

In conclusion it is evident that the addition of nucleic acid and/or their components to elemental or chemically defined diets can be beneficial in improving bodily and immunologic functions. Although the liver is a major site for the formation of nucleic acids and/or their components in adequate amounts, in certain clinical conditions the endogenous supply may not be sufficient for maximal function. This requirement is apparent with stress such as during periods of infection. These hosts are often immunocompromised and may be likely to develop complications of sepsis and infections caused by opportunistic pathogens, particu-
larly MRSA; the incidence of which perhaps may be reduced or prevented by provision of adequate amounts of diets containing nucleic acids and/or components. This report confirms the growing evidence that provision of elemental or semi-purified diets supplemented or enriched with nucleic acids and/or their components may be one such modality for the enhancement of the immune system of immunocompromised hosts.

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