

EDITORIAL

Dietary nucleic acids and Th1/Th2 balance: a clue to cow's milk allergy?

Nucleotides consist of a nitrogenous base (either a purine or a pyrimidine), a pentose sugar (either ribose or 2'-deoxyribose) and one or more phosphate groups; nucleosides lack the phosphate groups. The major purine bases are adenine, guanine, hypoxanthine and xanthine and the major pyrimidine bases are uracil, thymine and cytosine. Purine and pyrimidine nucleotides play a major role in cellular functions (Table 1). Purines and pyrimidines can be formed by *de novo* biosynthesis, using amino acids as the source of the nitrogen and some of the carbon atoms. Biosynthesis of nucleotides consumes a significant amount of energy. Bases and nucleosides are generated in the digestive tract largely by bacteria-mediated hydrolysis of DNA, RNA and nucleotides which have been consumed in the diet; these products of digestion are readily absorbed [1] and provide a source of precursors for nucleotide synthesis which has a lower energy cost than *de novo* synthesis. However, on a typical Western diet the bulk of purines consumed in the human diet are excreted as uric acid [2]. Clearly all cellular material that is consumed in the diet will contain nucleic acids and nucleotides. Milk too contains nucleic acids (mainly RNA) and nucleotides. Carlson [3] reported a nucleic acid concentration of 1400 $\mu\text{mol/L}$ in human milk and free nucleotide and cyclic nucleotide concentrations of 210 and 2–14 $\mu\text{mol/L}$, respectively, although the precise nucleotide content and composition changes during lactation [4,5]. It is often stated that the concentrations of nucleotides are much lower in cow's milk than human milk, but close examination of the literature [6], indicates that this is not strictly so (Fig. 1); indeed at some points during lactation the concentration of some of the common nucleotides is higher in cow's milk than human (Fig. 1). Sheep milk contains very high nucleotide concentrations, several-fold higher than human milk [6]. Orotate is a major nucleotide of cow's milk [7] and this nucleotide is, apparently, poorly salvagable by human infants [7].

Given the wide range of cellular functions of nucleotides (Table 1), it comes as no surprise that activation of T lymphocytes causes a rapid increase in the synthesis of nucleotides [8], which are required immediately for the increase in metabolism to provide energy and later for synthesis of nucleic acids (RNA for protein synthesis and DNA for cell division). Thus, exogenous nucleotides supplied by the diet could potentially increase immunity by contributing to the pool of nucleotides available to lymphocytes, and other

leucocytes, and so sparing the need for complete *de novo* synthesis. Because nucleic acids and nucleotides are hydrolysed to nucleosides before cellular uptake, it is not strictly necessary to discriminate between nucleic acids, polynucleotides, nucleotides and nucleosides when surveying the literature.

Yeast RNA increased the number of antibody-producing cells and the production of immunoglobulin (Ig) M and IgG in response to T-cell-dependent stimuli but not to T-cell-independent stimuli or B-cell activators *in vitro* [9–11]. Depleting cell preparations of CD4⁺ cells nullified the action of RNA [10]. Adding RNA or nucleotides to the diet of mice enhanced T-cell-dependent antibody responses [12], increased survival to bacterial and fungal challenges [13–17], and promoted allogeneic graft rejection, alloantigen-induced lymphocyte proliferation and delayed cutaneous hypersensitivity [18,19]. These data suggest that dietary nucleotides influence immune responsiveness primarily by enhancing the function of helper T (Th) cells. In accordance with this, spleen cells from mice fed a nucleotide-rich diet produced more interleukin-2 (IL-2) [20] and expressed higher levels of the IL-2 receptor [21] than those from mice fed a nucleotide-free diet. A nucleotide/nucleoside-enriched diet resulted in much higher interferon- γ (IFN γ) and IL-5 production by keyhole limpet haemocyanin (KLH)-stimulated murine spleen cells taken from KLH-sensitized mice than a nucleotide/nucleoside-free diet [22]; the levels of IFN γ and IL-5 production were enhanced by culturing the cells from mice fed the nucleotide-free diet in the presence of polynucleotides [22]. The nucleotide-free diet resulted in much lower levels of mRNA for IL-2, IFN γ , IL-4 and IL-5 than did the diet containing the nucleotide/nucleoside mix [22]. This study indicates that addition of nucleotides to the diet promotes the activity of both Th1 and Th2 lymphocytes. Close examination of the data of Jyonouchi *et al.* [22] leads to the view that adding nucleotides/nucleosides to the diet promotes preferential development of the Th1 phenotype (compared with the nucleotide-free situation), although this was not discussed in that paper. A paper in the current issue examines this further [23]. In this study mice were fed a nucleic acid-free diet or a diet containing nucleic acids (a 50:50 mix of salmon DNA and yeast RNA at the level of 1.2 or 2.4% by weight of the diet) for 4 weeks. Mice fed the nucleic acid-rich diets had lower circulating concentrations of IgM, IgG, IgE, and IgG₁ but not of IgG_{2a} than mice fed

Table 1. Cellular roles of nucleotides

Role	Example(s)
Structural component of DNA (cell division)	2'-Deoxyriboadenosine 5'-monophosphate (dAMP) 2'-Deoxyriboguanosine 5'-monophosphate (dGMP) 2'-Deoxyribocytidine 5'-monophosphate (dCMP) 2'-Deoxyribothymidine 5'-monophosphate (dTMP)
Structural component of RNA (protein synthesis)	Adenosine 5'-monophosphate (AMP) Guanosine 5'-monophosphate (GMP) Cytidine 5'-monophosphate (CMP) Uridine 5'-monophosphate (UMP)
Source of chemical energy	Adenosine 5'-triphosphate (ATP)
Intracellular signal transduction	Cyclic adenosine 3',5'-monophosphate (cAMP) Cyclic guanosine 3',5'-monophosphate (cGMP) Guanosine 5'-triphosphate (GTP) Guanosine 5'-diphosphate (GDP)
Regulation of enzyme activity	ATP Adenosine 5'-diphosphate (ADP) AMP
Formation of activated intermediates in biosynthetic reactions	Uridine 5'-diphospho-glucose (glycogen synthesis) Cytidine 5'-diphospho-diacylglycerol (phospholipid synthesis) Cytidine 5'-diphospho-choline (phospholipid synthesis) Uridine 5'-diphospho-galactose (ceramide and sphingolipid synthesis)
Component of co-enzymes	Co-enzyme A (includes adenosine 5'-diphosphate) Nicotinamide adenine dinucleotide (NAD) and related compounds (include adenosine 5'-monophosphate) Flavin adenine dinucleotide (FAD) and related compounds (include adenosine 5'-monophosphate)
Covalent modification of proteins	Poly ADP-ribosylation (involved in apoptosis)

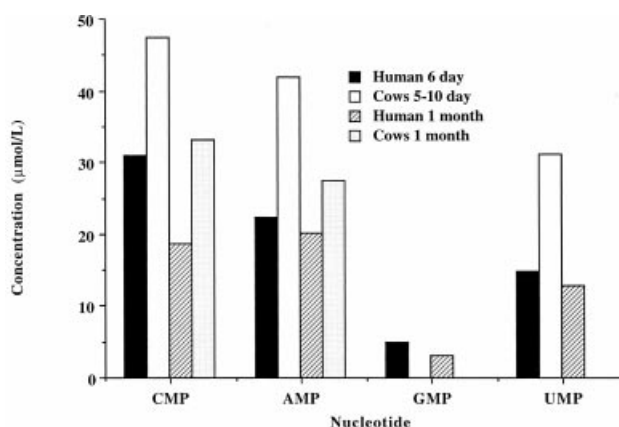


Fig. 1. Concentrations of nucleotides in human and cow's milk. Data are taken from [4,6]. GMP was not found in cow's milk at 5–10 days or 1 month lactation and UMP was not found in cow's milk at 5–10 days or 1 month lactation.

the diet without added nucleic acids. Mice fed the nucleic acid-rich diets and challenged with ovalbumin had lower circulating concentrations of ovalbumin-specific IgE and IgG₁ but not of IgG_{2a} than mice fed the diet without added nucleic acids. Ovalbumin-stimulated spleen cells from ovalbumin-sensitized mice fed the nucleic acid-rich diets produced less IL-4 and IL-10 and more IFN γ than cells from mice fed the diet without added nucleic acids; IL-2 production was not different among the dietary groups. It is concluded that a nucleic acid-free diet results in skewing towards a Th2-dominated response and that addition of nucleic acids to the diet promotes a shift towards a Th1-dominant response. Using the IFN γ /IL-4 ratio after ovalbumin stimulation as an indicator of the Th1/Th2 balance it can be calculated that the addition of nucleic acids to the diet at the level of 1.2% increased the ratio from 0.13 to 0.75, while addition of nucleic acids to the diet at the level of 2.4% further increased the ratio to 1.17. Thus, moving from a diet devoid of nucleic acids to a diet rich in nucleic acids promotes a strong shift away from the Th2 and towards the Th1 phenotype.

How do the dietary regimens used in these animal feeding studies relate to the human situation? Most human diets are rich in nucleic acids and in nucleotides [2]. The few exceptions to this would include patients receiving nucleic acid-, nucleotide- or nucleoside-free total parenteral or enteral nutrition or infants receiving solely nucleic acid-, nucleotide- or nucleoside-free formula. It is not immediately apparent what the absolute intake of nucleic acids/nucleotides/nucleosides is in humans, but the content of purine bases in common foods and beverages is known [2] and many foods are very rich in these compounds. On a typical mixed diet most humans would consume significant amounts of nucleic acids and nucleotides. In addition, through dietary protein they consume the substrates for *de novo* synthesis of purines and pyrimidines (it should be noted that, in contrast to a statement made by Sudo *et al.* [23] nucleic acids are not essential dietary components). Is there any evidence in the human setting for an immunological effect of dietary nucleic acids, nucleotides or nucleosides? Carver *et al.* [24] compared IL-2 production by blood mononuclear cells from breast-fed infants or those fed formulae with and without added nucleotides. Generally, IL-2 production was higher in the breast-fed and formula plus nucleotides groups than in the group fed nucleotide-free formula. While this study agrees with the general conclusion of Sudo *et al.* [23] of nucleotides promoting a Th1 response, it should be noted that Sudo *et al.* [23] found no difference in IL-2 production by ovalbumin-stimulated spleen lymphocytes from mice fed nucleic acid-free or nucleic acid-containing diets. Carver *et al.* [24] did not examine IFN γ or IL-4 production, which would have provided a direct comparison between human infants and this new animal study.

The paper of Sudo *et al.* [23] is important because it examines the influence of dietary nucleic acids on the balance of Th1 and Th2 responses using an appropriate model of allergen (ovalbumin) sensitization. It suggests that one dietary component, nucleic acids (which will provide the components for nucleotide synthesis), plays a role in altering the balance between Th1 and Th2 responses. The role of individual nucleotides in altering this balance deserves attention. Allergic diseases often develop due to feeding of cow's milk [25,26] and can be protected against by breast feeding [27]. As indicated above, cow's milk reputedly contains lower concentrations of some nucleotides than human milk and certainly has a different profile of nucleotides. Thus, the nucleotide level and profile in milk could play a role in protecting against development of allergy. During pregnancy the immune response becomes Th2 skewed [28]. One key factor in preventing the development of allergy in the offspring might be the efficiency by which immune responses become redirected towards the Th1 phenotype after birth. The paper by Sudo *et al.* [23]

suggests that components of milk (nucleic acids and nucleotides) may play a role in this redirection. The mechanism by which they might bring about this redirection is unclear.

Bifidobacteria grow better in the presence of nucleotides [29] and the faeces of human infants fed breast milk or formulae containing nucleotides contain more bifidobacteria, and less enterobacteria, than those of infants fed nucleotide-free formula [30–32]. This suggests that dietary nucleotides might act to alter the composition and balance of the microflora in the infant gut. This might in turn play a role in regulating development of Th1 vs Th2 phenotypes. It was recently shown that the percentage of faecal microflora contributed by bifidobacteria was lower in allergic than in nonallergic 2 year olds [33], although the absolute numbers of bifidobacteria were not different. Taken along with the data of Sudo *et al.* [23] these observations suggest an intriguing interaction of effects of dietary nucleotides to influence Th1/Th2 balance. Sudo *et al.* [23] have shown in their study that the effect of dietary nucleic acids on the Th phenotype is independent of any effect on gut microflora.

It is worth noting that most, if not all, semisynthetic diets fed to laboratory animals (e.g. standard laboratory chow) do not contain nucleic acids, nucleotides or nucleosides. Serum (including fetal calf serum) contains free bases, nucleosides and nucleotides, the medium used for lymphocyte cultures contains all of the precursors necessary for nucleotide synthesis (so long as glutamine is added) and lymphocytes have a high capacity for *de novo* nucleotide synthesis [8].

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P. C. CALDER
Institute of Human Nutrition
University of Southampton
Bassett Crescent East
Southampton
SO16 7PX
UK