

Immune Reactivities Against Gums

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ABSTRACT

Context • Different kinds of gums from various sources enjoy an extremely broad range of commercial and industrial use, from food and pharmaceuticals to printing and adhesives. Although generally recognized as safe by the US Food and Drug Administration (FDA), gums have a history of association with sensitive or allergic reactions. In addition, studies have shown that gums have a structural, molecular similarity to a number of common foods. A possibility exists for cross-reactivity.

Objective • Due to the widespread use of gums in almost every aspect of modern life, the overall goal of the current investigation was to determine the degree of immune reactivity to various gum antigens in the sera of individuals representing the general population.

Design • The study was a randomized, controlled trial.

Participants • 288 sera purchased from a commercial source.

Outcome Measures • The sera was screened for immunoglobulin G (IgG) and immunoglobulin E (IgE) antibodies against extracts of mastic gum, carrageenan, xanthan gum, guar gum, gum tragacanth, locust bean gum, and β -glucan, using indirect enzyme-linked immunosorbent assay (ELISA) testing. For each gum antigen, inhibition testing was performed on the 4 sera that showed the highest IgG and IgE immune reactivity

against the different gums used in the study. Inhibition testing on these same sera for sesame albumin, lentil, corn, rice, pineapple, peanut, pea protein, shrimp, or kidney bean was used to determine the cross-reactivity of these foods with the gum.

Results • Of the 288 samples, 4.2%-27% of the specimens showed a significant elevation in IgG antibodies against various gums. Only 4 of 288, or 1.4%, showed a simultaneous elevation of the IgG antibody against all 7 gum extracts. For the IgE antibody, 15.6%-29.1% of the specimens showed an elevation against the various gums. A significant percentage of the specimens, 12.8%, simultaneously produced IgE antibodies against all 7 tested extracts.

Conclusions • Overall, the percentage of elevation in IgE antibodies against different gum extracts, with the exception of carrageenan, was much higher than for the IgG antibody. The results of the current study showed that a subgroup of healthy individuals who produced not only IgG but also IgE antibodies against various gums may suffer from hidden food immune reactivities and sensitivities. Further study is needed to examine the clinical importance of gums and cross-reactive food antibodies in symptomatic individuals. (*Altern Ther Health Med*. 2015;21(suppl 1):64-72.)

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Humans have been using gum since the first caveman discovered that he derived pleasure from chewing the hardened sap that came out of trees and other plants. Since then, gum use has evolved into far more than only exercising jaws, blowing bubbles, or sticking shoes to the pavement. Today, gums have very wide industrial uses, including being employed in the food industry as stabilizers, thickening

agents, gelling agents, emulsifiers, and fixing agents in foods and soft drinks and in the printing, textile, pottery, lithography, cosmetics, and candy manufacturing industries as adhesives and binding agents. Together with natural resins and latexes, gums form a group of perhaps the most widely used and traded category of nonwood forest products that are not consumed directly as foods, fodders, or medicines.¹ In 1993 alone, the world market for gums as food additives was approximately \$10 billion. This amount does not include nonfood uses for gums.¹

As early as 1982,² it had already been demonstrated that various gums are highly antigenic, meaning that these substances are capable of eliciting an immune response comparable with those of protein antigens. Despite this fact, no serious attempt has been made to measure immunoglobulin G (IgG) and immunoglobulin E (IgE) antibodies in the blood of healthy subjects. Due to the extensive use of gums in almost every single product made, the overall goal of this

investigation was to determine the degree of immune reactivity to various gum antigens in 288 healthy individuals representing the general population.

Currently, gums that are generally recognized as safe by the US Food and Drug Administration (FDA) are labeled as such by the industries that submit materials to that agency. However, in conjunction with the extensive use of gums in almost every single product made, many work-related illnesses have been reported in the literature. For example, as early as 1933, a case of asthma caused by acacia gum was described.³ Later, occupational asthma among printers exposed to gum dust was repeatedly reported in more than 50 patients, some of whom exhibited positive skin tests to acacia, tragacanth, and gum arabic but not to gum karaya.⁴⁻⁷ Airborne exposure to these gums causes sensitization leading to allergic rhinitis, asthma, and urticaria.

The first thoroughly authenticated case report of clinical allergy caused by sensitivity to gum tragacanth was released in 1941,⁸ detailing how in 1939 a perfectly healthy 26-year-old female went to work in a New York gum factory where gum tragacanth dust freely proliferated in the air. Starting with what at first appeared to be a persistent head cold, the woman's health grew increasingly worse until, in 1941, she was suffering severe attacks of asthma that sometimes lasted for hours. Direct skin tests and passive transfer tests proved strongly positive for gum tragacanth and gum arabic but not gum karaya.

A case report from 2007 featured a 52-year-old male who took a slimming aid that contained guar gum.⁷ He first experienced generalized urticaria, then severe anaphylactic reaction, including circulatory collapse. Skin prick tests pinpointed guar and/or carob gum as the likely culprits. This variation in skin-testing results was described to be associated with antigenic variability between the gums.⁵

To document the prevalence of immune reactivity and allergic symptoms in the work environment, 162 workers of a carpet manufacturing plant were tested; results demonstrated a 5% prevalence of skin reactivity to guar gum.⁷ Despite this IgE-mediated immune reactivity, no serious attempt was made to measure the IgG, IgA, or IgM isotype antibodies to discover the degree of immune reactivities against various gums with which the human body is in contact on a daily basis. One study, however, described 3 individuals, one who worked for a pharmaceutical company and a second and third who were employed at a carpet manufacturing plant. For the 3 individuals, occupational asthma caused by exposure to guar gum was demonstrated, together with IgE and IgG specific to the product.⁹

Various types of natural gums are used in industry. Gum tragacanth is widely used in the printing industry, whereas guar gum is used extensively in numerous pharmaceutical and food products, such as lotions and creams, soups, salad dressings, ice cream, cheese, baby foods, and preserved foods.⁹⁻¹² The pharmaceutical industry also uses gums as excipients or drug delivery agents and focuses particularly on natural gums because they are nontoxic, stable, easily available, less expensive, and less subject to regulatory issues than their synthetic polymer counterparts.^{13,14}

THE STRUCTURE OF GUMS

From a chemical, structural point of view, most gums are composed of complex and variable mixtures of oligosaccharides, polysaccharides, and glycoproteins, with a polysaccharide that has an extremely high molecular weight that is attached to a hydroxyproline-rich, polypeptide backbone that accounts for approximately 2% of the molecular size.¹⁵ Together, these substances form a huge molecular structure with the size of 200 to 2000 kDa, consisting of 4000 to 40 000 amino acids. For comparison, insulin is composed of 110 amino acids; β -casein, the major protein found in human milk, is composed of 199 amino acids; human serum albumin (HSA) consists of 609 amino acids; and α -gliadin ranges from >200 to >300 amino acids. Therefore, if the partially digested molecules of certain gums manage to get into the circulatory system, they would induce a very strong immune response that would result in very high levels of IgG, IgA, or IgE antibodies against the gum molecules.

TYPES OF GUMS

Natural gums, such as mastic gum, gum arabic, carrageenan, guar gum, locust bean gum, and gum tragacanth are mainly derived from the woody elements or seed coatings of various plants.² These gums may be grouped into seed gums, exudate gums, marine gums, and microbial gums. Synthetic gums are basically the products of chemistry and laboratories.

Seed gums such as guar, carob, mesquite, and tara are derived from plant seeds.² Exudate gums such as gum arabic, karaya, and tragacanth are made from plant sap. Tragacanth is derived from *Astragalus gummifer*, a legume grown in Asia, whereas guar gum comes from the Indian legume *Cyamopsis tetragonolobus*. Marine gums such as carrageenan and agar are made from seaweed.¹ Microbial gums such as xanthan and gellan are fermented from plants by bacteria, causing some to refer to microbial gums as *synthetic gums* because they are the products of biosynthesis. However, truly synthetic gums, such as styrene-butadiene rubber, butyl rubber, and polyisobutylene are synthesized from chemicals in a laboratory.

Seed Gums

Seed galactomannans, generally known as seed gums, are vegetable, heterogeneous, storage polysaccharides composed of galactopyranosyl (Gal) and mannopyranosyl (Man) residues.¹⁰ Galactomannans have attracted considerable academic attention as well as industrial interest due to their property of forming viscous solutions or gels in aqueous media. They can thus be used in different forms for human consumption. The different chemical properties of these gums make them versatile materials for many different applications. Some of these important properties include holding H₂O, thickening, gelling, binding, suspending, and emulsifying as well as forming films. These characteristics have opened avenues for gums to be used in various industries, such as paper, textiles, petroleum, drilling, pharmaceuticals, food, cosmeceuticals, and explosives.¹⁶

The 2 galactomannans of major commercial importance are guar gum from *C tetragonolobus* and carob or locust bean

gum from *Ceratonia siliqua*.¹⁷ Carob gum forms gels at higher concentrations. The interaction of carob with a little xanthan produces a gelling that is used in commercial applications. The scope of the varied applications using seed gums that are obtained from different sources is huge.¹⁶

Guar Gum. Guar gum, also called *guaran*, is primarily the ground endosperm of guar beans. The guar seeds are dehusked, milled, and screened to obtain the gum.¹ It is typically produced as a free-flowing, off-white powder and is not self-gelling, although it forms viscous solutions.¹ However, it can be cross-linked with either borax or calcium to cause it to gel.¹⁸ Its chemical structure consists of repeating units of guar galactomannan.

Carob Gum. Carob or locust bean gum is the whitish powder obtained from grinding the endosperm of the seeds of *C siliqua*, a tree widely cultivated in the Mediterranean region.¹ It consists mainly of galactomannan-type polysaccharides, with a galactose:mannose ratio of approximately 1:4.¹⁹ It is employed in a wide range of products, among the most important of which are ice cream, baby foods, and pet foods. It is also used in soups, sausage products, soft cheeses, bakery products, pie fillings, powdered desserts, sauces and salad creams, and dairy products other than ice cream. It can be added during the paper-making process to improve the physical characteristics of the paper. In the textile industry, locust bean is used either alone or in combination with starch and synthetics as a sizing agent for cotton and other natural fibers. It is also used as a print-paste thickener in both roller and screen-printing to help provide greater purity and uniformity of shades and deeper penetration of dyes. Other minor uses include incorporation in oil-drilling fluids and some pharmaceutical and cosmetics applications.¹

Exudate Gums

The previous reference in this article to early humans deriving pleasure from chewing the hardened sap that oozed out of trees referred to exudate gums. Exudate gums are obtained as the natural secretions of different tree species; when the bark of certain trees and shrubs is injured, an aqueous gum solution is exuded to seal the wound, preventing infection and dehydration of the plant.²⁰ The exudates exhibit unique properties in a wide variety of applications. Five thousand years ago, they were already being used as thickening and stabilizing agents. Exudate gums have been important items of international trade in the food, pharmaceutical, adhesive, paper, textile, and other industries for centuries.²⁰

Gum Arabic. The exudate known as gum arabic is the oldest and most well known of all natural gums, deriving its name from its place of origin. As far back as the 3000 BC, the ancient Egyptians were shipping it as an article of commerce. It was used as a binder and adhesive for pigments, paints, inks, and cosmetics and even for flaxen wrappings for mummies.²⁰ Strictly speaking, *gum arabic* is defined by the Food and Agriculture Organization and World Health Organization's Joint Expert Committee for Food Additives as "a dried exudate obtained from the stems and branches of *Acacia Senegal* or

Acacia seyal."²¹ The term is now applied more loosely to other gums produced by other *Acacia* species and, thus, the substance is sometimes also referred to as *gum acacia* or *acacia gum*.

Gum Tragacanth. Tragacanth gum is the dried exudate produced by tapping the taproot and branches of certain shrubby species of *Astragalus*, particularly those that occur wild in Iran and Turkey. The gum is exported from the country of origin in ribbon or flake form and has a rather horny texture.¹ Chemically, it is a complex, highly branched, heterogeneous polysaccharide that occurs naturally as a slightly acidic, calcium, magnesium, and potassium salt. It has a molecular mass of approximately 8.4×10^5 Da.²⁰ The most important applications of tragacanth are now in foods and pharmaceuticals. Gum tragacanth consists of 2 fractions. Tragacanthic acid or bassorin is insoluble in water but has the capacity to swell and form a gel. The other fraction is called tragacanthin and is water soluble. Both fractions contain small amounts of proteinaceous material. The tragacanthic acid fraction that swells in water has a high molecular weight and a rod-like molecular shape. The water-soluble tragacanthin is a neutral, highly branched arabinogalactan with a spherical molecular shape.

Mastic. Although usually termed a gum, mastic is a hard resin, produced by tapping the stem bark of the small tree *Pistacia lentiscus*, which is cultivated on the Greek island of Chios. Mastic is produced in the form of small tears that are pale yellow in color, clear and glassy in nature, and liable to fracture. Its age-long use in Arab countries has been for chewing, where it sweetens the breath and helps preserve the teeth and gums. Its aromatic properties also make it suitable as a flavoring agent for alcoholic beverages. In the past, it was also used in the manufacture of high-grade varnishes for paintings and in medicinal applications. An essential oil can be distilled from the gum and finds some use for fragrance and flavoring purposes.¹

Marine Gums

Marine gums are extracted from seaweeds. The cell walls of many seaweeds contain phycocolloids (algal colloids) that can be extracted by hot water. The 3 major phycocolloids are alginates, agars, and carrageenans. These phycocolloids are polymers of chemically modified sugar molecules, such as galactose in agars and carrageenans, or organic acids, such as mannuronic acid and glucuronic acid in alginates. Most phycocolloids can be safely consumed by humans and other animals, and many are used in a wide variety of prepared foods, such as ready-mix cakes, instant puddings and pie fillings, and artificial dairy toppings.²¹

In the general market for polysaccharides, phycocolloids compete with seed gums, such as guar gum and locust bean gum; plant exudates, such as gum arabic, pectin, and other plant extracts; starch and cellulose derivatives; and various biosynthetic gums. They frequently offer distinct chemical and economic advantages.²²

Carrageenans. Carrageenans are extracted from various red algae, including *Eucheuma* in the Philippines; *Chondrus*, also called *Irish moss*, in the United States and the Canadian

maritime provinces; and *Iridaea* in Chile. They are used for thickening and stabilizing dairy products, imitation creams, puddings, syrups, and canned pet foods.²¹ They are also used in the manufacture of shampoos, cosmetics, and medicines.²¹

Microbial Gums

Microbial polysaccharide gums are as natural as seed, exudate, and marine gums. They are the product of the natural process of fermentation, involving bacteria, fungi, and some plants.²³ However, this natural process is generally induced, augmented, and highly controlled in a laboratory, so that some refer to microbial gums, such as xanthan gum, as synthetic or at least semisynthetic.²⁴ For example, xanthan gum is a polysaccharide produced by a pure culture fermentation of a carbohydrate with *Xanthomonas campestris*, and it is composed of glucose, glucuronic acid, 6-acetylmannose, and 4,6-pyruvylated mannose residues.

Xanthan Gum. Xanthan gum is a natural polysaccharide and an important industrial biopolymer. It was discovered in the 1950s at the Northern Regional Research Laboratories of the United States Department of Agriculture.^{25,26} The polysaccharide B-1459, or xanthan gum, produced by the bacterium *X. campestris*, was extensively studied because of its properties that allow it to supplement other known natural and synthetic water-soluble gums. Extensive research was carried out in several industrial laboratories during the 1960s, culminating in semicommercial production as Kelzan by Kelco. Substantial commercial production began in early 1964.

β -Glucan. β -Glucans are produced by fungi, yeasts, grains, and seaweed. β -Glucans are the constituents of the cell wall of certain pathogenic bacteria—*Pneumocystis carinii* and *Cryptococcus neoformans*—and fungi—*Aspergillus fumigatus*, *Histoplasma capsulatum*, *Candida albicans*, and *Saccharomyces cerevisiae*. The main components of the fungal cell wall are polysaccharides and glycoproteins. It is used both in the formulation of cosmetics and as a direct food additive.^{24,27}

CROSS-REACTIVITY

Because sensitization to gums, such as gum arabic, occurs through a carbohydrate-rich structure and a repetitive polysaccharide sequence, cross-reaction to other carbohydrate-containing molecules has been reported.²⁸⁻³⁰ For example, when preliminary investigation about the structure of gum arabic was made by high-performance liquid chromatography, it was shown that the gum arabic glycoprotein had an 8% to 15% similarity to oligosaccharides of horseradish peroxidase that is found in the roots of horse radish and pineapple bromelain.³⁰ Further, these cross-reactive carbohydrate determinants (CCDs) that contain fucose and xylose exist in almost all plant extracts.

The existence of the CCDs containing fucose and/or xylose in the pollen of the olive tree, Japanese cedar, and Bermuda grass and in glycoproteins of celery, potato, tomato, bean, soybean, and pea have been shown in numerous studies.³¹⁻³⁵ Based on these and more recent studies,^{30,36,37} it was concluded that immune reactivity to gum carbohydrate

structures occurs in patients allergic to pollen, without obvious exposure to gums. This cross-reactivity between the repetitive polysaccharide sequences of gums with plant enzymes, such as horseradish peroxidase or bromelain, pollens, trees, celery, potato, tomato, beans, and pea led the research team to believe that individuals may produce IgG or IgE antibodies against the CCD of gums that cross-react with many other food and environmental antigens.

MATERIALS AND METHODS

Sera from 288 healthy individuals of different ethnicities, aged 18 to 65 years, were obtained from Innovative Research, Inc (Southfield, MI, USA). Of that total, 144 were males with a median age of 35.5 years, and 144 were females with a median age of 36.2 years. These individuals were qualified to donate blood based on a health questionnaire provided by the FDA. Each individual at the time of the blood draw did not exhibit any health complaints. Prior to shipping, each blood sample was tested according to FDA guidelines for the detection of hepatitis B surface antigen, antibodies to HIV, antibodies to hepatitis C, HIV-1 ribonucleic acid (RNA), hepatitis C RNA, and syphilis.

Process

Mastic Gum, carrageenan, xanthan gum, guar gum, gum tragacanth, locust bean gum, and β -glucan were purchased from Sigma Aldrich (Saint Louis, MO, USA). Ten grams of each gum were extracted in 500 mL of buffer pH 4.6 by mixing it for 8 hours at 25°C on a magnetic stirrer. The solution was centrifuged at 20 000 $\times$ g, and supernatant was removed and concentrated by a factor of 10 using an Amicon filter. The protein concentration was measured using a kit provided by Bio-Rad (Hercules, CA, USA). All extracts were aliquoted and stored frozen at -20°C until used.

Different gum extracts were dissolved in 0.1M phosphate buffered saline (PBS). These antigens were diluted 1:50 in 0.1M carbonate buffer pH 9.2, and 100 μ L of each gum antigen was added to different wells of the microtiter plate coated with gum-specific antibodies and incubated overnight at 4°C. After washing, the unoccupied sites in the wells were saturated by adding 200 μ L of 2% bovine serum albumin (BSA) and incubated overnight at 4°C. Plates were washed again, and 100 μ L of the 288 sera, with a dilution of 1:4 for determination of the level of IgE and 1:400 for level of IgG, were added to duplicate wells of each microtiter plate and incubated for 4 hours at room temperature.

This procedure was followed by washing and addition of optimal dilution of alkaline phosphatase-labeled antihuman IgE to 1 set and antihuman IgG to a different set of plates, followed by incubation for 1 hour at room temperature. After another washing and the addition of the substrate para-nitrophenyl phosphate to the wells, the color development was measured at 405 nM. Four different sera from patients with known allergy to gums and 4 individuals with no known allergy to gums were used as positive and negative controls. Several wells coated with unrelated proteins such as HSA, rabbit serum albumin, and BSA were used only for the

determination of background in the enzyme-linked immunosorbent assay (ELISA).

Inhibition Study

ELISA inhibition was performed with 4 different sera that showed the highest IgG and IgE immune reactivity against different gums used in the study. A total of 2 mL of each serum prediluted 1:4 for IgE and 1:100 for IgG determination was preincubated with 100 µg of either HSA or 100 µg extracts of mastic gum, carrageenan, xantham gum, guar gum, gum tragacanth, locust bean gum, β-glucan, sesame albumin, lentil, corn, rice, pineapple, peanut, pea protein, shrimp, or kidney bean for 2 hours at 37°C and 2 hours at 24°C. After centrifugation at 3000 × g, 100 µL of each supernatant was transferred to the microtiter plate coated with the specific gum extract. After 4 hours incubation at 24°C the ELISA procedure was continued according to the standard procedure. Results were expressed as percent inhibition after subtraction of optical density (OD) from HSA.

Statistical Analysis

Coefficients of intra-assay variation were calculated by running 5 samples 8 times within a single assay. Coefficients of interassay variation were determined by measuring the same samples in 6 consecutive assays. This replicate testing established the validity of the ELISA assays, determined the appropriate dilution with minimal background, and detected serum IgG and IgE against different gums. Coefficients of intra- and interassay variations for IgG and IgE against all tested antigens and peptides were lower than 15%.

RESULTS

ELISA Testing

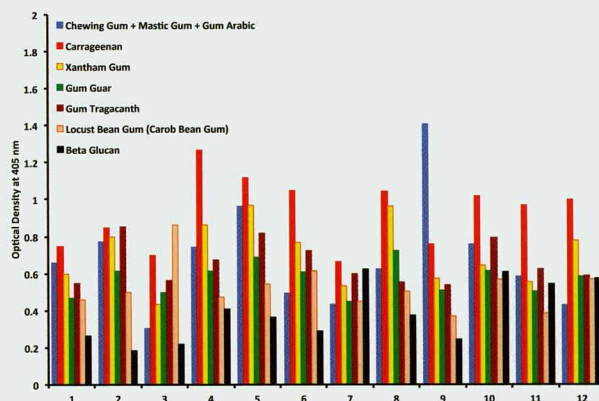
Using indirect ELISA testing, the 288 samples of sera were screened for IgG and IgE antibodies against extracts of mastic gum, carrageenan, xantham gum, guar gum, gum tragacanth, locust bean gum, and β-glucan.

IgG Antibody Response. For 60 of the 288 tested specimens, the data for the IgG antibody with the 7 groups of antigens is summarized in Figures 1 through 5.

These figures show a significant variation in IgG antibody response against various gum extracts. At 2 standard deviations above the mean of the 288 specimens, 4.2% to 27.1% of them showed a significant elevation in IgG antibodies against various gums (Table 1). The lowest percentage of IgG elevation was observed against guar gum in 12 of the 288 individuals (4.2%). The highest percentage of elevation of IgG was detected against carrageenan. Seventy-eight of 288 specimens (27.1%) reacted against carrageenan. Interestingly, although some individuals reacted against 1 or more gum antigens, only 4 of 288 (1.4%) showed simultaneous elevation in IgG antibody against all 7 gum extracts.

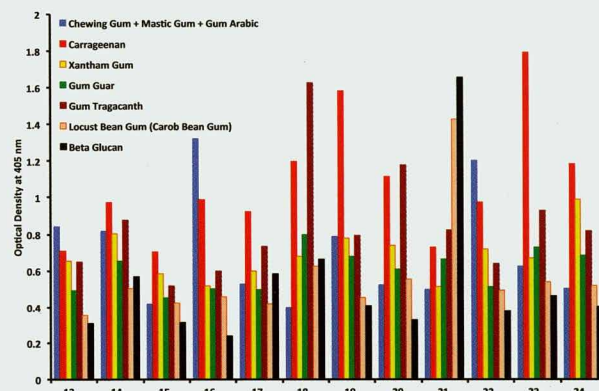
IgE Antibody Response. IgE-specific antibody reactivity against various gum extracts for the same 60 individuals is presented in Figures 6 through 10. At the cutoff of 2 standard deviations above the mean, 29.1%, 19.8%, 16.6%, 17.7%,

Figure 1. Optical density at 405 nM for samples from patients 1 through 12, tested for IgG against chewing gum + mastic gum + gum arabic, carrageenan, xantham gum, gum guar, gum tragacanth, locust bean gum (carob bean gum), and β-glucan.



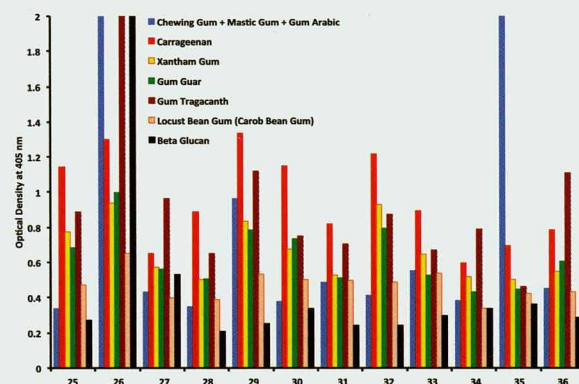
Abbreviation: IgG, immunoglobulin G.

Figure 2. Optical density at 405 nM for samples from patients 13 through 24, tested for IgG against chewing gum + mastic gum + gum arabic, carrageenan, xantham gum, gum guar, gum tragacanth, locust bean gum (carob bean gum), and β-glucan.



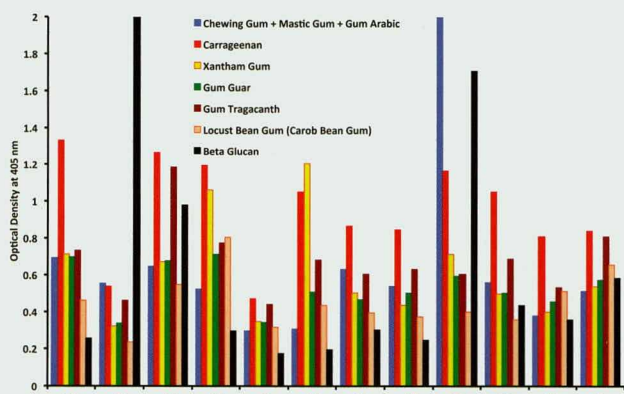
Abbreviation: IgG, immunoglobulin G.

Figure 3. Optical density at 405 nM for samples from patients 25 through 36, tested for IgG against chewing gum + mastic gum + gum arabic, carrageenan, xantham gum, gum guar, gum tragacanth, locust bean gum (carob bean gum), and β-glucan.



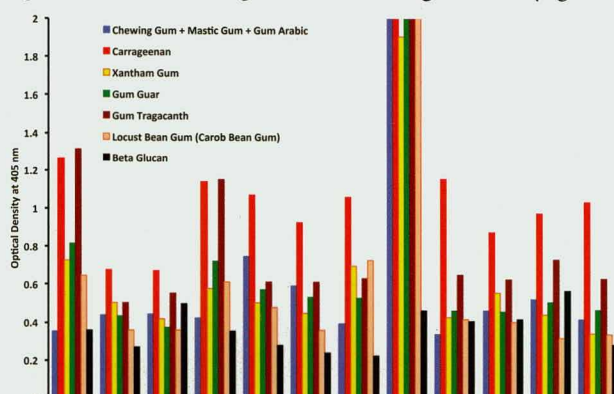
Abbreviation: IgG, immunoglobulin G.

Figure 4. Optical density at 405 nM for samples from patients 17 through 48, tested for IgG against chewing gum + mastic gum + gum arabic, carrageenan, xantham gum, gum guar, gum tragacanth, locust bean gum (carob bean gum), and β -glucan.



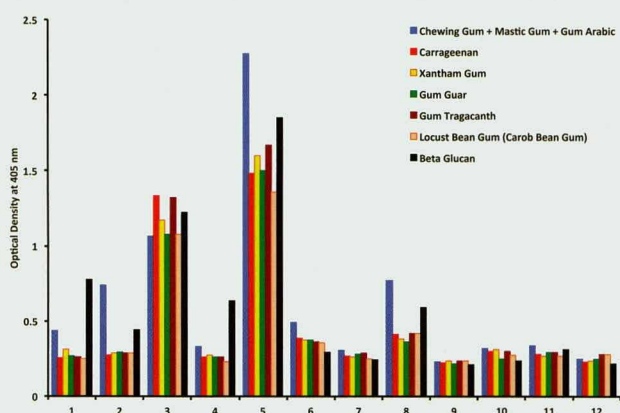
Abbreviation: IgG, immunoglobulin G.

Figure 5. Optical density at 405 nM for samples from patients 49 through 60, tested for IgG against chewing gum + mastic gum + gum arabic, carrageenan, xantham gum, gum guar, gum tragacanth, locust bean gum (carob bean gum), and β -glucan.



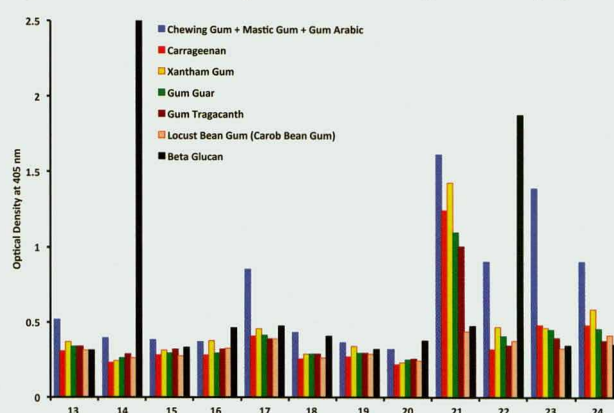
Abbreviation: IgG, immunoglobulin G.

Figure 6. Optical density at 405 nM for samples from patients 1 through 12, tested for IgE against chewing gum + mastic gum + gum arabic, carrageenan, xantham gum, gum guar, gum tragacanth, locust bean gum (carob bean gum), and β -glucan.



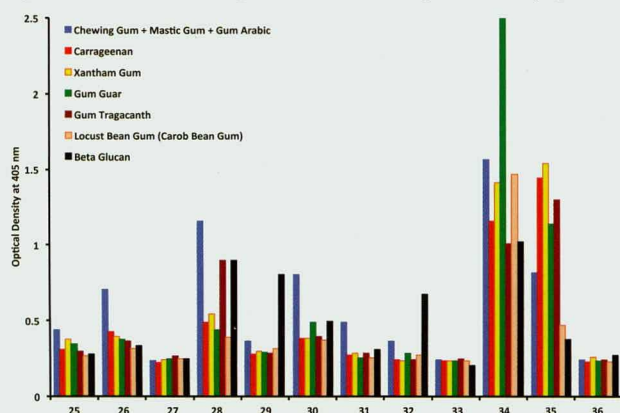
Abbreviation: IgE, immunoglobulin E.

Figure 7. Optical density at 405 nM for samples from patients 13 through 24, tested for IgE against chewing gum + mastic gum + gum arabic, carrageenan, xantham gum, gum guar, gum tragacanth, locust bean gum (carob bean gum), and β -glucan.



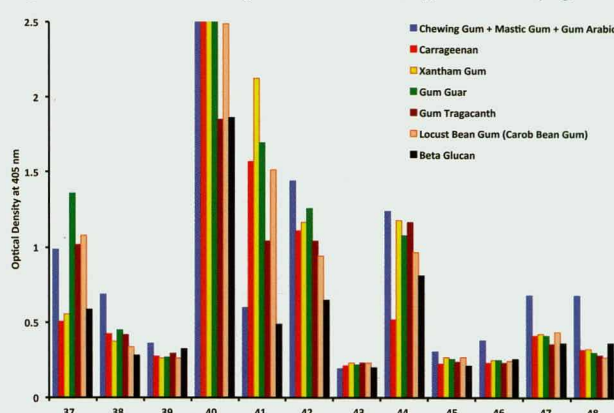
Abbreviation: IgE, immunoglobulin E.

Figure 8. Optical density at 405 nM for samples from patients 25 through 36, tested for IgE against chewing gum + mastic gum + gum arabic, carrageenan, xantham gum, gum guar, gum tragacanth, locust bean gum (carob bean gum), and β -glucan.



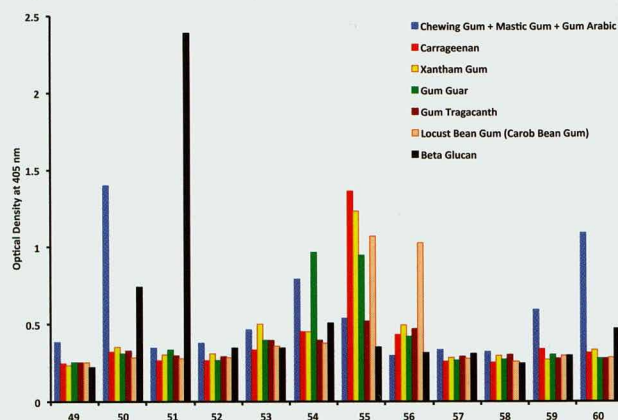
Abbreviation: IgE, immunoglobulin E.

Figure 9. Optical density at 405 nM for samples from patients 37 through 48, tested for IgE against chewing gum + mastic gum + gum arabic, carrageenan, xantham gum, gum guar, gum tragacanth, locust bean gum (carob bean gum), and β -glucan.



Abbreviation: IgE, immunoglobulin E.

Figure 10. Optical density at 405 nM for samples from patients 49 through 60, tested for IgE against chewing gum + mastic gum + gum arabic, carrageenan, xanthan gum, gum guar, gum tragacanth, locust bean gum (carob bean gum), and β -glucan.



Abbreviations: IgE, immunoglobulin E.

16.6%, 15.6%, and 22.9% of specimens showed an elevation in IgE antibody against mastic gum, carrageenan, xanthan gum, guar gum, gum tragacanth, locust bean gum, and β -glucan, respectively (Table 1). A significant percentage (12.8%) simultaneously produced IgE antibodies against all 7 tested extracts. Overall, this percentage elevation in IgE antibodies against different gum extracts was much higher than elevation in IgG antibodies ($P < .011$), with the exception of carrageenan.

Inhibition Study for IgE Antibodies

Inhibition studies were performed with specific, non-specific gum extracts and other possible cross-reactive food antigens such as sesame albumin, lentil, corn, rice, pineapple, peanut, pea protein, shrimp and kidney bean, to demonstrate the specificity of the IgG and IgE antibodies detected against different gum extracts. In comparison with HSA, which caused only between 1.3% and 7.2% inhibition of IgE antibody reactivity, the addition of specific gum antigens to the sera resulted in 76.9% to 86.5% inhibition in antigen-antibody reaction (see Table 2). Because the sera used in inhibition studies were found to react to many gum extracts simultaneously,

we performed similar inhibition studies with these gum extracts as well. We found that addition of different gum extracts also caused a significant inhibition in IgE reactivity against specific gum extracts. For example, addition of mastic gum to a highly-reactive sera with mastic gum, resulted in 82.3%. Inhibition when the same sera was absorbed with mastic gum and reacted with mastic gum coated plate. Repeating similar experiments by replacing mastic gum with carrageenan during absorption and then reacting the sera against mastic gum coated plated resulted in 56.7% inhibition of IgE reactivity of mastic gum antibody against mastic gum antigens (Table 2). Similarly, mastic gum caused 48.5% inhibition in carrageenan-anticarrageenan antibody reactivity. This is in comparison with 85.2% inhibition when carrageenan was added to sera with highly IgE-reactive against carrageenan. Similar to inhibition study with different gums, different food antigens were used for absorption of gum-specific IgE antibodies. Addition of these food antigens to the liquid phase and testing gum IgE reactive sera against gum antigen-coated plates resulted in a significant inhibition of antigen-antibody reaction. This inhibition of gum IgE antibody by different food antigens was the highest when sera were absorbed with pineapple. As is shown in Table 2, pineapple caused between 39.7% and 53.7%; sesame, 28.6% and 41.2%; and kidney bean, 19.5% and 26.8% in inhibition of IgE reactivity against various gum antigens.

Inhibition Study for IgG Antibodies

Similar inhibition studies were conducted with sera highly reactive against different gum antigens. Addition of each specific gum antigens to sera with very high IgG titer against various gums resulted between 70% and 80% IgG immune reactivity against gums extracts. However, addition of nonspecific gum or possible cross-reactive food antigens to sera with high IgG against specific gum resulted only in 5% to 14% inhibition in IgG antibody levels (data not shown).

DISCUSSION

Inspired by the detection of IgG against guar gum by Lagier et al,⁹ the current study measured IgG and IgE antibodies against different gum extract preparations in 288 healthy individuals.

Table 1. Percentage Elevation in IgG and IgE Antibodies Against Various Gum Antigens in Sera of 288 Healthy Individuals, at 2 SDs Above the Mean

	Mastic Gum	Carrageenan	Xanthan Gum	Guar Gum	Gum Tragacanth	Locust Bean Gum	β -Glucan	Simultaneous Elevation
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
IgG	42/288 (14.6)	78/288 (27.1)	30/288 (10.4)	12/288 (4.2)	15/288 (5.2)	33/288 (11.5)	18/288 (6.2)	4/288 (1.4)
IgE	84/288 (29.1)	57/288 (19.8)	48/288 (16.6)	51/288 (17.7)	48/288 (16.6)	45/288 (15.6)	66/288 (22.9)	37/288 (12.8)

Note: $P = .011$.

Abbreviations: IgG, immunoglobulin G; IgE, immunoglobulin E; SD, standard deviation.

Depending on the gum's structure, the research team found significant immune reactivity against various gum extracts. For example, an IgG response against gum tragacanth was found in 5.2% of the tested individuals and against carrageenan in 27.1% (Table 1).

These findings indicate that not only is a significant percentage of the healthy population exposed to various gum products, but also that these individuals immunologically react against them and produce non-IgE-mediated immune reactivity. In relation to IgE antibodies, a much higher percentage of the sera tested not only reacted immunologically against various gum antigens but also produced a significant level of IgE antibodies. Elevations in levels were detected against locust-bean gum extract in 15.6% of tested individuals and against mastic gum in 29.1% (Table 1). Although the research team did not have information about hypersensitivity to various allergens and antigens for the individuals whose sera was tested, it seems that these IgE antibodies could have contributed to allergy and sensitivity in them.

Indeed, when the team conducted inhibition studies to demonstrate the specificity of these antigen-antibody reactions, the addition of specific gums to sera with high titers of IgG antibody caused more than 69% inhibition in the antibody titers, whereas the addition of nonspecific food antigens caused only 5% to 14% inhibition of these reactions. Therefore, it would seem that sesame, lentil, corn, rice, pineapple, peanut, pea protein, shrimp, and kidney bean do not share any epitopes with various gum antigens that could result in production of IgG cross-reactive antibodies.

However, when similar experiments were conducted with gum-specific, IgE-containing sera, in addition to inhibition of 76.9% to 86.5% with gum-specific antigens, significant inhibition of IgE antibody reactivity occurred with nonspecific gum antigens or possible cross-reactive food antigens (Table 2). For example, the addition of HSA to sera

Table 2. Demonstration of specificity through percentage of ELISA inhibition of antigen-antibody reaction by the addition of specific and nonspecific inhibitors to the serum prior to testing. The addition of a specific antigen to a serum results in a corresponding inhibition to that same substance, as shown by the boxed numbers when a specific inhibitor reacts with its specific antigen. For example, mastic gum inhibits the reaction to mastic gum antigen by 82.3%.

INHIBITORS	ANTIGENS															
	Mastic				Xanthan				Locust				Sesame			
	Gum	Carrageenan	Gum	Tragacanth	Gum	Carrageenan	Gum	Tragacanth	Gum	β-Glucan	Albumin	Lentil	Corn	Rice	Pineapple	Pea Protein
HSA	1.5	2.2	3.6	4.1	1.3	5.6	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5
Mastic Gum	82.3	56.7	33.1	24.7	29.6	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5
Carrageenan	48.5	85.2	36.8	20.6	33.3	29.2	29.2	29.2	29.2	29.2	29.2	29.2	29.2	29.2	29.2	29.2
Xanthan Gum	43.8	54.6	76.9	22.5	30.5	38.6	38.6	38.6	38.6	38.6	38.6	38.6	38.6	38.6	38.6	38.6
Guar Gum	23.1	48.5	38.6	80.8	29.7	36.8	36.8	36.8	36.8	36.8	36.8	36.8	36.8	36.8	36.8	36.8
Gum	33.5	44.8	32.6	23.6	78.4	31.7	31.7	31.7	31.7	31.7	31.7	31.7	31.7	31.7	31.7	31.7
Tragacanth																
Locust Bean	41.3	47.9	33.8	26.9	25.3	81.6	81.6	81.6	81.6	81.6	81.6	81.6	81.6	81.6	81.6	81.6
Gum	24.7	28.3	21.7	23.8	22.1	26.4	26.4	26.4	26.4	26.4	26.4	26.4	26.4	26.4	26.4	26.4
β-Glucan																
Sesame	39.5	31.6	34.2	31.4	32.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7
Albumin																
Lentil	33.5	29.7	38.4	35.7	30.6	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5
Corn	35.7	33.6	36.5	35.1	32.9	36.8	36.8	36.8	36.8	36.8	36.8	36.8	36.8	36.8	36.8	36.8
Rice	28.1	31.4	29.7	26.8	29.1	30.2	30.2	30.2	30.2	30.2	30.2	30.2	30.2	30.2	30.2	30.2
Pineapple	46.3	40.5	41.1	44.5	41.6	43.9	43.9	43.9	43.9	43.9	43.9	43.9	43.9	43.9	43.9	43.9
Peanut	35.2	36.7	39.4	35.3	30.2	34.5	34.5	34.5	34.5	34.5	34.5	34.5	34.5	34.5	34.5	34.5
Pea Protein	29.6	33.4	34.7	31.6	32.8	34.3	34.3	34.3	34.3	34.3	34.3	34.3	34.3	34.3	34.3	34.3
Shrimp	31.5	28.2	32.2	34.6	27.7	28.5	28.5	28.5	28.5	28.5	28.5	28.5	28.5	28.5	28.5	28.5
Kidney Bean	21.3	18.7	16.9	21.3	19.2	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6

Abbreviation: ELISA, enzyme-linked immunosorbent assay.

containing β -glucan IgE antibody caused only a 6.3% inhibition of anti-IgE binding to β -glucan, which is within the accepted variation of the ELISA assay. But when β -glucan was added to the sera, it caused an inhibition of 86.5% in the binding of the IgE to the β -glucan that was bound to the microtiter plate. The addition of other gum extracts resulted in inhibition of 28.7% to 39.1% of the antigen-antibody reaction.

Similarly, when possible cross-reactive food antigens were added to the sera with the IgE antibody containing β -glucan, the inhibition was found to be 34.4% for the sera with kidney bean, 42.9% with shrimp, 40.2% with pea protein, 53.7% with peanut, 52.7% with pineapple, 43.5% with rice, 48.1% with corn, 42.4% with lentil, and 34.1% with sesame albumin.

This significant inhibition of the IgE antibody against various gums by the addition of nonspecific gums as well as food extracts indicates that specific epitopes are shared first between various gums and then between foods and different gum extracts, which results mainly in cross-reactive IgE responses (Table 2). Therefore, exposure to gums through different products may cause hidden food allergies and immune reactivities.

CONCLUSIONS

The research team concluded that the subgroup of individuals who appear to be symptom free but have IgG- and IgE-mediated immune reactivities against gums should be put on diets that remove these gums, and that serious consideration should also be given to removing foods that cross-react with these gums. Otherwise, patients may continue to suffer from hidden allergies and food immune reactivities, even after the elimination of the specific offending food antigens.^{7,38-41}

The team strongly encourages further clinical research with symptomatic individuals to examine whether gum reactivity contributes to multiple food and chemical reactivities and strongly urges that the correlation between inhalation-induced reactivity and ingestion-induced reactivity be given serious study.

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