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Etiology of Crohn's disease: Do certain food additives cause intestinal inflammation by molecular mimicry of mycobacterial lipids?

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Summary Crohn's disease is a chronic granulomatous inflammation of the gastrointestinal tract which was first described in the beginning of the 20th century. The histological similarity with intestinal tuberculosis has led to the assumption of an involvement of mycobacteria and mycobacterial antigens, respectively, in the etiology. A major defense mechanism against mycobacterial lipid antigens is the CD1 system which includes CD1 molecules for antigen presentation and natural killer T cells for recognition and subsequent production of cytokines like interferone-gamma and tumour necrosis factor-alpha. These cytokines promote granulomatous transformation. Various food additives, especially emulsifiants, thickeners, surface-finishing agents and contaminants like plasticizers share structural domains with mycobacterial lipids. It is therefore hypothesized, that these compounds are able to stimulate by molecular mimicry the CD1 system in the gastrointestinal mucosa and to trigger the pro-inflammatory cytokine cascade. The understanding of Crohn's disease as a CD1-mediated delayed-type hypersensitivity to certain food additives would lead to strong emphasis on a dietary treatment. Related aspects of pathology, physiology and epidemiology of Crohn's disease are presented.

Background

Crohn's disease is a granulomatous inflammation of yet unknown etiology

After the first description of Crohn's disease (CD) by Dalziel and Crohn et al. [1,2] as an apparently rare intestinal condition of young adults, there has been a steady rise in the number of cases recognized during the last century [3]. CD is now one of the major gastrointestinal problems in industrialized countries. Substantial research has been done to identify the cause and the main interest has been directed towards genetic predisposition, infectious agents, cell-mediated immunity, and dietary factors. However, the etiology of CD remains still unclear.

One of the characteristic histological features in CD are non-caseating granulomas containing epithelioid cells and giant cells [4]. Therefore and for the

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similarities to intestinal *Mycobacterium paratuberculosis* infection of ruminants (Johne's disease), mycobacteria were suspected to be involved in the etiology of CD [4]. However, the results of extensive search for infecting microorganisms are inconsistent and do not meet the Koch's postulates [5,6].

A hypothesis of interaction between dietary components and immune system is presented in the present article. Certain food additives like emulsifiants are esters of long-chained fatty acids and share domains with immunologically active glycolipids of the cell wall of microorganisms, especially mycobacteria. It is thus conceivable that one or more of those substances fit in special receptors of antigen-presenting cells by means of molecular mimicry and are able to trigger the specific cytokine release in the pre-immunisized gut-associated lymphoid tissue (GALT).

Immunological considerations

Apart from caseation, granulomatous inflammation in CD resemble closely that in intestinal tuberculosis [4]. However, the formation of granulomas does not require living microorganisms. Several investigators demonstrated in animals a specific granulomatous inflammation after administration of various glycolipid containing cell wall preparations from mycobacteria [7–9]. The defense mechanism against mycobacterial lipid antigens includes CD1 molecules expressed by antigen presenting cells [10]. Human CD1 molecules (CD1a–CD1d) are highly conserved and – analogous to major histocompatibility complex (MHC) molecules and peptide antigens – appear to be predicated on the presentation of lipids [10]. Lipids presented by CD1 molecules are mainly recognized by a CD4, CD8-double negative subset of T lymphocytes called natural killer T cells (NKT) with a T cell receptor repertoire of limited diversity [10–12]. These oligoclonal cells phenotypically resemble cytolytic T cells and are capable of producing large amounts of interferone-gamma (IFN-γ) [10,13,14]. IFN-γ plays a key role in macrophage activation and subsequent release of the whole cascade of proinflammatory and chemotactic cytokines belonging predominantly to type-1 (interleukin-1, interleukin-12, IFN-γ, tumour necrosis factor-alpha, and the regulatory counterpart interleukin-10) which promotes delayed-type hypersensitivity and granulomatous transformation [15,16]. This characteristic pattern of cytokines is seen in both mycobacterial infection and CD [14,17,18].

Various ligands for the CD1 system so far were identified (Fig. 1). Mycolic acids, glucosemonomycolate and phosphatidyl inositol mannosides, for instance, are presented by human CD1b [14,19,20] (Fig. 1A,B). These compounds are substructures of mycobacterial cell wall lipids, which are known to stimulate the secretion of type-1 cytokines [14,15,21]. CD1d is capable of presenting glycosylceramides, phosphatidyl ethanolamine, and phosphatidyl inositol compounds, which are found in cell membranes of both prokaryotic and eukaryotic organisms [10,22,23] (Fig. 1C).

Food additives as potential CD1-restricted antigens?

An antigen potentially presented and recognized by the CD1 system requires two long-chained hydrophobic residues for the binding in hydrophobic pockets of the ligand-binding groove of the CD1 molecule and a hydrophilic backbone to serve as target for the T cell receptors [10] (Fig. 1). Several emulsifiants and thickeners fulfil these structural requirements: diglycerides of long-chained fatty acids esterified with acetic, lactic, citric, or tartaric acid (E 472a–d) and fatty acid esters of propylene glycol (E 477), polyglycerol (E 476), or polyoxyethylene sorbitan (E 432–E 436) (Fig. 1D–F). They are frequently used in the industrialized production of sweets, cream desserts, sauces, spreads and margarine. Increased consumption of the latter already has been suspected to play a role in the etiopathogenesis of CD [24]. Furthermore waxes of animal or vegetable origin like beeswax, carnauba wax and candelilla wax (E 901–E 914) used as surface-finishing agents for sweets and citrus fruits contain long-chained fatty acid esters [25]. The antigenic potential of beeswax was described in a case report of contact dermatitis [26]. Other candidates are dialkyl phthalate esters (di[2-ethylhexyl] phthalate, DEHP; diisononyl phthalate, DINP (Fig. 1G)), which are used as plasticizers for polyvinyl chloride (PVC) since the 1930s [27,28]. They were found in concentrations up to 45% in children's toys and teething rings made of soft PVC [27] and migrate into the saliva during chewing and mouthing activities [27,29]. Ready-to-serve meals are heavily contaminated with plasticizers when PVC gloves were worn during cooking and packaging [30].

Physiological and pathological observations

Supportion of the idea of a possible involvement of artificial or natural long-chained esters in the

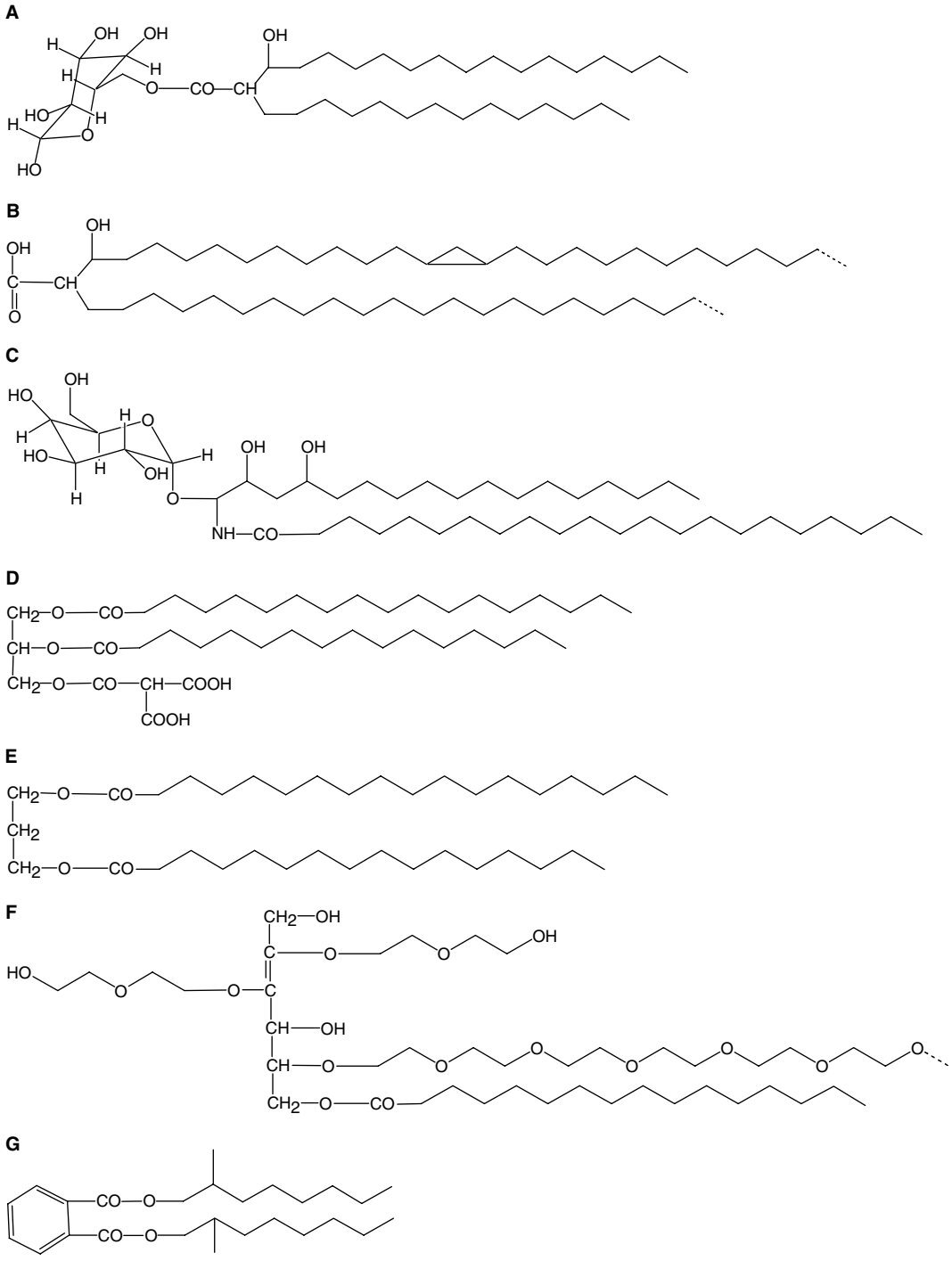

Figure 1 Molecular structures of natural ligands of human CD1b (A,B) and CD1d (C), of three emulsifiants (D—F) and of a widely used plasticizer (G). (A) glucose monomycolate, (B) mycolic acid, (C) α-galactosyl ceramide, (D) diglyceride esterified with citric acid (E472c), (E) propylene glycol ester of edible fatty acids (E477), (F) polyoxyethylene sorbitan monopalmitate (E434), (G) diisononyl phthalate (DINP). Dotted ends symbolize longer side-chains than shown.

etiology of CD comes from the physiology of the digestive tract. Fatty acid esters are partly cleaved in the duodenum and processed in the enterocytes of the small intestine [31]. The absorption of even natural occurring edible fatty acid esters enhances migration of T lymphocytes to Peyer's patches and stimulate cytokine release [32].

CD has a predilection for this part of the gut. The earliest microscopic lesion in CD consists of a focal accumulation of mononuclear cells and formation of epitheloid cells [17]. Both lymphatic cells and epithelials of the ileum express CD1 molecules [10,33,34]. Interestingly, ileal epithelial cells of CD patients were shown to contain lysosomes packed with lamellar layers of a material, myelin-like on light-microscopy and with the characteristics of lipids on electron-microscopy [4,35,36].

Epidemiological evidence

After a period of steady increase, the incidence of CD with around 6.0 per 100,000 seems to have reached a plateau in North and West Europe and North America with regional differences [3]. Much less cases have been reported from African and Asian populations, even when they have European ancestors [37]. The disease is more common in urban than in rural areas [3,37]. No cases of CD are reported from native tribes with traditional lifestyle [38]. The emergence of CD parallels the rapidly expanding food industry since the beginning of the 20th century. Wherever the disease is highly prevalent much of the foodstuff is altered from its natural state and manufactured with the aid of a variety of food additives. In previous epidemiological studies, the most consistent difference between CD patients and controls appeared to be in relation to consumption of pastries and sweets [39—41], which may contain up to 60% emulsifiants and stabilizers [42]. When controls were matched in age, sex, social class and marital status, the pre-illness dietary intake of sweets was up to three times higher in CD patients [39,43].

Hypothesis

The proposed pathomechanism of CD is as follows: environmental and/or pathogenic mycobacteria (which can pass the digestive system because of its acid-fast cell wall) repeatedly stimulate the CD1 system of the intestinal epithelials and the GALT. Once the gut is pre-immunized, food-additives and -contaminants, respectively, with structures related to that of mycobacterial lipid antigens mimic these antigens and booster the immunological reaction. A clone of killer T cells bearing T cell receptors with special ability to recognize the food additive is upregulated and eventually starts the type-1 cytokine cascade by molecular mimicry leading to granulomatous inflammation of the intestine.

Maybe only a defined mycobacterial species is able to elicit T killer cell clones with receptors cross-reactive with certain food additives. Also, the hypothesis does not disclose a genetic predisposition of the patients immune system. Current information regarding the CD1 system in the human intestinal mucosa suggests that there are two genotypically different forms of CD1d molecules as well as different subsets of NKT cells [33].

One can object that food additives like medicaments undergo extensive tests for their toxicological safety in animal experiments before approval by the Expert Commitees (FAO/WHO Expert Committee on Food Additives and EEC Scientific Committee for Food) [44]. However, the CD1 system of different mammalian species varies from that of humans [10] and the conditions of husbandry of laboratory animals (mainly rats and beagle dogs) may prevent pre-immunization with mycobacteria. Moreover, the duration of even long-term studies (up to 2 years) may not be sufficient to reveal possible immunogenic actions of the substance.

Testing the hypothesis and implications

There are few data about a possible role of the CD1 system in the pathogenesis of CD [18,33,45]. Quantitative assessment of CD1d demonstrated an over-expression in the epithelial cells of affected mucosa in CD patients [33]. First of all, this relationship should further be clarified. In vitro stimulation experiments should be conducted with lymphatic cells removed from affected gut tissue to reveal type-1 cytokine response to one or more food additives of lipid nature. The ability of CD1 molecules to present these compounds could be tested in binding assays as described [46,47].

The previously described presence of lipid-like lamellar bodies in enterocytes of CD patients [4,35,36] should be confirmed in larger trials and investigated with improved physical or biochemical methods. Furthermore, new animal models are to be developed where intestinal pre-immunization with attenuated mycobacteria or mycobacterial cell wall preparations should precede the feeding with food containing the suspected additive. Finally, CD patients could agree to keep special diets free from food additives and changes in the course of their disease should be documented over months or years.

The demonstration that CD is a CD1-mediated immunological cross reaction of the GALT against

mycobacteria and certain food additives with resulting delayed type hypersensitivity and granulomatous inflammation would lead to strong emphasis on a dietary treatment of CD and would spare the patients long-term immunosuppression and straining surgery.

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