Autoimmunity Reviews 14 (2015) 479–489

Contents lists available at ScienceDirect

Autoimmunity Reviews

journal homepage: www.elsevier.com/locate/autrev

Review

Changes in intestinal tight junction permeability associated with industrial food additives explain the rising incidence of autoimmune disease

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ARTICLE INFO

Article history:
Received 2 January 2015
Accepted 18 January 2015
Available online 9 February 2015

Keywords:
Autoimmune disease
Food additive
Tight junction
Permeability
Intestine
Industry

ABSTRACT

The incidence of autoimmune diseases is increasing along with the expansion of industrial food processing and food additive consumption.
The intestinal epithelial barrier, with its intercellular tight junction, controls the equilibrium between tolerance and immunity to non-self-antigens. As a result, particular attention is being placed on the role of tight junction dysfunction in the pathogenesis of AD. Tight junction leakage is enhanced by many luminal components, commonly used industrial food additives being some of them.
Glucose, salt, emulsifiers, organic solvents, gluten, microbial transglutaminase, and nanoparticles are extensively and increasingly used by the food industry, claim the manufacturers, to improve the qualities of food. However, all of the aforementioned additives increase intestinal permeability by breaching the integrity of tight junction paracellular transfer. In fact, tight junction dysfunction is common in multiple autoimmune diseases and the central part played by the tight junction in autoimmune diseases pathogenesis is extensively described. It is hypothesized that commonly used industrial food additives abrogate human epithelial barrier function, thus, increasing intestinal permeability through the opened tight junction, resulting in entry of foreign immunogenic antigens and activation of the autoimmune cascade. Future research on food additives exposure-intestinal permeability–autoimmunity interplay will enhance our knowledge of the common mechanisms associated with autoimmune progression.

Contents

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http://dx.doi.org/10.1016/j.autrev.2015.01.009

1. Introduction

The incidence of autoimmune diseases (AD) is increasing world-wide, mainly in western countries and the role of the environment in AD development is gradually becoming clear [1]. Similarly, industrial food processing and food additive consumption is expanding. The recent increased knowledge on the functions, mechanisms and abnormalities of intestinal permeability and the specific relationship between some common food additives and their deleterious effects on the tight-junction, prompted us to review these observations and put forward the hypothesis that increased intestinal permeability induced by the industrial food additives explains the observed surge in autoimmune disease.

2. Autoimmune diseases are on the increase

Epidemiological data provide strong evidence of a steady rise in AD throughout westernized societies over the last three decades [2]. Multiple sclerosis, type 1 diabetes, inflammatory bowel diseases (mainly Crohn's disease), systemic lupus erythematosus, primary biliary cirrhosis, myasthenia gravis, autoimmune thyroiditis, hepatitis and rheumatic diseases, bullous pemphigoid, and celiac disease are several examples [2–6, Fig. 1, Ref.: 4,7–24]. Fig. 1A shows the cumulative net fold increases in AD incidence/prevalence worldwide in those countries where it was reported. Increased folds\year of type 1 diabetes, bullous pemphigoid, and autoimmune thyroiditis, of 0.77, 0.35 and 0.24, respectively, were noted [14,18,20]. Type 1 diabetes increased 3–4% per annum and undiagnosed celiac disease mortality increased 4 fold in the USA [21,25]. Grouping the ADs to major disease classes, the highest net increase % per year was noted in the neurological followed by gastrointestinal, endocrine, and rheumatic diseases (Fig. 1B). The geoepidemiological distribution of AD, the world-wide North–south and West–east gradients in Europe, their relationship to socioeconomic status, their rapid increase in developed countries and observations in migrant populations all indicate some form of environmental impact, rather genetic factors, driving these recent and rapid evolutionary processes [1–3]. Among many others, two major environmental factors, strongly related to socioeconomical status are suspected to driver these phenomena: infections and nutrition. The present review will not expand on the debate of the interrelationship between AD and infections [26]. A survey of the recent changes in industrial food additives processing and the effect of food additives on intestinal permeability, resulting in increased tight junction leakage, local and systemic immune stimulation and potentiating AD induction, is presented here.

3. Increased usage of industrial and consumer food additives

The changes in agricultural and industrial practices over the past decades have increased the world's capacity to provide food through

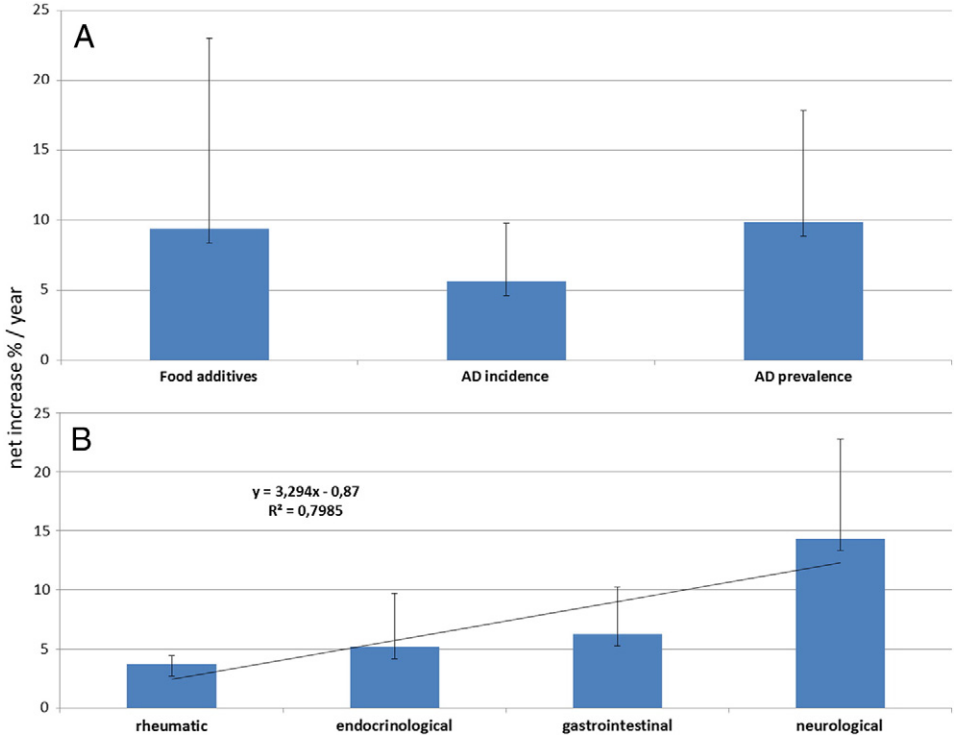

Fig. 1. A. The mean net % increase per year of industrial food additives usage and AD incidences and prevalences. B. The mean net % increases of different classes of ADs. Adapted from: Ref.: [4,7–24].

increased productivity and diversity, decreased seasonal dependency and seasonal prices [27]. Increased consumption has been facilitated by rising income, urbanization, food industry marketing, media advertisement and trade liberalization, mainly in developed countries. Major shifts in dietary patterns are continually occurring, even in basic staples consumption towards more diversified and industrially processed food products. Living in westernized countries has a strong impact on nutritional patterns collectively termed the "Western diet" including high fat, trans fatty acids, cholesterol, proteins, sugars, salt intake, as well as frequent consumption of processed and "fast food" [28]. Influenced by this reality, populations of developing countries undergo a rapid change towards "transition nutrition". The traditional dietary pattern is gradually being replaced by the Western one [29]. Many studies, throughout the world, have documented the abovementioned changes, but only Brazil will be sited as an example. Between 1987–8 and 2002–2003 the contribution of ultra-processed food to total household energy availability increased from 19.2 to 28% (an increase of 46%), replacing the intake of unprocessed and minimally processed foods [30]. This world-wide process is happening in both developing as well as in developed countries [31,32]. Even today, the transition in nutritional habits starts in child day care centers [33]. In the first year of life, the percentages of adequacy for carbohydrates and sodium were respectively more than twice and 20 times, higher than that recommended.

The following paragraphs will deal with 7 nutritional ingredients that are being increasingly added during industrial food processing and find their way to the market product shelves, impacting human health.

3.1. Sugars

Sugars are a major additive used in food. Analyzing the calories from main commodities (kcal/capita/day) in developing countries and China, the percentage change in 4 decades for sugars was +127 and +305 respectively [27]. The mean increase in availability of sugar and sweeteners (kcal/person/day) during the last 4 decades, around the Mediterranean and in Central Europe increased 145 and 123% respectively [27]. More so, countries that traditionally had the highest adherence to the Mediterranean diet (less processed, lower calories, higher fiber) like Greece, experienced the greatest fall (63%) in the Mediterranean adequacy index, consuming ever increasing amounts of energy-dense and sweet products. A comparable decrease was depicted in other Mediterranean countries and in Central Europe [27]. In Asia, sugar consumption has rapidly increased in lower-middle and upper-middle-income countries, carbonated soft drinks are the most significant vector for increased sugar consumption [34].

3.2. Salt

Salt is considered a silent killer since increased consumption is associated with hypertension, strokes, left ventricular hypertrophy, renal diseases, obesity, renal stones and stomach cancer. Over consumption of salts is real, spanning multiple populations, ages, gender and continents.

Sodium intakes around the world are well in excess of physiological need (i.e. 10–20 mmol/day). Most adult populations have mean sodium intakes >100 mmol/day, and for many (particularly the Asian countries) mean intakes are >200 mmol/day. Sodium intakes are commonly >100 mmol/day in children over 5 years old, and increase with age. In European and Northern American countries, sodium intake is dominated by sodium added in manufactured foods (approximately 75% of intake). Cereals and baked goods are the single largest contributor to dietary sodium intake in UK and US adults. In Japan and China, salt added at home and in soy sauce remains the largest sources. The salt content in processed foods can be more than a 100 times higher that that in similar homemade meals [35].

In Brazil, as mentioned above, infants below the age of 1 year consume 20 fold the recommended intake of sodium [33]. Based on these data, the national annual production of petit-Swiss cheese category, where each 30 g contains 412 mg of sodium, increased from 10,000 tons in 1992 to 24,000 tons in 2005 [33]. More so, even in Europe, in South London, salt intake is 66, 73, and 73% above the maximum daily recommended intake in 5–6, 8–9, and 13–17 year age-groups, respectively [36]. The estimated sodium intake for the Brazilian population for processed foods with added salt increased by 3.1% in 6 years when studied in 2008–2009 [37]. In the nutrition transition parts of the world, like Asia, salt intake is increasing rapidly and processed baked goods are the main vector [34]. In fact, 80% of consumed salt comes from manufactured products in developed countries [38]. On the face of it, a voluntary decrease in salt consumption seems to be an easy policy to implement, but good sense and good health face the formidable opposing forces of flavor, habit, and culture.

3.3. Emulsifiers

Emulsifiers are a group of substances that concentrate at the interface between oil and water and reduce the surface or interfacial tension, thereby making the emulsion more stable. In many industries emulsifiers are referred to as "surfactants", which is an abbreviation of surface active agents. They can interact with other ingredients like starch, protein and fat. They are widely used in the bakery, confectionary, dairy, fat and oil, sauces, butter and margarine, ice cream, cream liqueurs, meat, coffee, gum, beverages, chocolate and convenient food industries. As intestinal barriers can inhibit oral drug bioavailability, absorption enhancers are generally applied. In many cases the surfactants added to foods are exactly the same as the ones used in pharmaceutics as absorption enhancers. The most important synthetic surfactants used in the food industry are: mono-and di-glycerides of fatty acids, sucrose esters of fatty acids, polyglycerol esters of fatty acids, sodium/calcium stearoyl-2-lactylate and sorbitan esters of fatty acids. But many others groups like: lecithins, glycolipids, saponins, fatty alcohols, saturated/unsaturated/trans fatty acids, proteins, polysaccharides and microbial surfactants are used in the food and beverage industries [39].

The global food emulsifier market is a promising segment within the food ingredients market. The food emulsifier market is considered to be the fastest growing segment of the food additives market due to the growing trend towards reducing fat content in food products. The emulsifier market is largely driven by di-glycerides and derivatives, lecithin, stearoyl lactylates, and other emulsifiers such as polyglycerol esters (PGE), polyglycerol polyricinoleate (PGPR), polysorbate and sucrose esters, however, and many other synthetics as well as natural emulsifiers are expected to gain momentum in the near future. In terms of application, bakery items have been dominant, although research in various fields has opened up new avenues for the application of these substitutes.

The "marketresearch.com/food-emulsifiers-market" report [40] estimates the market size of the global food emulsifier market in terms of revenue and volume. According to the published report the food emulsifier market will grow from an estimated $2.108.9 million in 2012 to $2.858.6 million by 2018, an increase of 35%. The global food emulsifier market is expected to reach 933.4 KT by 2018, due to increasing demand for emulsifiers. Di-glyceride & derivatives dominate the market in terms of volume. In terms of geography, Europe is the major player, followed by North America, Asia-pacific and the rest of the world. With the huge market potential and the growing preference for their use, the market is likely to witness considerable growth in the years to come.

3.4. Organic solvents

Per definition, a solvent is a liquid capable of dissolving another substance. The organic solvent chemicals are genuinely dangerous.

Most of them have warning information on the labels as poisons. Examples of organic solvents used in industry are: benzene, xylene, toluene, turpentine, acetone, methyl/ethyl acetates, hexane, ethanol and several detergents. Several of them are used during industrial manufacturing processes for extraction/solubilization/cleaning/disinfection or nanoemulsion fabrication and not normally as food additives. The use of solvents for extraction of active ingredients or removal of undesirable substances is one of the more common practices in the food industry. This process generally involves use of commercial Hexane. It is one of the cheapest organic solvents available. It is estimated that about 60 million tons of edible oils are produced by Hexane extraction technology, mainly for soy oil. Many other organic solvents are considered as food additives mainly as antioxidants, stabilizers, preservatives, flavoring, etc [41]. Recently, in a meta-analysis, they were described as risk factors for AD [42]. An entirely novel area is the use of organic solvents in nanotechnology, creating new molecules, some of them potentially could be applied to the food industry. For example, nanocomplexation-assisted solubilization to improve microencapsulation [43]. Time will tell, if the new nanotechnology will enter the food chain. According to market-reports, the solvent market is expected to grow at a CAGR of 5.0% over the next five years to reach $43.4 billion, by 2018. Asia-Pacific, with its flourishing economy and rapidly expanding industrial sectors, is the leading consumer of solvents and will experience the highest growth in demand from 2013 to 2018, after North America [44]. The market experts from Ceresana forecast the global solvent market to earn revenues of about US$33 billion in 2019. Especially the dynamic economic development in emerging markets like China, India, Brazil, or Russia will continue to boost demand for solvents. The market research institute expects worldwide solvent consumption to increase at an average annual rate of 2.5% over the next years. Accordingly, the growth rate seen over the last eight years will be surpassed.

3.5. Gluten

Gluten is the major constituent in wheat, comprising 80% of the proteins. Wheat is grown on more than 216,000,000 ha (530,000,000 acres), larger than for any other crop and the world trade in wheat is greater than that for all other crops combined. Along with rice, wheat is the world's most favored staple food. In addition to agronomic adaptability, wheat offers ease of grain storage and ease of converting grain into flour for making edible, palatable, interesting and satisfying foods. Wheat is the most important source of carbohydrate in a majority of countries [45]. In the 20th century, global wheat output expanded by about 5-fold, whereby until about 1955 most of this reflected increases in wheat crop area, with limited (about 20%) increases in crop yields per unit area. Since 1955 however, there has been a dramatic ten-fold increase in the rate of wheat yield improvement per year, and this has become the major factor associated with increases in global wheat production. An average 2.5 tons wheat of was produced on one hectare of cropland in the world in the first half of 1990s, but this had increased to about 3 tons in 2009. According to the Australian Bureau of Statistics, the world production of wheat was 679 and 696 million tons in the years 2009–10 and 2011–12, respectively [46].

3.6. Microbial transglutaminase (mTG)

Transglutaminase (EC 2.3.2.13), i.e. protein-glutamine γ-glutamyltransferase, belongs to the class of transferases. It catalyzes the formation of an isopeptide bond between the group of γ-carboxamides of glutamine residues (donor) and the first-order ε-amine groups of different compounds, for instance, proteins (acceptors of an acyl residue).

The development of bread process was an important event for mankind, resulting in bread becoming a commodity within almost everyone's reach. Introduction of industrial enzymes in the baking process has, over the last 14 years, led to the development of a significant segment of the industry, as reflected by increased market value and the growth predictions for the next 6 years. In fact, the value of the baked goods industrial enzyme market doubled between 2000 and 2010. The prediction for 2015–2020 is an additional increase, mounting to 144% [47].

Microbial transglutaminase (mTG) occupies a substantial segment of this market. The application of isolated transglutaminase enzymes from a microbiological source has allowed for simplification of certain processes and has provided energy and economic savings. Thanks to established transgenesis procedures, gene transfer became possible and the expression of genes gave rise to massive microbial transglutaminase production. Multiple applications of mTG in the food industry exist: improvement of meat texture, appearance, hardness and preservability, increased fish product hardness, improved quality and texture of milk and dairy products, decreased calories, improved texture and elasticity of sweet foods, protein film stability and appearance and improve texture and volume in the bakery industry. Applications of mTG as a biological glue in the biomedical and biotechnology domains are constantly expanding. This is probably one of the fastest-growing areas, as reflected by the increasing number of patent applications filed on mTG. The mTG treated food industry is expanding on a great scale and mTG is ingested in large amounts in the common Western diet [48–50]. The demand for baked goods, food and beverage enzymes is forecasted to grow by 0.22 to 0.32 fold per year, between 2000 and 2020 (47). Altogether, a maximum daily intake of mTG could range up to 15 mg. Dosing for restructuring is about 50–100 mg of mTG for each kilogram of treated food [51].

3.7. Nanoparticles

Nanotechnology encompasses the understanding and control of matter at dimensions between 1 and 100 nm. At these dimensions, materials may acquire unusual physical, chemical and biological properties and functions that are remarkably different from those observed at the macro- or microscale. The future implications of nanotechnology are outstanding as it can offer more solutions to technological problems than conventional systems. Nanotechnology is a multi-disciplinary field, which can create materials and devices that can be applied to multiple domains including foods production and packaging. Nanoemulsions fabricated from food-grade ingredients are being increasingly utilized in the food industry to encapsulate, protect, and deliver lipophilic functional components, such as biologically-active lipids and oil-soluble flavors, vitamins, preservatives, and nutraceuticals. The small size of the particles in nanoemulsions means that they have a number of potential advantages over conventional emulsions: higher stability to droplet aggregation and gravitational separation, high optical clarity, ability to modulate product texture, and increased bioavailability of lipophilic components [52]. Oil-in-water nanoemulsions, which consist of oil droplets dispersed within aqueous phase, have the greatest potential for application within the food industry. Nevertheless, water-in-oil ones are used in some types of food products. Nano-carrier-based delivery presents an appropriate choice of protein-based nutrient carriers owing to their property to protect proteins from degradation by the low pH conditions in the stomach or by proteolytic enzymes in the gastrointestinal tract, without affecting their taste or appearance. Additionally, carbohydrates, salts, lipids, emulsifiers, organic solvents, gluten and mTG are also used during nanoparticle assembly or for enhanced delivery. Nanomaterials are being developed to improve the taste, color, uniformity and texture of foods, as well as in food packaging so as to minimize leakage of CO_2 from bottles or kill bacteria (silver nanoparticles embedded in plastic). Nanosensors in plastic packaging can detect gases given off by food when it is spoiled. Nanoliposomes can carry nutrients, nutraceuticals, enzymes, food antimicrobials and food additives [53].

The total nano-enabled food and beverage packaging market in the year 2008 was $4.13 billion, and was expected to grow to $7.30 billion

by 2014, at a CAGR of 11.65% [54]. The Helmut Kaiser Consultancy Group estimated that the global nanofood market was worth US$5.3 billion in 2005 and had grown to US$20.4 billion by 2010. It predicted that nanotechnology will be used in 40% of food industries by 2015 [55].

The fold net increases/year of the abovementioned, industrial food additives actual and forecast sales and consumption during the last 4–6 decades, can be seen in Fig. 1A. The more detailed bars (Fig. 2) show the past compared to the actual fold increase of consumption or sales of the various industrial additives, during the last decades, where the use of industrial enzymes and nanoparticles has surged. The differential geographical distribution of the net increased % of food additives is shown in Fig. 3. The use in Europe is ahead of the world average, and that of USA and Brazil.

4. Intestinal tight junction regulation

Only a single layer of epithelial cells separates the luminal contents from effector immune cells in the lamina propria and the internal milieu of the body. Breaching this single layer of epithelium can lead to pathological exposure of the highly immunoreactive subepithelium to the vast number of foreign antigens in the lumen. The permeability of the intestinal epithelium depends on the regulation of the mucosal immune system and intercellular tight junction (TJ). Research carried out over the last decade has demonstrated that the TJ is composed of a complex network of proteins, the interaction of which dictates its competency.

Zonulins, occludins, claudins and junctional adhesion molecules are a few examples that modulate movement of fluid, macromolecules and leukocytes from intestinal lumen to the blood stream and vice versa. In addition, these TJ proteins are involved in protecting the epithelial cells of the intestine against colonization by microorganisms. It is now apparent that TJs are dynamic structures that are involved in developmental, physiological and pathological processes. They regulate the trafficking of macromolecules between the environment and the host through a barrier mechanism. Together with the gut-associated lymphoid tissue and the neuroendocrine network, the intestinal epithelial barrier, with its intercellular TJs, controls the equilibrium between tolerance and immunity to non-self antigens. As a result, particular attention is being placed on better understanding the role of TJ dysfunction in the pathogenesis of several diseases, particularly AD.

Pathophysiological regulation of tight junctions is influenced by many factors, including: secretory IgA, enzymes, neuropeptides, neurotransmitters, dietary peptides and lectins, yeast, aerobic and anaerobic bacteria, parasites, proinflammatory cytokines, free radicals and regulatory T-cell dysfunction [56]. Given the complexity of intracellular structure and function of TJ proteins, it is not surprising that when affected, the physiological state of epithelial and/or endothelial cells is dramatically changed as well.

TJ dysfunction seems to be a primary defect in AD [57]. Intestinal permeability is decreased in many AD: Ulcerative colitis, Crohn's disease, celiac disease, inflammatory joint disease, ankylosing spondylitis, juvenile onset arthritis, psoriatic arthritis, type 1 diabetes mellitus and primary biliary cirrhosis. In fact, in addition to genetic predisposition and exposure to triggering non-self antigens, the loss of protective function of mucosal barriers that interact with the environment is necessary for autoimmunity to develop.

5. Commonly ingested food ingredients increase intestinal permeability

The seven food additives and their increased usage, described above, induce or are associated with increased intestinal permeability:

5.1. Sugars

Glucose is known as an absorption enhancer. It is known that a major portion of intestinal glucose absorption occurs through tight junctions and not by saturable transcellular active transport. The absorption of a significant portion of glucose through tight junctions requires increased junctional permeability, a very high intraluminal glucose concentration, and a sufficient osmotic gradient to promote volume flow [58]. Glucose was found to increase permeability and produce changes in distribution of the main protein of the tight junction in the human cell line Caco-2, indicating intercellular leakage. Addition of luminal glucose to segments of rodent small intestine, mounted in Ussing chambers caused significant increase in paracellular permeability to small molecules. The same observation was seen in rats and even in human [59–61]. Most recently, glucose, as an absorption enhancer, was shown to increase Caco-2 cell permeability, parallel to abnormal distribution of TJ proteins [61]. In fact, Crohn's patients (increased intestinal permeability) have a higher dietary intake of sucrose and refined carbohydrates, compared to controls [62].

5.2. Salt

The consumption of processed foods containing high amounts of salt may in part be responsible for the increasing incidence of autoimmune diseases. In a recent study it was demonstrated that an excess uptake of salt can affect the innate immune system, in particular macrophage function, and affects the differentiation of naïve CD4+ T cells into

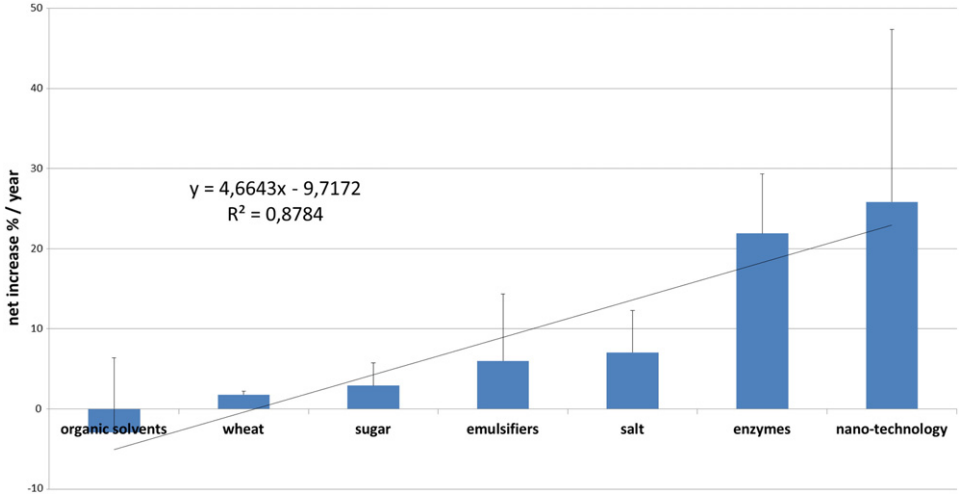

Fig. 2. The net fold % increases/year of the different industrial food additives, actual and forecasted sales and consumption over the last 4–6 decades. Adapted from: [24,26,28,30,34,37,41–43,47,51,52].

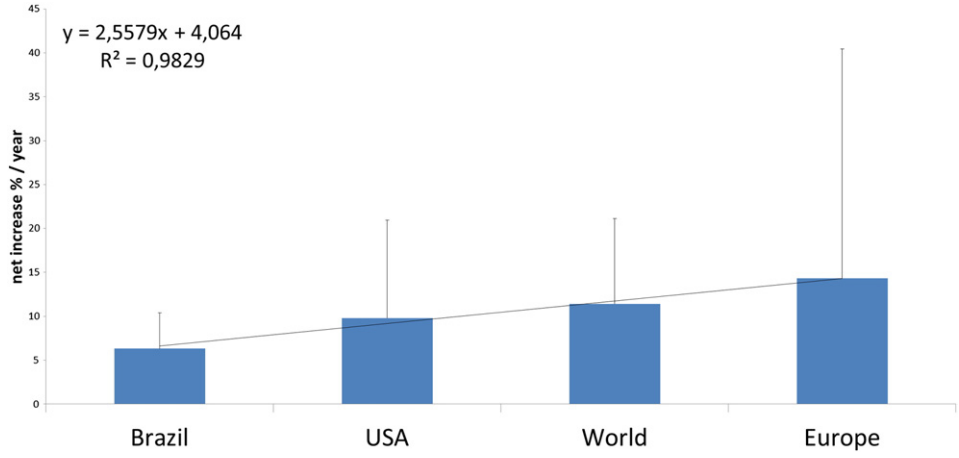

Fig. 3. The mean net % increases per year of industrial food additives, in various geographical areas.

a greater number of T_H17 cells. High salt concentration, change in osmolarity, the influence of IL-23 and IL-23 receptor signaling, and the activation of various enzymes drive the expression of T_H17-associated cytokines and the formation of the pathogenic T_H17 phenotype. This immune pathway plays a pivotal role in autoimmune disease [63]. Most recently, increased salt concentrations were shown to drive neuropathy in a mouse model of multiple sclerosis by induction of pathogenic T_H17 cells [64].

More so, initiation of Na^+-glucose cotransport leads to activation of NHE3 (a major route of Na^+ absorption in the small intestine), increased phosphorylation of myosin light chain, contraction of the perijunctional actomyosin ring and ultimately, increased permeability of intestinal tight junctions [59]. Finally, and most recently, the importance of the tight junction proteins claudin2 and 15, in paracellular Na^+ flow associated nutrient transport was elucidated. Loss of these claudins leads to death of the mice from malnutrition [65]. Thus, increased salt consumption is an enhancer of intestinal permeability through the TJ machinery.

5.3. Emulsifiers and surfactants

Numerous synthetic surfactant food additives have been shown to increase the intestinal permeability through paracellular and/or transcellular mechanisms and some of these additives were also shown to inhibit P-glycoprotein. Additionally, based on the general characteristics of surfactants it can be predicted that they decrease the hydrophobicity of the mucus layer, which has also been shown to associate with increased intestinal permeability. Previously, Csáki KF, Ilbäck et al. and Roberts CL et al., hypothesized that synthetic surfactant food additives can cause intestinal barrier dysfunction [66–68]. Two hypotheses were proposed supporting food additive induction of Crohn's disease [68,69]. They summarized the observations on the deleterious effects of surfactant additives on the hydrophobic intestinal mucus layer, on the epithelial cell membranes and on the membranous transport protein p-glycoprotein. The present review will expand on the emulsifier-induced alterations in intracellular events causing destabilization of tight junctions between the GI epithelial cells, thus increasing intestinal leakage.

Sucrose monoester fatty acids, major and potent surfactants used in the food industry, induced actin disbandment and structural separation of TJ, in Caco-2 cells [70]. They showed those effects even at a concentration of 50 mg/L. Surprisingly, the same surfactant is permitted in infant milk formulae at concentrations of up to 120 mg/L, bringing the subject of early life environmental effects on AD induction to the fore [66]. Surface active compounds, like oleic and docosahexaenoic acids, compromised the integrity of the intestinal epithelium and enhanced the paracellular absorption of poorly absorbed hydrophilic substances [71]. In general, fatty acids like EPA, DHA, γLA, capric and lauric acids increase TJ permeability [72]. More recently, Na-cholate, an additional absorption enhancer was shown to disturb TJ protein distribution resulting in increased Caco-2 inter-cell permeability [61]. Other surfactants, used in pharmaceutical and food industries like: Cremophor EL, Gelucire 44/14 or sodium taurocholate were found to disrupt the intestinal barrier [73]. Self-microemulsifying systems that are used to improve drug delivery via a microemulsion achieved by chemical rather than mechanical means, were found to open the TJ and change the distribution of ZO-1 and actin [74]. Their food applications are pending. Similarly, when Caco-2 monolayers are exposed to benzalkonium chloride and saponin at nontoxic levels, paracellular flux increases [75].

In Japan, a major player in the food industry utilization of emulsifiers, a positive correlation was shown between the annual sales of emulsifiers for food and beverage production and an increased incidence of Crohn's disease [68]. It can be summarized that there are enough scientific observations concerning the emulsifier effects on breaching the integrity of the TJ, even at concentrations used in industrial food processing.

5.4. Organic solvents

The health hazards associated with organic solvent exposure are extensively described. However, the present review highlights the potential risks in using them in the food and beverage industry, in the face of enhancing TJ permeability. Some nutrients like glutamine and polyphenols protect TJ barrier integrity, in contrast, several organic solvents used in the food and beverage industries, like alcohol and its metabolites impair the TJ barriers [63].

Ethanol increases paracellular permeability and induces alterations in TJ proteins, as shown in Caco-2 cells [76]. Acetaldehyde, dissociates the PTP1B-E-cadherin-beta-catenin complex in Caco-2 cell monolayers resulting in increased permeability [77]. Fermented food and many alcoholic beverages can also contain significant amounts of acetaldehyde. Acetaldehyde, derived from mucosal or microbial oxidation of ethanol and diet, appears to act as a cumulative carcinogen in the upper digestive tract of humans. In isolated rabbit jejunal mucosa, ethanol enhanced the transport of electrolytes and organic substances [78]. Ethanol and methanol were shown to increase permeability and disturb TJ protein arrangement in the Caco-2 model [61].

In a clinical study, paradichlorobenzene, a mothball and toilet cleaner, was found to induce leucoencephalopathy and deterioration in multiple sclerosis patients [79,80]. Furthermore, in a recent meta-analysis, organic solvents were described as risk factors for AD [42]. Exposure to organic solvents was found to be associated with systemic

sclerosis, primary systemic vasculitis and multiple sclerosis individually and also with all the ADs evaluated and taken together as a single trait. The authors concluded that individuals with non-modifiable risk factors should avoid any exposure to organic solvents in order to avoid increasing their risk of ADs.

5.5. Gluten

Tissue transglutaminase is the autoantigen of celiac disease and gluten is the environmental inducer of the disease [81,82]. Phenotypically, gluten represents the offending nutrient at the origin of the classical and the extra intestinal manifestations of the disease [83–85]. Evidence exists that intestinal barrier defects have a role in initiating celiac disease [72,86,87]. A number of in vitro studies have confirmed the cytotoxicity of gluten's main antigen, gliadin. Gliadin has agglutinating activity, reduces F-actin content, inhibits cell growth, induces apoptosis, alters redox equilibrium and causes a rearrangement of the cytoskeleton through the zonulin pathway and the loss of TJ competence in the gastrointestinal mucosa. When IEC6 and Caco-2 cells are exposed to gliadin in vitro, interaction between occludin and ZO-1 is compromised and the cytoskeleton is rearranged, leading to increased monolayer permeability.

The mechanism for this has been linked to zonulin, known to modulate TJ. Gliadin induces zonulin release, leading to PKC-mediated cytoskeletal reorganization. Ex vivo human intestinal samples from celiac patients in remission also showed zonulin release when exposed to gliadin, causing cytoskeletal rearrangement and ZO-1 reorganization, leading to increased permeability. Gliadin causes zonulin release by binding to the CXCR3 receptor in intestinal cells via a MyD88-dependent pathway and subsequent transactivation of EGFR by PAR2, leading to small intestine TJ disassembly. Clinical studies demonstrated that the expression of CXCR3 and zonulin in the intestinal mucosa of CD patients is highly elevated. Abnormal increased permeability results in substantial exposure of immune cells to gliadin. The mucosal events become more complex since gliadin binding to CXCR3 expressed in T cells induces an inflammatory cascade further augmenting inter-epithelial permeability. Despite alternative intracellular pathways of gluten passage through the apical membranous CD71 and antigliadin SIgA TJ-independent protected transport, most probably the TJ abnormalities in CD are the major immunogenic force driving gluten induced enteropathy. Most recently, a gene expression study confirmed the involvement of tight junction genes related to permeability, polarity and cell proliferation in the epithelial destruction observed in CD. Coexpression patterns of several genes support the idea of a common regulatory mechanism that seems to be altered in active CD [88,89].

5.6. Microbial transglutaminase (mTG)

Three reactions are catalyzed by transglutaminase: acyl-transfer reaction, cross-linking reaction between Gln and Lys residues of proteins or peptides (transamidation) and deamidation [48].

Transglutaminase is an extracellular enzyme and is biosynthesized by several microbes. It has been isolated from *Streptoverticillium* sp. Contrary to human TG, microbial TG is calcium independent, has a lower molecular weight, has a single structural domain and exhibits a different reactivity to some food proteins. These characteristics make mTG a very useful tool for modifying the functionality of proteins in food products [48,90].

The following are some observations where mTG may increase intestinal permeability by cross-linking amino acids or protein:

1. If mTG imitates the protective and trophic functions of human TG on infectious agents and facilitates their survival in the gut lumen, the tight junction may leak since infections increase intestinal permeability [91].
2. Glutamine and sulfur-containing amino acids regulate Caco-2 cell tight junction proteins. Deprivation of glutamine (Gln) from cell culture medium and inhibition of Gln synthetase using methionine sulfoximine, led to significant decreases in transepithelial resistance of Caco-2 cell monolayers and increased permeability [92]. Also the sulfur-containing amino acids, cystine, cysteine and methionine enhance epithelial TJ permeability [93]. It can be speculated that the mTG mediated nonspecific linking of these amino acids to other molecules can induce a state of deprivation/surplus at the intestinal epithelial level, thus indirectly affecting TJ performance.
3. Cross-linking of different proteins acts as a major modifier of the physical and chemical properties/secondary and tertiary structure/antigenicity/and epitope repertoire of the linked complex. New nutritional immunogenic epitopes are a potential result of the cross-linking by mTG, presenting potential for TJ aberrations. It should be mentioned that meat products have been found to contain variable amounts of mTG, indicating that the mTG used in the food industry finds its way onto the market shelf to be ingested by the consumers, directly exposing their intestinal lumen to mTG [94].
4. mTG has emulsifying properties by cross-linking different proteins [95]. The deleterious effects of emulsifiers have been described above.
5. mTG has the ability to catalyze lipidation of protein, thus providing them with emulsifying activity [96]. Moreover, recently mTG induced cross-linking of various dietary proteins originating from casein, pork myofibrils, peanut and fish, was shown to improve their emulsifying capacity [95,97]. One wonders if gluten cross-linked to comparable proteins, will impact on its emulsifying property, since hydrolyzed gluten by itself improves emulsification, regardless of mTG treatment [98].
6. A new aspect of mTG usage is in nanoparticle cross-linking [99] or using the enzyme for designing new luminal delivery systems [100]. There are few limits to the possible usage of mTG in nanotechnology. The next section will deal with nanoparticles as potential enhancers of intestinal permeability.
7. Additionally, multiple mTG linked proteins, including bakery products, are immunogenic to celiac disease patients [101] and most recently we observed specific anti-mTG and mTG-gliadin neo complex antibodies only in CD sera and not in healthy controls (personal communication, unpublished).

5.7. Nanoparticles

Nanoparticles, due to their unique properties and surface characteristics, can protect drugs from the destructive factors in the GI tract and can increase the permeability of macromolecules through the gastrointestinal barrier [102,52]. However, the question arises as to whether such advantages to the pharmaceutical industry represent disadvantages to the food and beverage industries. Many recent reports confirm that nanoparticulate systems with unique properties can increase the transport of poorly water-soluble compounds across the GI barrier by enhancing paracellular transport via opening of the TJ:

There are many reports on the application of nano-based thiolated chitosan for enhancing permeability, mucoadhesivity and intestinal absorption of active agents. Permeation studies showed that nanoparticles opened the tight junctions of monolayer Caco-2 cells and increased paracellular transportation [103]. The signaling mechanism initiating the cascade of disruption of the TJ, was elucidated recently [104]. Nanoparticles composed of chitosan and over sulfated fucoidan opened the TJ and induced redistribution of ZO-1 TJ protein [105]. Chitosan by itself, a potent paracellular permeation enhancer, induced clustering of integrin $\alpha(V)\beta(3)$ along the cell border, F-actin reorganization and claudin4 down-regulation, eventually disrupting TJ integrity [106]. Hydrophobic nanoparticles opened the

TJ in Caco-2 cells and resulted in increased bioavailability of hydrophilic substance in rats [107]. Nanoparticles prepared by nanoprecipitation using poly(lactic-co-glycolic acid) as carrier material and surface modified by methoxy poly(ethylene glycol) and chitosan, were shown to reduce the distribution of ZO-1 protein, thus impacting paracellular entry [108]. Very recently, Zhang et al. showed that goblet cell-targeting CSK peptide modified nanoparticles strongly opened epithelial TJ via a C-Jun Nh_2-terminal kinase-dependent pathway [109]. Finally, natural polymer, synthetic, polymer and synthetic lipid-polymer based nanoparticles increased TJ permeability in a surface charge depended way, implicating electrostatic interactions with the TJ proteins [110].

The critical cut-off point for TJ mediated transfer has been estimated to be relatively small (a few nm) under normal circumstances, but certain nanoparticles such as some surfactants, polymers, chelating agents and mainly the small size lipid nanoparticles, may increase the gap dimensions [111].

There are health concerns associated with increasing the oral bioavailability of bioactive components that exhibit deleterious effects when consumed at too high levels. If one of these bioactive components normally has a very low bioavailability but its absorption by the human body is increased substantially by encapsulating it within lipid nanoparticles, then it could exhibit toxic effects that could not be predicted from data obtained on the same material in microscopic or macroscopic form. This is particularly true if the bioactive component is incorporated into a product that is consumed regularly in large volumes, such as a soft drink or beverage [111]. In summary, the small size, high surface area, and high surface energy of nano-sized lipid particles may lead to effects in the GI tract that are not predictable from our knowledge on the behavior of micro- or macro-sized lipids. Nanotechnology usage in the food sector has been hindered by concerns about the safety of the engineered nanoparticles, as well as ethical, policy, and regulatory issues [112]. Their safe use in food requires knowledge of their absorption, distribution, metabolism, excretion and toxicological profiles.

It is practical to study separately the different food additives mentioned above, but reality is much more complex since in nanotechnology many of the additives can be combined, thus potentially enhancing their effect on TJ permeability. Several examples of these combinations are: food-grade nanoemulsion using surfactants, salt nanowires, nanostructured lipid carriers, nanotechnology using organic solvents, sugars and bacterial enzymes. Many other nutrients or food additives, not classified in the 7 categories mentioned above, have been shown to increase intestinal permeability: L-alanine, tryptophan, the polysaccharide chitosan, epigallocatechin galat (polyphenol in green tea) and many more [113].

6. The hypothesis

The diet of the industrialized and urbanized parts of the world today is vastly different from what it was even two or three decades ago, with a whole new range of novel food experiences that come from new food component sources, new breeds of food plants and food animals, genetic modifications, chemical ingredients, flavors, preservatives and new nanotechnologies. Over recent decades, a significant increase in the incidence of autoimmune diseases in industrialized countries has led to the postulation that diet is a potential environmental risk factor for such disorders [63]. Although, causality has not been proven, increases in the usage of the abovementioned food additives have paralleled increased incidences and prevalences of AD during the last decades, as evidenced by Fig. 4A and B ($r^2 = 0.9829, 0.886$, respectively).

We put forward the hypothesis that modern food additives (such as sugars, salt, organic solvents, emulsifiers, gluten, microbial TG, and nanoparticles) increasingly used in the food and beverage industries are a major environmental factor for AD induction. All the food ingredients mentioned here abrogate human epithelial barrier function and increase intestinal permeability through the opened TJ, resulting in entry of foreign immunogenic antigens and activation of the autoimmune cascade.

7. Potential mechanism of food additive and TJ crosstalk and autoimmune induction

- Molecular mimicry between food ingredients and TJ self-antigen.
- Change of immunogenicity following industrial or luminal transformation of a nutrient, exposing neo-epitopes to the TJ.
- Food composition changes the luminal microbiota. Typical abnormal microbiota composition is allocated to specific AD and infections are a major driver of TJ increased permeability.
- Epigenetics can provide a plausible link between nutritional ingredients and autoimmunity [114]. Nutrients affect DNA methylation and histone modification [115]. Glucose, folic acid, vitamin B12, β hydroxibutyrat, threonine, homocysteine, gentistein, polyphenols and more nutritional factors have been shown to affect epigenetics. Recent studies have revealed that nutrients and their metabolites exert an important influence on the epigenome, as they serve as substrates and/or coenzymes for epigenetic-modifying enzymes [116]. The multiple genes involved in TJ regulation might be affected by nutrient-epigenetic cross-talk [57,58,114].
- The hapten hypothesis proposes that certain chemical products may react with self-components of the body to generate novel antigenic molecules. Food additives may combine to self TJ proteins.

The precise mechanisms responsible for the development of nutrient-induced autoimmune disorders are unknown. Although many hypotheses for the occurrence of autoimmune phenomena due to various environmental exposures have been proposed, none of these is completely supported by direct causal evidence. Additionally, mechanisms thought to be involved in the initiation of the disease process might differ from the mechanisms believed to exacerbate or maintain an established illness.

8. Limitations and biases

- Additives were studied separately. In reality food composition is complex with many inter-nutrient interactions.
- Most of the studies were performed in vitro, which is a far distance from the multicomplex situation in vivo.
- Immortalized, epithelial cell-lines of human origin cannot replace the human small bowel in permeability studies.
- Study of multiple food composition, in vivo, in the presence of microbiota is more accurate.
- Many bioavailability studies originate in the pharmaceutical domain and data is of little relevance to nutritional.
- The observations presented have stronger associative than cause and effect relationships.

9. Conclusions

The food and beverage industries are constantly changing and transforming our food composition through new food processing technologies. The result is neo-linked, transformed molecules and delivery systems, representing intestinal mucosal load with altered physicochemical and immunogenic properties.

Glucose, salt, emulsifiers, organic solvents, gluten, mTG, and nanoparticles are extensively and increasingly used by these industries to improve the qualities of the food (as claimed by manufacturers and some consumers). However, all these food additives increase intestinal permeability by bringing about TJ paracellular transfer. In fact, TJ

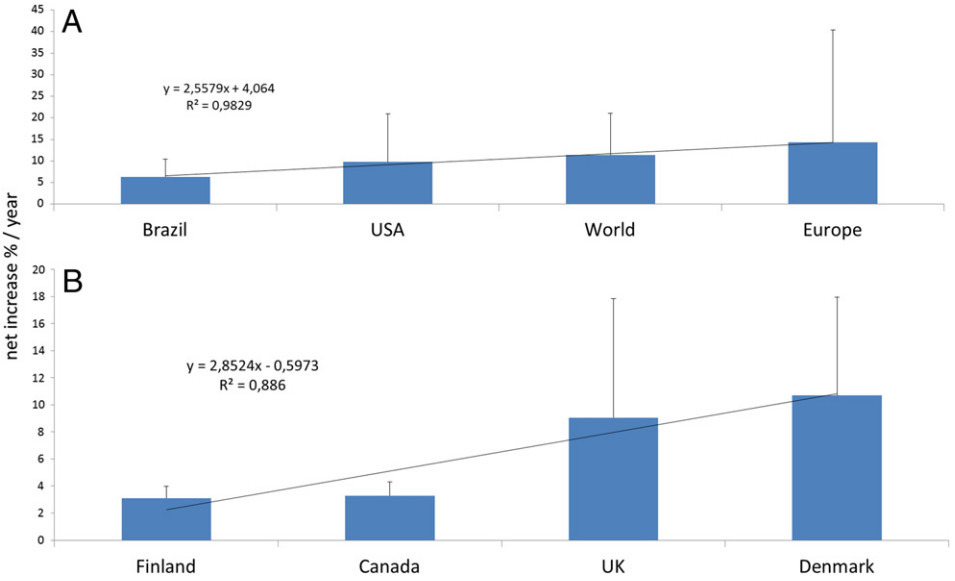

Fig. 4. The parallel net increase % per year in various countries of: A. Food additives usage and B. AD frequencies over the last decades. Adapted from: Ref. of Fig. 1+2.

dysfunction is common in multiple AD and the central part played by the TJ in AD pathogenesis has been extensively described. It is hypothesized that commonly used industrial food additives abrogate human epithelial barrier function, thus, increasing intestinal permeability through the opened TJ, resulting in entry of foreign immunogenic antigens and activation of the autoimmune cascade (Fig. 5). Future research on food additives exposure-intestinal permeability–autoimmunity interplay will enhance our knowledge of the common mechanisms associated with AD. As a corollary, individuals with non-modifiable risk factors (i.e. familial autoimmunity or carrying shared autoimmune genes) should consider decreased exposure to some food additives in order to avoid increasing their risk of AD. The U.S.A. Food and Drug Administration recently proposed a revision to the nutrition facts label that must appear on virtually all packaged foods [117,118]. Further studies on the effects of industrial food additives on intestinal permeability functions resulting in enhanced autoimmune, allergic and cancer diseases will impact on the food industry additive policy, food products labeling, consumer awareness, regulatory authorities and public health implementation.

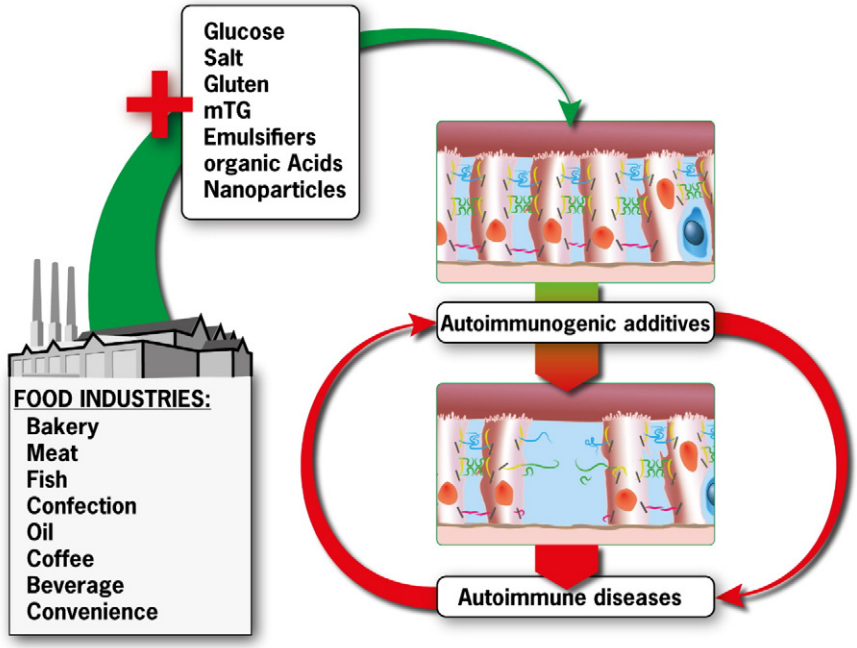

Fig. 5. A schematic representation of the sequential steps through which industrial food additives induce autoimmune diseases. Commonly used industrial food additives abrogate human epithelial barrier function, thus increasing intestinal permeability through the opened TJ, resulting in entry of foreign immunogenic antigens and activation of the autoimmune cascade.

Take-home messages

- Incidence of autoimmune diseases and food additive consumption is increasing.
- Commonly used industial food additives enhance intestinal junction leakage.
- Glucose, salt, emulsifier, gluten, microbial TG,nanoparticle increase TJ leakage.
- Intestinal entry of foreign antigen activates the autoimmune cascade.

Acknowledgments

The authors would like to acknowledge Ms. Patricia Jeremias for creating the figures and Dr. Peter Trinder for editing the manuscript.

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