Natural Antibodies to Dietary Components

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Abstract: Natural antibodies (NAbs), present in the serum of vertebrates, are defined as antibodies that are produced without any previous infection, vaccination, foreign antigen exposure or passive immunization. They can activate the classical pathway of complement leading to lysis of enveloped viral particles long before the adaptive immune response comes into play. Many NAbs are directed against the disaccharide Gal-α-1,3-Gal (abbreviated as α-Gal), which is found as a terminal sugar on glycosylated cell surface proteins, and are generated in response to production of this disaccharide by the human gut bacteria. NAbs have been found to several self-antigens including the human ABO blood group antigens. NAbs have been shown to exhibit 'polyreactivity' or 'oligoreactivity' with several antigens including some self-antigens. Since human gastrointestinal system is exposed to a wide variety of food antigens, study of NAbs to various dietary components in humans is important as it may provide information about food components that are more likely to be immunogenic. Further, NAbs to dietary components may provide immediate immune response for combating bacterial and viral infections. Among the dietary proteins, several lectins/agglutinins (garlic, soybean, peanut, banana and wheat germ) have been shown to induce NAbs in healthy humans, besides other proteins such as avidin, bromelain, lactoferrin and alliinase. Polysaccharides which have been reported to induce NAbs are pectic polysaccharides from some plants, β-glucans from yeast and teichoic acid from Gram-positive bacteria; in the case of cod, polymannuronic acid present in seaweed and chitosan induce NAbs. Foods supplemented with ω-3 fatty acids, phospholipids or soy components have been shown to increase the levels of NAbs to certain endogenous mediators and proteins in humans. The review also covers a short account of the significance of NAbs to dietary components.

Keywords: Carbohydrates, dietary components, dietary lectins, food proteins, natural antibodies, polysaccharides.

1. INTRODUCTION

The immune system elicits two types of effector responses, viz., innate and adaptive immune response. The degree of adaptive immune response against a foreign molecule is based on the innate immune response against it [1]. In adaptive immune response, the antigens are usually neutralized by two different pathways: (a) cellular immune response wherein the antigen is neutralized by cellular pathways (mostly by T-cells), and (b) humoral immune response wherein the antigen is neutralized or removed by antibodies (produced by activated B-cells). Antibodies play a vital role in adaptive immune response by recognition of the antigen and elicit a response against it by way of complement activation, activation of macrophages, and other processes [2].

2. NATURAL ANTIBODIES AND THEIR PROPERTIES

The designation of natural antibodies (NAbs) is currently given to antibodies, natural or spontaneous antibodies (circulating in the sera of normal or non-immunized humans or animals) that have been produced in the absence of overt specific antigenic stimulation or passive immunization. The production of NAbs seems to be basically independent of internal or external antigenic stimuli. NAbs are thought to play an important role in host immunity; however, assessing the role of NAbs in immunity has been difficult, because in most cases their specificity is unknown [3, 4]. In some cases, food is found to be one of the important sources of antigenic stimuli for production of NAbs [5]; it has also been suggested that additional sources including exogenous components from the mother and the intestinal gut flora participate in this process. Interestingly, most of the NAbs are found to be polyreactive (multi-specific or cross-reactive) in nature [6, 7]. The cross-reactivity of NAbs towards molecules other than the actual stimuli (antigen) was found to be higher with increasing electronegative potential of proteins, mostly acetylated proteins [8]. The polyreactivity of NAbs could also be explained by the strength of the antigen (larger molecules are easily encountered by the immune system) and its stability in the digestive process [9]. It has been shown experimentally that the epitopes for natural polyreactive antibodies are rich in proline [10].

Controversially, in some studies the production of NAbs is triggered or augmented by commensal bacteria in the intestine, as these bacteria interact with cells of the gut-associated lymphoid tissues (GALT). Among the cells of the GALT, the vital cells which produce the NAbs are the well-known B-1 cells [11]. Generally, NAbs belong to the IgM class, although some belong to the IgG and IgA classes [12]. The

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major source of natural IgM antibodies that are generated during fetal or neonatal development is the long-lived, self-renewing B-1 subset of B-cells [13]. Recent studies have shown that some NAbs are synthesized by splenic $CD5^-$ B2 cells and peritoneal $CD5^+$ B1 cells [14]. The flow chart for the production of NAbs is shown in Figure **1**. NAbs are mostly encoded by germ-line variable (V) genes without extensive somatic mutations. Broadly, most of the NAbs displaying 'multi-reactivity' do show low affinity towards a wide range of self or non-self antigens. The binding avidity of NAbs ranges widely from 5×10^{-3} to 5×10^{-11} M [14].

2.1. Functions of Natural Antibodies

Several biological roles have been proposed for NAbs; however, till date there is no general agreement on their putative functions. NAbs may participate in a variety of physiological activities, from immune regulation, homeostasis and repertoire selection, to resistance to infections, transport and functional modulation of biologically active molecules [15]. Humans and other vertebrates contain 'natural antibodies' which are present in serum prior to viral or bacterial infections. The broad reactivity pattern of the NAbs may help to protect against a variety of pathogens not previously encountered [6]. NAbs help in avoiding the viral infections to vital organs, and are involved in elimination of viruses by enhancing the trapping of antigen in secondary lymphoid organs such as spleen and lymph nodes. Due to the high avidity of polymeric IgM, NAbs may contribute to the initial immune defense and to the control of invading pathogens until the immune system has time to launch a specific adaptive response [16, 17]. Recently, Panda *et al*. [18] showed that natural IgG recognizes a spectrum of bacteria through lectins like ficolin (group of oligomeric lectins usually specific for N-acetylglucosamine with subunits consisting of both collagen-like long thin stretches and fibrinogen-like globular domains) and mannose-binding lectin (MBL). Infection/inflammation condition markedly increased the affinity of natural IgG for bacteria associated with ficolins; after opsonization with IgG:ficolin complex, the bacteria were phagocytosed by monocytes *via* FcγRI. Their findings have provided a fresh perspective on NAbs in that they are not quiescent, but play a vital and immediate role in immune defense.

NAbs from the milk of healthy mothers were found to show catalytic activities [19]; hence, these antibodies function as 'abzymes'. It was demonstrated for the first time that the light chain of IgG catalyzes the reaction of DNA hydrolysis [19]. Polyclonal sIgA purified from the milk of healthy human mothers showed catalytic (protein kinase) activity which was mediated by the light chain of sIgA [20]. The secretory IgA antibody from human milk showed catalytic activity like phosphorylation of lipids [21]. IgG antibodies from human milk, their Fab fragments, and secretory IgA isolated from human milk possessed amylolytic activity towards malto-oligosaccharides and several artificial substrates [22]; however, secretory IgA also showed kinase activity towards oligo- and polysaccharides as substrates, i.e., the ability to transfer the phosphoryl

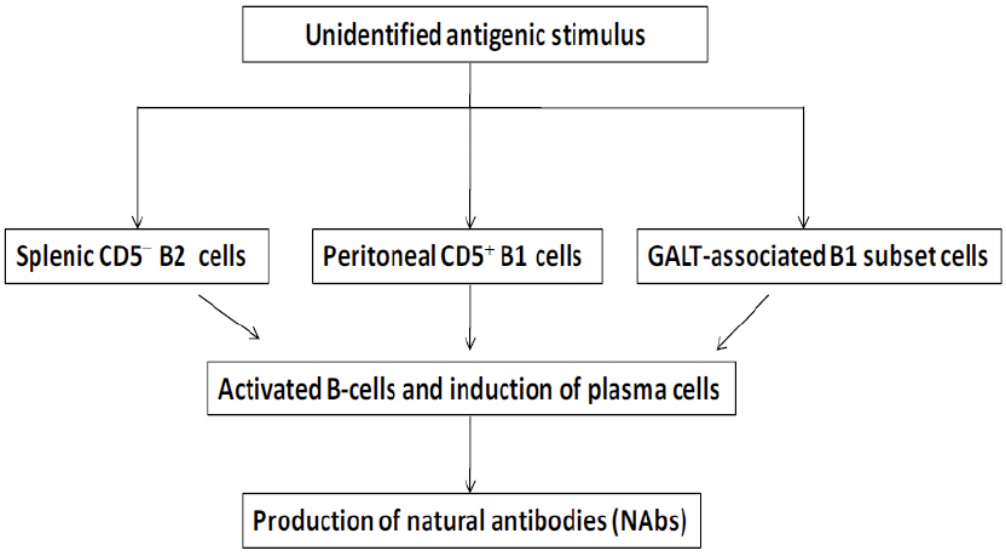

Figure 1: Flow chart depicting the production of natural antibodies (NAbs) in secondary immune organs by unidentified antigenic stimulus. GALT, gut-associated lymphoid tissue.

group from ATP; in fact, orthophosphate is a better substrate than ATP for this reaction [23].

NAbs to various self-antigens including the ABO blood group system, β-amyloid, senescent red blood cells, apoptotic cells and endogenous mediators have been investigated in depth and described in the literature. Further, vast amount of literature is available on the role of natural autoantibodies in various pathological conditions. Extensive coverage of these aspects of NAbs has recently been compiled in a volume on "Naturally Occurring Antibodies (NAbs)" [24]. The present review specifically focuses on the description of NAbs to foods or food components described till now by various researchers in this field.

3. NATURAL ANTIBODIES TO FOOD COMPONENTS

Humans are exposed to several foods in the course of a day, and food represents the largest antigenic load for the immune system. However, NAbs to only some foods or food components have been investigated in detail. These are compiled below with respect to the two major dietary components, viz., proteins and carbohydrates.

3.1. Natural Antibodies to Dietary Proteins

In the last two decades, several researchers have reported the presence of 'natural antibodies' in the serum of normal (non-immunized) humans against various dietary proteins which are present in their daily intake of foods. The dietary proteins that have been identified, till date, to react with NAbs in human serum are compiled in Table **1**.

3.1.1. Natural Antibodies to Dietary Non-Lectin Proteins

Egg is consumed widely, as part of the daily food intake, all over the world. A report on NAbs to the glycoprotein, avidin present in egg white was described from Israel [25]. In this particular study, human serum from healthy individuals (n = 270) contained NAbs to avidin to different extents; the affinity-purified NAbs to avidin were found to belong to the IgG and IgM classes [25]; the NAbs to avidin are capable of activating the complement system. The glycans present on avidin are not an absolute requirement for the interaction with natural anti-avidin antibodies.

The existence of NAbs in human serum against the garlic (*Allium sativum*) major protein alliinase (alliin lyase) has been reported from Israel [26]. Garlic is present in many cooked and processed foods as a flavor enhancer; traditionally, garlic is used as a medication for various diseases in the Indian system of medicine, Ayurveda. Normal human sera from a pool of 40 healthy adult donors of both sexes contain antibodies to garlic alliinase. The NAbs against garlic alliinase was purified by affinity chromatography using the corresponding immobilized antigen; the purified antibodies were mainly of the IgG and IgM classes. Anti-alliinase antibodies were highly specific. The immunogenic determinants present on garlic alliinase are shared by the human NAbs as well as polyclonal

Table 1: Dietary Proteins for which NAbs have been Detected in Vertebrate Serum

Sl. No.	Reactivity of NAbs towards	Class of Ig[a]	Antigenic source	Route of antigenic exposure	Ref.
1	Avidin	IgG, IgM	egg	ingestion	[25]
2	Alliinase	IgG, IgM	garlic	ingestion	[26]
3	*Allium sativum* agglutinin (ASA)	IgG, IgM	garlic	ingestion	[26]
	ASA-I	IgG (mice)	ASA-I	oral administration	[35]
	ASA-II	IgG (mice)	ASA-II	oral administration	[35]
4	*Erythrina corallodendron* lectin (EcorL)	IgG	*Erythrina corallodendron* (coral tree beans)	ingestion	[33]
5	Peanut agglutinin (PNA)	IgG	peanut	ingestion	[33]
6	Soybean agglutinin (SBA)	IgG	soybean	ingestion	[33]
7	Wheat germ agglutinin (WGA)	IgG	wheat	ingestion	[33]
8	Lactoferrin (LF)	IgM	human milk	ingestion	[30]
9	BanLec-1	IgG4	banana	ingestion	[32]

[a]Refers to human serum in all cases except where indicated otherwise.

antibodies evoked during the induced (experimental) immunization in rabbits [27].

Proteolytically active antigens such as bromelain (from pineapple stem) can stimulate both systemic and mucosal immune responses following repeated oral exposure in mice *sans* adjuvant; its enzymic activity is not lost in the gastrointestinal tract [28, 29]. NAbs to bromelain do not neutralize the proteolytic activity of bromelain.

The IgM NAbs in normal human sera reacted with a component of the protein coat of morphologically normal, human spermatozoa (SP80); this component has been identified as lactoferrin (LF), a bi-lobed 80 kDa glycoprotein of broad occurrence in body fluids including seminal plasma [30]. The IgM NAbs from a large cohort of normal human sera reacted with human milk LF and SP80 in the denatured state only. Further, the antibody reactivity was localized to the 88-residue peptide representing the C-terminal sequence of human lactoferrin [30]. These authors speculate that because LF has been shown to be multifunctional and to be capable of assuming a different configuration to serve each function, it is possible that the antibodies are related to the function served by LF in the context of its configuration in the sperm coat.

3.1.2. Natural Antibodies to Dietary Lectins (Agglutinins)

A landmark study by de Aizpurua and Russell-Jones [31] has revealed that among the classes of proteins tested for eliciting antibody responses upon oral administration, proteins with "lectin or lectin-like" binding activities were most effective. Generally these proteins, by virtue of their ability to bind to glycolipids and/or glycoproteins on the intestinal mucosal cells stimulate them to transport the proteins into the systemic circulation, thereby eliciting a systemic immune response. Some studies have suggested that either carbohydrate-binding activity or proteolytic activity as an important parameter for the immunogenicity of proteins.

Typical human diet includes many substances of plant origin, several of which contain the commonly occurring proteins such as the specific carbohydrate-binding proteins known as lectins. IgG4 antibodies to banana were found to occur in human serum far more frequently than expected; the most important antigen involved was identified as the mannose-specific lectin, BanLec-1 [32]. The immune nature of binding was proved by 3 observations: (a) the binding of BanLec-1 to IgG4 is mannoside-resistant, (b) only a minor fraction of the IgG4 in human serum was bound, and (c) the lectin binds to the Fab fragment of IgG4. These results reinforce the earlier suggestion that some lectins are particularly prone to induce an immune response upon oral feeding. Koshte *et al*. [32] advocate the usefulness of banana lectin as a potential carrier protein for oral anti-hapten immunization in humans.

Tchernychev and Wilchek [33] demonstrated the presence of NAbs towards dietary lectins (three structurally related legume lectins: *Erythrina corallodendron* lectin (ECorL), peanut agglutinin (PNA), and soybean agglutinin (SBA) and one cereal lectin, wheat germ agglutinin (WGA). NAbs against these lectins were purified by affinity chromatography from human sera and an examination of their binding specificity showed that anti-SBA, anti-ECorL and anti-WGA antibodies exhibited high specificity, whereas the anti-PNA antibodies were polyreactive. Though anti-WGA antibodies were highly specific for WGA, they also cross-reacted slightly with some other proteins. The anti-ECorL antibodies bound to native SBA, but the anti-SBA antibodies failed to bind to the native ECorL. Although the anti-SBA and anti-ECorL antibodies both exhibited specificity when interacting with native lectins, they bound to a wider range of denatured lectins, indicating a common or universal epitope which is recognized by many NAbs. Interestingly, the NAbs did not interfere with the agglutination properties of the lectins. These properties may provide a basis for studying the *in vivo* biological effects of anti-dietary protein antibodies, including those against carbohydrate-binding proteins [33].

The detection of NAbs in human serum against the garlic major protein, mannose-specific lectin, also known as ASA (*Allium sativum* agglutinin), was first reported from Israel [26]. Normal human sera (n = 40 from both sexes) contained antibodies to garlic lectins. The affinity-purified NAbs against ASA were mainly of the IgG and IgM classes. In contrast to anti-alliinase antibodies which were highly specific, anti-ASA antibodies were polyreactive. The difference in specificity of the NAbs against the two major proteins from the same dietary source could explain the strength of antigen, mainly based on their size, i.e., bigger molecules are easily encountered by the immune system and their stablility in the digestive process [26].

Two protein components of ~13 kD (QR-1, QR-2 in the ratio 1:4) were separated by Q-Sepharose

chromatography of 30 kD ultrafiltrate of raw garlic extract; these proteins exhibited mitogenic activity towards human peripheral blood lymphocytes, murine splenocytes and thymocytes [34]. Both proteins displayed mitogenicity, hemagglutination and mannose-binding activities; in all these functional studies, QR-2 was more potent than QR-1. Clement and Venkatesh [34] concluded that the two major proteins QR-2 and QR-1 present in a ratio of 4:1 in raw garlic contribute to garlic's immunomodulatory activity, and their characteristics are markedly similar to the abundant *Allium sativum* agglutinins – ASA I and II, respectively.

In view of the reported presence of NAbs to garlic lectins in human serum [26], Clement and Venkatesh investigated the immunogenic response of orally-administered garlic lectins in BALB/c mice [35]. The immunogenicity of garlic lectins ASA I and ASA II in the absence of an adjuvant at 10 and 100 µg doses was studied in 12-week-old BALB/c mice. The time-course of humoral response followed the normal course of immunization with an IgG response by 12 days after the primary oral dose on day 1; further oral exposure (on days 14, 21, 28, 35, 42 and 49) resulted in booster effects to the administered test proteins as evidenced by IgG responses on days 35 and 55 (Figure **2**). Compared to control, the garlic lectins ASA I and ASA II show a 3-4 fold increase in IgG response for both dose groups and on different days. The model weak antigen, ovalbumin (OVA), shows a very weak IgG response, while the oral immunogenic lectin, phytohemagglutinin (PHA) shows a 3-fold increase in IgG response vs. control. Thus, garlic lectins are as immunogenic as PHA, and there is no significant difference in the IgG response between the two doses (10 and 100 µg) or between booster doses.

Overall, it appears that garlic lectins ASA I and ASA II are important immunomodulatory proteins of garlic that are able to withstand the harsh conditions of the gastrointestinal tract, perhaps taken up by the enterocytes, and modulate the immunoresponder cells to evoke immunogenicity [34, 35], and could possibly represent one of the critical components responsible for a host of beneficiary immunomodulatory responses including the production of NAbs described in garlic till date.

3.2. Natural Antibodies to Carbohydrates

Interestingly, NAbs are produced not only towards proteins but also to carbohydrates. The presence of NAbs against carbohydrates in human sera could be explained by the presence of NAbs against the saccharide groups of blood group antigens (blood group hemagglutinins), their cross-reactivity and possible immune response (production of NAbs) against a variety of carbohydrate structures shared by

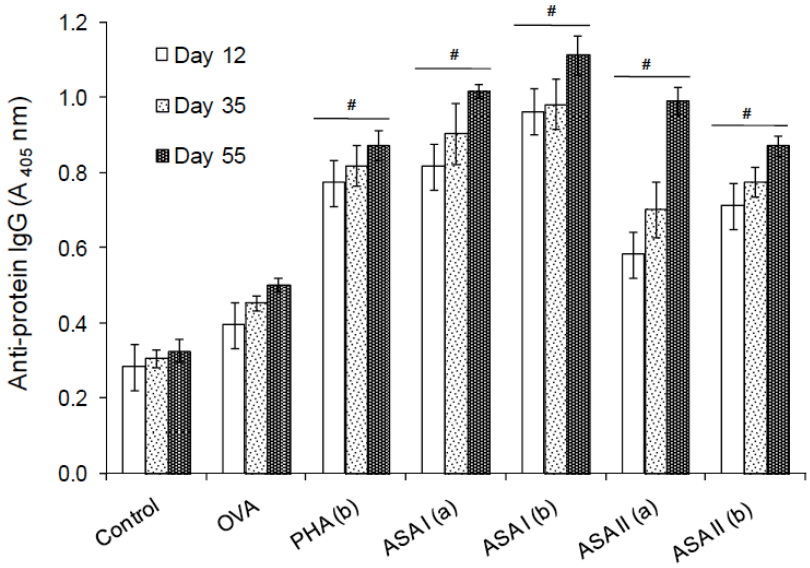

Figure 2: Oral immunogenicity of garlic lectins in BALB/c mice. Mice were administered two doses of garlic lectins or PHA by gavage feeding: (a) 10 µg and (b) 100 µg. Ovalbumin (OVA; 100 µg dose) represents a weak immunogen and PHA a prototype antigen representing the lectin category. Anti-protein IgG response in the sera (1:10 dilution) of BALB/c mice was measured by ELISA using the individual administered proteins as coating antigens. Serum IgG response to PHA(a) representing 10 µg protein is identical to PHA(b), and hence is not shown. Values represent mean of triplicates ± SE. Significant difference (# $p < 0.05$) was observed in IgG response of ASA I and ASA II groups at both doses vs. control group. Reproduced from Clement and Venkatesh [35] with permission from Elsevier, Inc., ©2010.

gastrointestinal bacterial flora [36]. The predominant carbohydrate epitope was found to be α-linked galactose (α-galactosyl) epitope; the α-galactosyl antibodies constitute nearly 1% of total circulating IgG [37]. The predominant production of NAbs against the α-galactosyl epitopes provides an explanation that the pre-existing α-galactose-specific antibodies somehow increase the efficiency of T- and B-cell priming to α-Gal-modified antigens. The presence of α-Gal-specific antibodies may allow immune complex formation or efficient antigen presentation by B cells to occur, which increases the efficiency of priming to the carrier [3].

Dietary carbohydrates including those of herbal medicines which react with NAbs in human serum are compiled in Table **2**. An interesting study from Japan concerns the finding of NAbs to various carbohydrates like pharmacologically active pectic polysaccharides from herbal medicines including the traditional Japanese herbal (Kampo) medicines [38]. Normal human sera and human colostrum were shown to contain IgM, IgG, IgA and secretory IgA class antibodies which react with the active pectic polysaccharides to different degrees. The reacting IgG antibody recognized the ramified regions (rhamnogalacturonan core with carbohydrate side-chains) of the pectic polysaccharides as the active sites for complement-activating activity. Further, it was observed that a significant positive correlation was observed between reactivity with the reacting IgG antibody and the degree of complement-activating activity of the active polysaccharides. The reacting IgG class antibody (specific for the pectic polysaccharide, bupleuran 2IIc, from the roots of *Bupleurum falcatum*) showed cross-reactivity with other pharmacologically active pectic polysaccharides from other medicinal herbs as well as auto antigens such as single-stranded DNA from calf thymus, rabbit muscle myosin and bovine brain tubulin [38].

The presence of IgM NAbs to advanced glycation end products (AGEs), a heterogeneous and complex group of compounds that are formed when reducing sugars (including dehydroascorbic acid) react non-enzymically with amino groups of proteins, lipids and nucleic acids, has been reported in human sera [8]; the complex structures (AGEs) are more prevalent in age-related diseases. These findings suggest that the protein modification by the endogenous carbonyl compounds, generating electronegative proteins, could be a source of multispecific natural antibodies.

The environmental origin of NAbs to teichoic acid (a cell-wall constituent of Gram-positive bacterial polysaccharides of glycerol phosphate or ribitol phosphate linked *via* phosphodiester bonds) has been studied by Rozmiarek *et al*. [5]. They concluded that NAbs to teichoic acid (polyglycerophosphate specificity) that occur frequently in the rat without apparent antigenic stimulus are due to stimulation by living or dead Gram-positive bacteria in the food. It was suggested that natural responses of the polyglycerophosphate specificity, which also occur in humans and several other species, likewise may be elicited by Gram-positive bacteria in the environment. In an interesting study on human subjects examined for caries activity, Bolton [39] found that subjects free of active caries produced higher levels of anti-glycerol-teichoic acid (naturally-occurring IgA) in their saliva than subjects with one or more active carious lesions.

BALB/c mice, raised in a germ-free environment and fed a chemically defined, ultrafiltered diet (GF-CD)

Table 2: Dietary Carbohydrates for which NAbs have been Detected in Vertebrate Serum

Sl. No.	Reactivity of NAbs towards	Class of Ig[a]	Antigenic source	Route of antigenic exposure	Ref.
1	Pectic polysaccharides	IgM, IgG, IgA sIgA	medicinal plants and citrus fruits	dietary intake	[38]
2	Advanced glycation end (AGE) products	IgM	citrus fruits	dietary intake	[8]
3	β-1,3-Glucan	IgM, IgG	*Candida* species	dietary intake	[41]
4	Branched 1,3/1,6-β-glucan	IgM, IgG (mice)	*Sparassis crispa* and *Candida* species	oral administration	[42]
5	Chitosan	IgM (cod)	cod fish feed	oral administration	[44]
6	Polymannuronic acid	IgM (cod)	*Durvillaea antartica*	oral administration	[44]
7	Teichoic acid	IgA (human saliva)	Gram-positive bacteria	dietary intake	[39]

[a]Refers to human serum in all cases except where indicated otherwise.

had normal serum IgM levels, but IgG and IgA levels were ~5% of conventionally reared littermates [40]. Antibodies against levan and dextran were lower in GF-CD than in conventional mice, but levels of anti-3-fucosyllactosamine (3-FL) antibodies were comparable to those in conventional animals. Peptidoglycan polysaccharide complexes (PPC) are carbohydrate antigens of bacterial origin, like levan and galactan. NAbs against PPC were found in the serum of conventional mice, but were severely reduced in GF-CD mice. These results indicated that most NAbs against carbohydrate antigens of bacterial origin found in conventional mice are caused by exogenous stimulation [40].

Normal human serum has also been reported to contain NAbs to the commercially available polysaccharide β-1,3-glucan from *Candida* species; NAbs to β-1,3-glucan do not show cross-reactivity to β-1,6-branched β-1,3-glucan, grifolan [41]. The titer of NAbs against β-1,3-glucan could be used as a diagnostic parameter for autoimmune diseases like rheumatoid arthritis and anti-neutrophil cytoplasmic antibody–associated vasculitis, because these patients contained significantly lower concentrations of NAbs to anti-β-1,3-glucan independent of any therapy [41]. Interestingly, NAbs to branched 1,3/1,6-β-glucan from *Sparassis crispa* and *Candida* species have been detected which showed significantly higher titer in naïve DBA/1 and DBA/2 mice strains compared to other strains indicating that these strains carried specific and unique immunological characteristics to branched 1,3/1,6- β-glucan [42].

Chiani *et al*. [43] showed that natural IgG and IgM antibodies to laminarin (β-1,3-glucan) are present at low levels in healthy humans as compared to other anti-β-glucans and, mostly, anti-mannan antibodies, and suggested that a protective antifungal vaccination in humans should attempt to tip the balance of antifungal antibodies in favor of the anti-laminarin antibodies.

A recent study on the NAbs of cod (*Gadus morhua* L.) has demonstrated that they have a broad, but characteristic specificity, primarily directed against haptenated proteins and possible food antigens [44]. Most sera from various groups of cod showed relatively strong specificity for 2,4,6-trinitrophenyl (TNP)–BSA, chitosan and polymannuronic acid (polyM from the brown seaweed (*Durvillaea antartica*). Chitosan, polyM and *Limulus polyphemus* hemocyanin are likely constituents of the natural feed of cod, and the specificity for the stomach antigens seemed to indicate another link between the NAbs of cod and its feed or food antigens [44]. Anti-hapten activity of NAbs has been observed in most vertebrates; it has been suggested that the 3-D structure of TNP hapten is similar to a common, pathogenic molecular pattern or to self-antigenic determinants, possibly proline-rich motifs [10].

4. INFLUENCE OF DIETARY COMPONENTS ON THE PRODUCTION OF NAbs

Addition of linseed oil (rich in ω-3 polyunsaturated fatty acids) to anti-atherosclerotic diet for 4 weeks increased the levels of NAbs to bradykinin and angiotensin II in the serum of patients with ischemic heart disease, hyperlipidemia and hyptertension [45]. In another study, addition of 'Vitol' preparation (biologically active food supplement enriched in phospholipids) to low-sodium anti-atherosclerotic diet for 4 weeks increased the levels of NAbs to adrenaline, noradrenaline, dopamine, thrombin, antithrombin III and α_2-macroglobulin [46]. Administration of an anti-atherogenic diet including soy protein isolate, soybean flour and phytoestrogens in patients with ischemic heart disease and hyptertension increased the production of NAbs to thrombin, antithrombin III, α_2-macroglobulin, angiotensinogen and noradrenaline [47].

An additional role of NAbs in regulating blood pressure is indicated by the presence of NAbs that recognize renin, angiotensin II and catecholamines [45, 48]. Therefore, diet appears to influence this natural humoral immunoreactivity [49]. NAbs are thought to function as immunotransporters; the binding of NAbs to hormones involved in vascular and blood volume control of arterial pressure may serve to modulate and maintain an appropriate hormonal level in the midst of physiological fluctuations. Dietary determinants that affect blood pressure may act through anti-oxidative/pro-oxidative factors and vascular modulators as well as the level of NAb activity [50].

Cheng and Sundram [51] found the presence of autoantibodies to cholesterol oxides in healthy individuals; also, they showed the common occurrence of NAbs to phospholipids including cardiolipin and phosphatidylserine. Antibodies to phospholipids were shown to cross-react with oxidized LDL. Although the immunologic origin of NAbs to phospholipids is still unknown, it was interesting to observe that the amounts of these antibodies could be affected by diet

in an experimental mouse model of autoimmune disease [52]. The authors speculate that the same dietary factors probably influence the NAb populations. The amount and activity of circulating oxidized LDL could possibly be controlled by regulatory mechanisms involving endogenous and exogenous antioxidants as well as NAb activity. The postulated protective role of NAbs also extends the spectrum of effectors that have been described in the immunologic control of atherogenesis [53].

A gluten-free vegan diet in rheumatoid arthritis induced changes that are atheroprotective and anti-inflammatory, including decreased LDL and oxidized LDL levels, and raised atheroprotective NAbs (IgM and IgA) against phosphorylcholine in serum [54]. Recently, Frostegård [55] hypothesized that low level of NAbs against phosphorylcholine represents a novel paradigm as a cause of chronic inflammatory diseases such as atherosclerosis and cardiovascular diseases.

5. SIGNIFICANCE OF NAbs TO DIETARY COMPONENTS

From the description of NAbs present in the serum of vertebrates, it is seen that NAbs have been detected for various dietary proteins including lectins and carbohydrates. Since NAbs to dietary components are mainly of the IgM, IgG and IgA classes, in our opinion, they certainly do not contribute to the development of food allergy since IgE antibodies are essential for this phenomenon. On the contrary, NAbs may exhibit beneficial antibacterial and antiviral activities in fighting pathogens due to their oligo-/polyspecificity; however, these aspects have not yet been proven experimentally in the case of NAbs to food components. Certain foods are known as 'immune-boosting' foods; some examples are garlic, guduchi (*Tinospora cordifolia*), black cumin (*Nigella sativa*), black pepper (*Piper nigrum*), mushrooms, yam, bitter gourd (*Momordica charantia*), moringa (*Moringa oleifera*), fenugreek (*Trigonella foenum-graecum*) and mushrooms. However, the mechanisms by which the various components of these foods cause immunostimulation are not known, and induction of NAbs by one or more components may be one of the mechanisms.

6. CONCLUSIONS

From the foregoing description of natural antibodies to dietary components, it is clear that NAbs have been detected to only a handful of dietary proteins and polysaccharides in the sera of vertebrates. Examples of dietary proteins which evoke NAbs in humans include avidin from egg white, bromelain from pineapple, lactoferrin from human milk, and dietary lectins/agglutinins (from banana, garlic, soybean, peanut and wheat germ). Dietary polysaccharides which have been reported to induce NAbs are pectic polysaccharides from some plants, β-glucans, polymannuronic acid and chitosan, to name a few. Foods supplemented with ω-3 fatty acids, phospholipids or soy components have been shown to increase the levels of NAbs to certain endogenous mediators and proteins in humans, and may help in the prevention of atherogenesis. Research in the area of NAbs to foods and dietary components is still in its infancy, and an extensive research is needed to completely understand as to why only certain foods induce NAbs in humans, and the putative functions of such antibodies in maintaining the normal health status.

ABBREVIATIONS

ASA = *Allium sativum* agglutinin

BSA = bovine serum albumin

EcorL = *Erythrina corallodendro*n lectin

GALT = gut-associated lymphoid tissue

Ig = immunoglobulin

NAbs = natural antibodies

PHA = phytohemagglutinin

PNA = peanut agglutinin

SBA = soybean agglutinin

WGA = wheat germ agglutinin

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