

Expression of CD1d in the Duodenum of Patients with Cow's Milk Hypersensitivity

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CD1 cell surface glycoproteins represent a family of non-major histocompatibility complex (MHC) encoded antigen-presenting molecules. All members of the CD1 family appear to mediate the recognition of microbial or endogenous lipid and glycolipid antigens. The recognition of CD1d by a unique subset of natural killer (NK) T cells that leads to rapid production of large amounts of both type 1 and type 2 cytokines can be augmented by some synthetic glycolipids. Because of the proposed role of such CD1d-restricted T cells in immunoregulation, we hypothesized that CD1d molecules participate in mucosal immune responses in patients with gastrointestinal symptoms owing to food hypersensitivity. Patients of that category represent a heterogeneous group in which poorly defined immunological mechanisms are believed to contribute to disease pathogenesis. The expression of CD1 in duodenal biopsy samples from six patients with verified intolerance to cow's milk and six healthy controls was studied by immunoperoxidase staining of cryostat sections using a panel of mouse monoclonal antibodies (MoAbs) specific for CD1a, b, c, and d. Large numbers of CD1d positive cells were found in the lamina propria of all the patients, both during the symptomatic and the asymptomatic periods, whereas healthy controls were virtually devoid of CD1d expression in the duodenum. The localization of CD1d positive cells corresponded to areas where B cells, plasma cells and dendritic cells (DC) were present. A positive correlation was found between the numbers of CD1d⁺ and CD19⁺ cells in the lamina propria. In contrast to previous reports, no CD1d expression was found on the epithelial cells. Although less numerous than CD1d⁺, the CD1c⁺ cells were also present in all the patients and in five out of six controls. No staining for CD1a or CD1b was detected in the duodenal biopsy samples from any of the subjects. The exclusive presence of CD1d in the duodenal lamina propria of the patients with cow's milk hypersensitivity might suggest the participation of these molecules in the pathogenesis of allergic reactions to food.

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INTRODUCTION

CD1 cell surface glycoproteins have been identified as a family of non-MHC encoded antigen presenting molecules [1]. The human CD1 family is encoded by five closely related genes residing on chromosome 1 [2]. The CD1 molecules are

structurally homologous to MHC encoded proteins and like MHC class I, they are heterodimers of an approximately 45-kDa glycosylated heavy chain associated with β 2-microglobulin (β 2m). In contrast to MHC, they are nonpolymorphic [3, 4].

The CD1 molecules are divided into two groups based on sequence homology: CD1a, b and c belonging to group 1, and

CD1d representing group 2 [3]. The group 1 CD1 proteins are absent from mice and rats, whereas group 2 is present in rodents. Mouse CD1 (mCD1) is a close homologue to human CD1d [3, 4]. Tissue distribution of these two groups differ substantially, current data also suggest that they differ in function (reviewed in [1]).

Human CD1 group 1 molecules are widely expressed by professional antigen-presenting cells (APC), including DC, Langerhans cells, activated monocytes, and B cells, as well as by immature thymocytes (reviewed in [5]). CD1a and c have also been found on intestinal intraepithelial lymphocytes [6]. CD1a, b and c molecules are capable of presenting lipid and glycolipid bacterial antigens to T cells [7–11].

Several earlier studies have shown CD1d molecules on gastrointestinal epithelial cells both in humans and mice [12–14]. However, this could not be confirmed by later studies possibly owing to the use of different panels of monoclonal antibodies (MoAb) for the detection [15–17]. It was demonstrated that a large fraction of CD1d expressed by human intestinal epithelial cells lacks both glycosylation and the associated $\beta 2m$ [18], which could account for some of the discrepancies.

The functional role of CD1d in the regulation of intestinal immune responses was first suggested by experiments showing that a mixed lymphocyte reaction between intestinal epithelial cells and CD8⁺ T cells was inhibited by a MoAb against CD1 [19]. Further studies revealed that CD1d represents a restriction element for a subset of NK T cells expressing an invariant V $\alpha 24J\alpha Q$ T-cell receptor (TCR) α chain, paired predominantly with V $\beta 11$ in humans. These V $\alpha 24+$ cells have been shown to specifically recognize CD1d and simultaneously produce large amounts of both Th1 and Th 2 cytokines [20–22]. Recent studies showed that some synthetic glycolipids are recognized by both human and mouse NK T cells in a CD1d-restricted manner, providing further evidence for a role of CD1d in immunoregulation and host immune responses [23–25]. Recognition of these antigenic compounds by human NK T cells triggered proliferation, cytokine release and cytotoxic activity [25]. Furthermore, this T-cell recognition system was shown to be highly conserved through mammalian evolution [24] implicating its fundamental importance for the immunological homeostasis. Recently published data suggested the role of CD1d mediated responses in polarizing NK T cells towards Th2 cytokine production [26]. Such mechanisms may be operating in patients with food hypersensitivity, as food-allergic patients have been shown to have an imbalance of interleukin (IL)-4 and interferon (IFN)- γ production by peripheral blood mononuclear cells (PBMC) after mitogen stimulation [27].

In the present study we used immunohistochemistry to compare the presence of CD1d in duodenal biopsy samples from patients with gastrointestinal symptoms owing to food hypersensitivity with healthy controls. We found large amounts of CD1d positive cells in the lamina propria of patients with cow's milk hypersensitivity both at the time of gastrointestinal manifestations and during asymptomatic periods in contrast to

healthy individuals who did not show any CD1d expression in the duodenum.

MATERIALS AND METHODS

Patients. Six female patients with cow's milk intolerance manifested with gastrointestinal symptoms were studied. In three of them some nonspecific general symptoms, or asthma also occurred. Their ages ranged from 35 to 65 years with an average of 54 years. Clinical data are shown in Table 1. The relation between gastrointestinal symptoms to cow's milk was proven in all the patients with double-blind placebo-controlled food challenge (DBPCFC) [28]. No case of lactose intolerance was detected. None of the patients had signs of immunoglobulin (Ig)E-mediated hypersensitivity to cow's milk when tested with skin prick test (SPT) and radioallergen sorbent test (RAST). However, one of the patients had atopic allergy to some other food and inhalant allergens as diagnosed by SPT and RAST. Six age- and sex-matched healthy volunteers without either a history of food intolerance or any kind of allergy to inhalant allergens (checked with the Phadiatop test, Pharmacia Diagnostics, Uppsala, Sweden) were studied as controls.

Food challenge. The duodenal biopsy samples were obtained from the patients with cow's milk hypersensitivity both at the time when they developed gastrointestinal symptoms after the open food challenge and 2–3 months later when they were free of symptoms owing to an elimination diet. In the open food challenge the patients were encouraged to eat all the suspected foods during a period of 7 days. On day 8, when the gastrointestinal symptoms had developed, the biopsies were taken.

Biopsy material. Endoscopic biopsy specimens from the proximal part of the duodenum were taken with a Fujinon EG 200-SP instrument (Fujinon Inc., Wayne, NY, USA). The tissue specimens were immediately frozen in an OCT compound (Histolab. Products AB, Göteborg, Sweden) on dry ice and kept at -70°C until the immunoperoxidase staining. Informed consent was obtained from the participants, and the study protocol was approved by the Ethic's Committee of the Medical Faculty, University of Göteborg.

MoAbs. For immunostaining the following MoAbs were used: anti-CD1a, clone 10H3.9.3, mouse IgG2a (produced from a hybridoma provided by Dr Daniel Olive, Marseille, France); anti-CD1b, clone BCD1b3.1, mouse IgG1 [29]; anti-CD1c, clone F10/21A3, mouse IgG1 [30]; anti-CD1d, clones CD1d42.1 and CD1d68.3, mouse IgG1 [21]. These MoAbs were used at a concentration of 5 $\mu\text{g}/\text{ml}$. Other mouse MoAbs used in the study were: anti-HLA-DP, DQ, DR antigen (CR3/43, IgG1 κ); anti-HLA-ABC (W6/32, IgG2a κ); anti-CD3 (UCHT1, IgG1 κ); anti-CD4 (MT 310, IgG1 κ); anti-CD8 (DK25, IgG1 κ); anti-CD19 (HD37, IgG1 κ); anti-CD68 (KP1, IgG1 κ) (all from Dako, Glostrup, Denmark); anti-CD14 (M ϕ P9, IgG2b κ , Becton Dickinson, San Jose, CA, USA); anti-HLA-DM (MaP.DM1, IgG1 κ); anti-CD38 (HIT2, IgG1 κ) (both from Pharmingen, San Diego, CA, USA); and antihuman IgA heavy chain (Mc24–2E11, IgG1, Serotec, Oxford, UK).

Immunoperoxidase staining of the tissue. The 5 μm tissue sections were fixed in ice cold acetone (30 s in 50% acetone followed by 5 min in 100% acetone) and then air dried for 30 min. After this, the endogenous peroxidase activity was blocked by incubation of the slides for 20 min at room temperature in a solution of 1 U/l glucose oxidase (Sigma, St. Louis, MO, USA), 10 mM glucose and 1 mM NaN_3 . Before use, this solution had been preheated for 15 min at 37°C . For immunostaining the slides were incubated overnight in a humid chamber at 4°C with a portion of mouse MoAb directed against

Table 1. Clinical characteristics of patients with food intolerance

No	Age/ sex	Positive results of DBPCFC*	Symptoms after food challenge	Time of onset of symptoms after challenge	Duration of symptoms
1	59/F	cow's milk	unspecific tiredness, abdominal pain, diarrhoea, flatulence, swollen abdomen	1 h	12–24 h
2	35/F	cow's milk, soy proteins, hen's egg	stomach pain, diarrhoea, obstipation, colic pain	1 h	12–24 h
3	65/F	cow's milk	swollen abdomen, skin itching, nausea, diarrhoea	1 h	12–24 h
4	65/F	cow's milk	swollen abdomen, nausea, diarrhoea, asthma	5 min	12–24 h
5	61/F	cow's milk	swollen abdomen, diarrhoea, mouth symptoms: small bubbles, pain in tongue and palate	5 min	12–24 h
6	41/F	cow's milk	vomiting, nausea, fever, flatulence, tiredness	24 h	24–48 h

* Double-blind placebo-controlled challenge.

CD1a, b, c, and d and other cell surface molecules diluted in phosphate-buffered saline (PBS)-Tween (0.05% Tween) containing 2% bovine serum albumin (BSA). Sections used as a negative control were incubated with isotype-matched irrelevant MoAb (mouse IgG1, IgG2a, Becton Dickinson or IgG2b κ , Dako) at a concentration of 5 μ g/ml. After washings with PBS (3 $\times$ 5 min), the sections were incubated for 1 h with a biotinylated F(ab')₂ fragment of rabbit antimouse immunoglobulins (Dako) diluted 1 : 400 in PBS-Tween + 2% BSA + 2% normal human serum. Thereafter, the slides were incubated with avidin-conjugated peroxidase (ABC-complex, Dako), for 30 min. The peroxidase staining was revealed with amino-ethyl-carbazole (Sigma), followed by a light counter staining with Mayer's hematoxylin, and mounting in Aquamount (BDH Laboratory, Poole, UK). Positively stained cells were counted using a computer supported image analysis system Leica Q500MC (Leica, Cambridge, UK). All the positive cells in the whole area of the mucosa were counted including the villi and the lamina propria regions. The positive cells were recorded as the number of stained cells/mm² tissue.

Statistics. The two-tailed Mann–Whitney *U*-test was used for statistical evaluation.

RESULTS

Increased expression of CD1d in the duodenal lamina propria of patients with cow's milk intolerance

Immunohistochemistry analysis revealed that all the duodenal biopsy sections of patients with cow's milk intolerance showed

large numbers of CD1d-positive cells in the lamina propria and in the basal parts of the villi (Fig. 1C,D, Table 2).

A high expression of CD1d was detected both after the food challenge when the patients had gastrointestinal symptoms and during the asymptomatic period after an elimination diet. The pattern of the expression and the number of CD1d-positive cells did not significantly differ ($P > 0.05$) between the symptomatic and asymptomatic periods (Table 2).

The histological examination of the biopsies revealed no signs of gross morphological changes like villous atrophy, crypt hyperplasia or oedema. However, signs of an inflammatory response were evident with moderate lymphocyte infiltration and eosinophilia in the lamina propria.

CD1d expression was not found in the healthy controls except for single weakly stained CD1d+ cells in the lamina propria in occasional sections (Fig. 1A,B, Table 2).

Similar results were obtained with both the CD1d42.1 and the CD1d68.3 (not shown) MoAbs used for immunostaining.

In contrast to previously reported data, no CD1d expression was detected on epithelial cells either in the patients or in the healthy controls, using either of the current MoAbs. An occasional weak staining of the epithelial cells was observed in some sections, however, a similar weak diffuse staining in these sections was also noticed using the isotype control (mouse IgG1) (not shown) suggesting that it was owing to nonspecific binding of MoAb to the epithelial cells. CD1d MoAb also had the ability to nonspecifically vividly bind to the mucus which

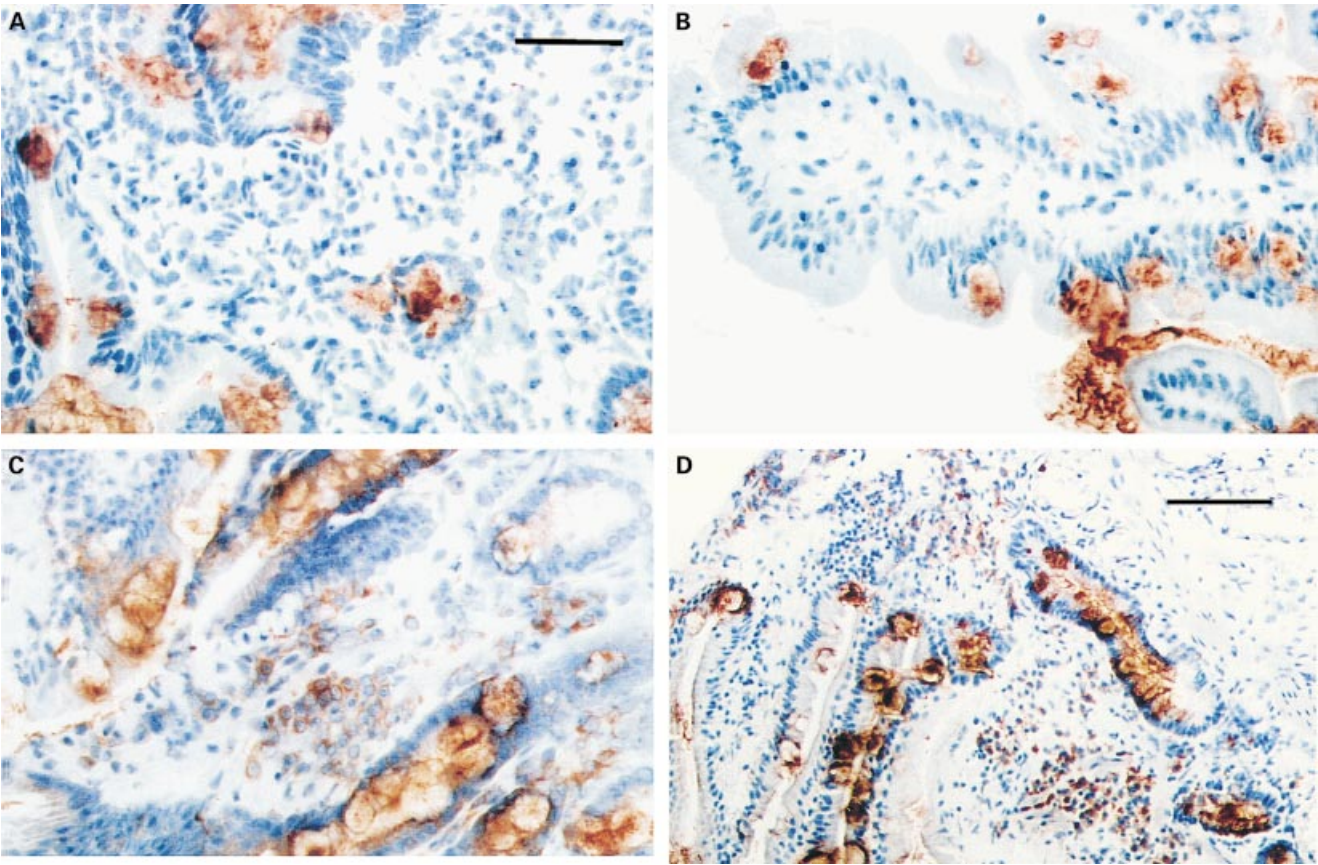


Fig. 1. Immunohistochemistry of duodenal biopsy sections using the monoclonal antibodies (MoAb) CD1d42.1 and the avidin-biotin complex peroxidase (ABC) technique. (A) section of a healthy control (lamina propria region); (B) section of a healthy control (villi region); (C) section of a patient with cow's milk hypersensitivity. The biopsy was taken during the asymptomatic period; bar = 50 μ m; original magnification 200 $\times$; (D) section of a patient with cow's milk hypersensitivity (symptomatic period); bar = 100 μ m; original magnification 100 $\times$. Cells stained brown in the lamina propria represent CD1d+ cells. Note strong nonspecific staining of mucus (goblet cells) in the epithelial layer in both the patient and the control samples. In contrast the CD1d+ cells in Fig. 1(C,D) are seen in the lamina propria and the basal portion of the villi.

Table 2. Number of CD1d, CD1c, and CD19 positive cells in the duodenal lamina propria in patients with cow's milk hypersensitivity and healthy controls (Median and range, cells/mm² tissue)

Positive cells	Patients, symptomatic period	Patients, asymptomatic period	Healthy controls
CD1d	183 57 – 279	158 84 – 266	single weakly stained cells
CD1c	10 4 – 77	10 0 – 112	15 0 – 120
CD19	57* 11 – 151	18 4 – 143	3 0 – 30

* $P < 0.05$ as compared to healthy controls.

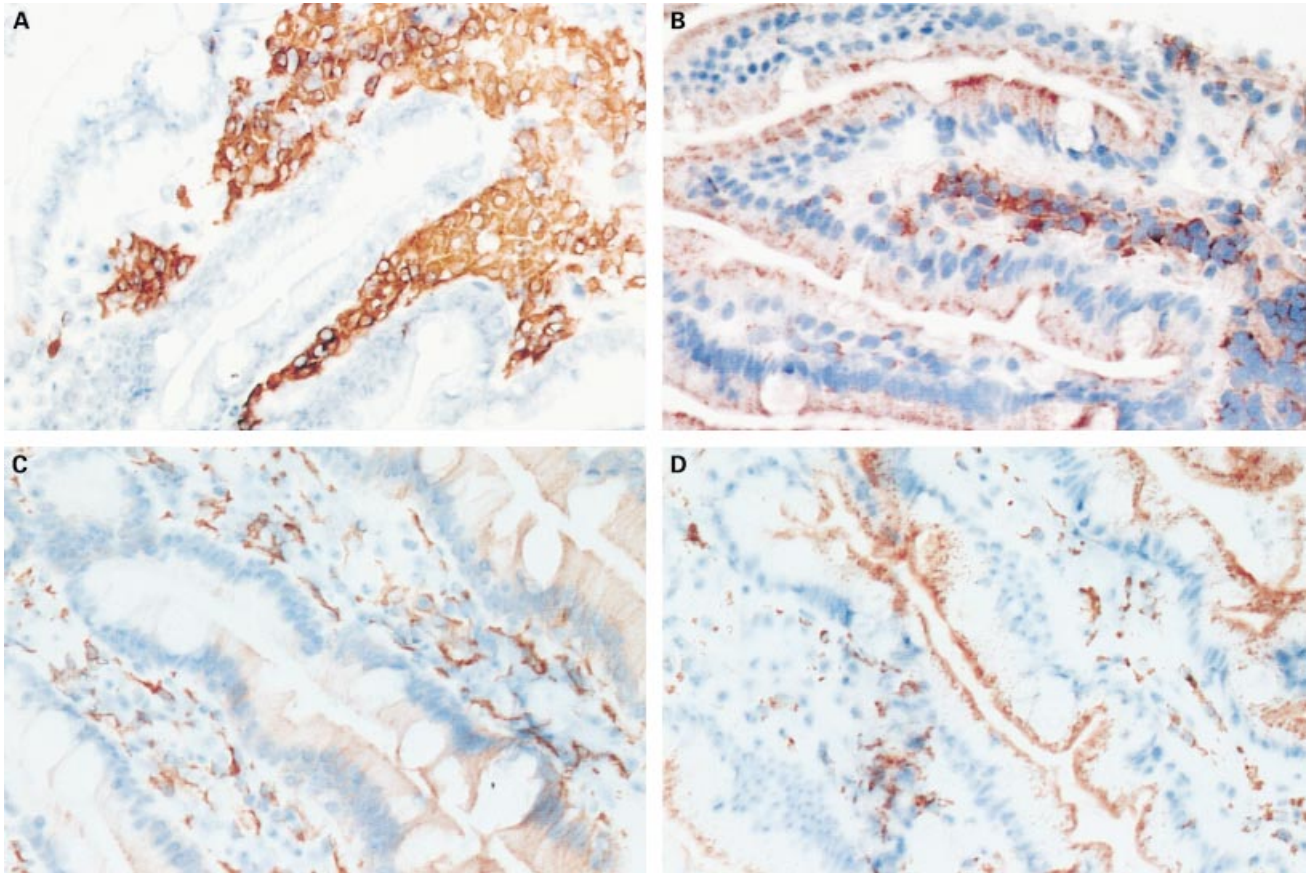


Fig. 2. Immunohistochemistry of sections from the duodenum of one patient with cow's milk hypersensitivity. The biopsy was taken during the symptomatic period. The immunostaining used the following MoAbs: (A) CD38, HIT2; (B) CD68, KP1(C) HLA-DP, DQ, DR, CR3/43; (D) HLA-DM, MaP.DM1; original magnification 200 $\times$.

led to intensely stained aggregates of mucus in the goblet cells in both the patients and the healthy controls (Fig. 1A-D).

Morphology and localization of CD1d positive cells in the duodenal mucosa

CD1d-positive cells were detected in the lamina propria and in the basal cores, but not in the apical parts of the villi (Fig. 1C,D). In four patients CD1d+ cells were found both in the villous and lamina propria areas (Fig. 1C). In the remaining two patients the CD1d+ cells were detected mainly in the basal lamina propria, with only few weakly stained cells scattered in the villi (Fig. 1D).

Some of the positive cells were large and had irregular shape with intensively stained cytoplasmic processes, similar to DCs. The rest of the cells morphologically appeared as lymphocytes (small, with nuclei densely stained by hematoxylin and a thin halo of cytoplasm) or plasma cells (large cells with large nuclei and extensive cytoplasm).

In an attempt to identify the type of cells expressing CD1d in the lamina propria, we sectioned biopsies serially and stained alternate sections with different MoAb recognizing cell surface

markers of T lymphocytes (CD3, CD4, CD8), B lymphocytes (CD19), monocytes/macrophages (CD4, CD14, CD68), DC (CD4, CD68) and plasma cells (CD38, IgA). Because the lamina propria shows a highly organized architecture of the lymphoid cells it was possible to establish in which compartments the CD1d+ cells colocalized. Thus the CD3+ and CD4+ cells, but not CD8+ cells partly colocalized with CD1d+ ones (not shown). The CD8+ cells were residing in the intraepithelial compartment where CD1d+ cells were never detected. The CD38+ (Fig. 2A) and IgA+ (not shown) cells were abundantly present in the same areas, as CD1d+ cells, but their numbers far exceeded those of CD1d+. The CD68+ cells often had an irregular shape with cytoplasmic processes and these cells were present in the lamina propria too, however, they were seen throughout the whole villi including the apical areas (Fig. 2B), where CD1d+ cells did not appear. CD14+ cells were occasionally found in the lamina propria in the samples taken during the symptomatic period, but were not detected in the samples obtained in the asymptomatic period (not shown). CD19+ cells appeared to be most closely located to the CD1d+ cells. However, the numbers of CD1d+ cells exceeded those of CD19+ cells (Fig. 3A,B, Table 2). Nevertheless, there was a

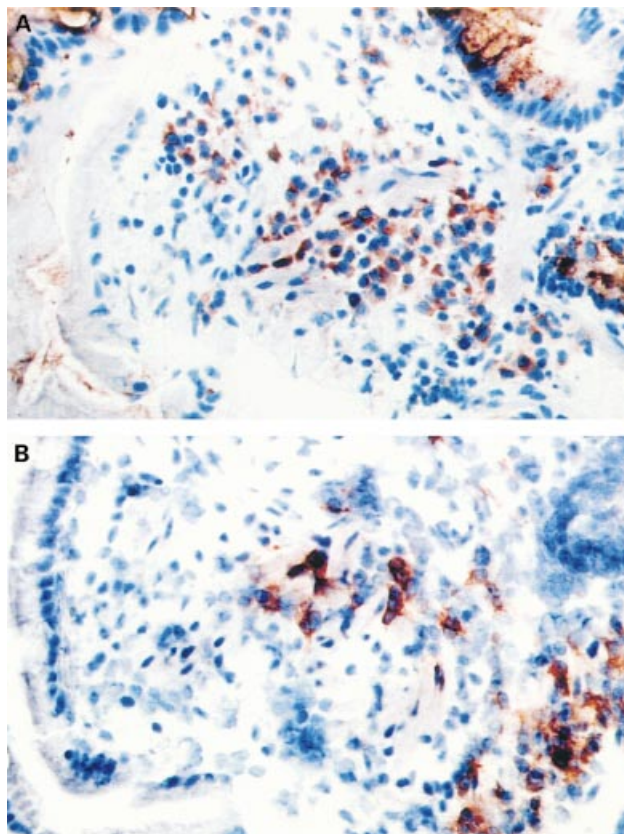


Fig. 3. Immunohistochemistry of serial sections from the duodenum of the same patient as in Fig. 1D and Fig. 2. The biopsy was taken during the symptomatic period. The immunostaining used the following MoAbs: (A) CD1d42.1; (B) CD19, HD37; original magnification 200 $\times$.

positive correlation between the numbers of CD1d+ and CD19+ cells ($r = +0.68$, $P = 0.014$).

Because some of the CD1d+ cells appeared as large irregular ones with cytoplasmic processes we stained the duodenal sections with MoAb specific for MHC class II and HLA-DM to see if DC occurred in the same area. It was, indeed, the case: cells showing dendritic-like morphology, intensively expressing MHC class II molecules (HLA-DR, -DQ, -DP) and HLA-DM were present in the same areas in the lamina propria and the basal parts of the villi as CD1d+ (Fig. 2C,D). There was, however, no difference in the expression of MHC II and HLA-DM between the patients and the controls. In contrast to CD1d, strong staining with HLA-DM and less intensive, but clear staining with HLA-DR, -DQ, -DP was detected on the apical parts of the epithelial cells.

Presence of CD1c+ , but not CD1a+ or CD1b+ cells in the duodenal lamina propria

Although less numerous as compared to CD1d-positive cells, CD1c-positive cells were found in the lamina propria and in the

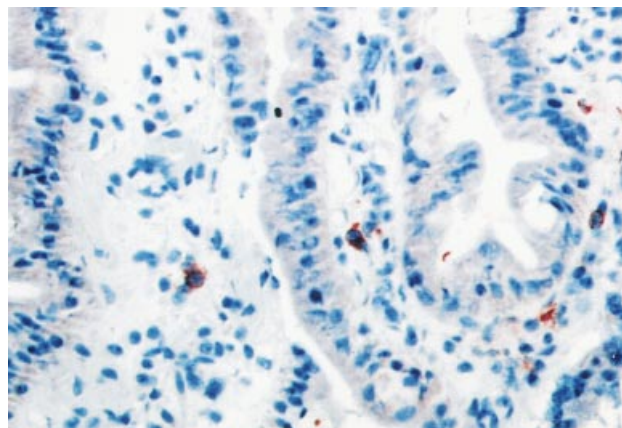


Fig. 4. Immunohistochemistry of a section from the duodenum of one patient with cow's milk hypersensitivity using the MoAb CD1c, F10/21A3. The biopsy was taken during the symptomatic period; original magnification 200 $\times$.

cores of the villi in all the cow's milk intolerant patients and in five out of six of the healthy controls. These cells were scattered in the mucosa and were not found in all the villi in the sections, but if present they occurred in the same areas and with similar appearance (rather large irregularly shaped cells) as CD1d+ cells (Fig. 4). Occasional intraepithelial lymphocytes stained with CD1c were detected in one patient (not shown). The numbers of CD1c-positive cells did not significantly differ between the patients and the healthy controls (Table 2). CD1a and CD1b expression was not found in any of the intestinal biopsy samples (not shown).

DISCUSSION

The role of immune mechanisms in the pathogenesis of adverse reactions to food in the gastrointestinal tract is still poorly understood. In particular, in many adult patients with staple food induced gastrointestinal symptoms no signs of IgE mediated responses to the relevant food have been found [28]. Indeed, in our patients with firmly proven diagnosis of gastrointestinal hypersensitivity caused by cow's milk (positive DBPCFC in all the cases) specific IgE to milk proteins were not found either by skin testing or in serum with RAST. However, in some of these patients a local increase of IgE on intestinal mast cells along with eosinophilia and T-cell infiltration was detected (31, X.P. Lin, unpublished data).

In the present study we found a high expression of CD1d in the duodenal lamina propria in patients with cow's milk hypersensitivity during both symptomatic and asymptomatic periods, while there were virtually no CD1d positive cells in healthy individuals.

The intestinal mucosa is a highly organized tissue compartment where specific cell populations are residing in special areas. While in a severely inflamed tissue this organization can be disrupted, our patients only showed signs of a mild inflammation in the mucosa that did not destroy the architecture

of the tissue. Therefore, in an attempt to identify the type of cells expressing CD1d in the lamina propria of the patients, we sectioned biopsies serially and stained alternate sections with MoAb recognizing different cell surface molecules.

According to the data from immunoperoxidase staining of these sections we suggest that CD1d in the duodenal lamina propria might be expressed by B lymphocytes (CD19+), plasma cells (CD38+ , IgA+), CD4⁺ T cells (CD3⁺ , CD38+) and DC-like cells (CD4⁺ , CD68, MHC class II+).

Although the CD38 antigen is known to be a marker of plasma cells, CD38+ cells in the lamina propria were much more numerous than the IgA+ and CD1d+ cells. This is not surprising given that CD38 is expressed by activated T cells and B blasts as well [32, 33].

CD1d+ cells were located in the same areas as CD3⁺ and CD4⁺ cells and therefore it is possible that T cells (but not CD8⁺) and macrophages can express CD1d as well. However, the common marker for monocytes/macrophages, CD14 was never detected in sections of asymptomatic patients and only occasionally found in the sections taken during the symptomatic period. This is in line with the finding that, in contrast to circulating monocytes, the CD14 molecule is only weakly expressed by macrophages residing in the noninflamed gut [34,35]. In contrast, we found strong staining with antibody recognizing CD68 antigen in the duodenal lamina propria and in the apical part of the epithelial cells both in the patients and the healthy controls. This molecule which belongs to the family of lysosomal associated glycoproteins is expressed by APCs like macrophages and DC [35–37]. Both populations of circulating monocytes and macrophages residing in the gut can express CD4 molecules [38, 39].

However, the cells in the lamina propria, stained with MoAb recognizing some macrophage markers, like CD4 and CD68, should be better defined as DC as a result of their typical dendritic morphology and constitutive high expression of HLA-DR, -DQ, -DP and -DM molecules.

Indeed, some of the CD1d+ cells appearing as DC with long cytoplasmic projections, occurred in the same area as CD4 and HLA-DR, -DQ, -DP and -DM positive cells. It should be noted that the CD4 antigen was previously detected on both tissue and blood DC [40,41] and that the prominent presence of HLA molecules is one of the most important characteristics of DC of any origin. Recent studies discovered two functionally different subsets of DC in the peripheral lymphoid organs: monocyte-derived and plasmacytoid DC which express different surface molecules [42, 43]. However, the tissue distribution of these subsets has not been studied yet. Studies of intestinal DC are very limited, particularly in humans. The phenotype of human gut DC was studied only in the colon [35], but to our knowledge, no publications describing the characteristics of the small intestinal lamina propria DC are available at present.

The increased expression of CD1d in the duodenum of the patients with cow's milk hypersensitivity may be owing to either an increased migration of CD1d+ cells from peripheral lymphoid organs and/or the occurrence of local factors inducing

the expression of CD1d on residing mononuclear cells (MNCs). The first possibility is supported by data showing the expression of CD1d in the circulation by the same cell types as in the lamina propria: by a subset of circulating T and B lymphocytes, and by resting monocytes [1, 44]. Although the expression of CD1d on peripheral blood MNCs in the patients with cow's milk hypersensitivity is similar to those in healthy individuals (M. Ulanova & S.A. Porcelli, unpublished data) it does not exclude this possibility. One can speculate that a subset of blood and/or other peripheral lymphoid organ CD1d+ cells could migrate to the intestine or alternatively upregulate CD1d and then migrate. Local inflammatory responses induced by food challenge can cause an increased influx of lymphocytes and monocytes to sites of inflammation that would then contribute to the occurrence of large numbers of CD1d+ cells among the lamina propria MNC. Our findings of the increased numbers of B cells in the lamina propria of the patients, as compared to the healthy controls, speak in favour of this suggestion. Nevertheless, we did not notice any significant differences between the numbers of CD1d+ and CD19+ cells in the intestinal biopsy samples taken at the time of gastrointestinal symptoms and during the asymptomatic period. However, lack of clinical manifestations 2–3 months after the food challenge does not exclude the possibility of ongoing local inflammatory responses in the gut.

The regulation of the expression of CD1d still remains unknown. None of the cytokines known to upregulate the expression of group 1 CD1 molecules were found to be involved in the regulation of the CD1d expression [1, 44].

One can speculate that some local factors causing the upregulation of the CD1d in the gut can be induced during inflammatory conditions.

In contrast to previously published data [13, 14] we could not detect any expression of CD1d on intestinal epithelial cells either in the patients, or in healthy persons. The discrepancies between these observations can be explained by the use of different MoAbs, as well as by the possible expression of different forms of CD1d (mature, fully glycosylated form, associated with β 2m, versus immature form lacking glycosylation and β 2m) [45] by different types of human intestinal cells.

The increased expression of CD1d in the duodenum of patients with cow's milk hypersensitivity appears to be selective for this member of the CD1 family. Group 1 CD1 molecules were either not present in the duodenal biopsy samples at all (CD1a and b), or were expressed in lower amounts both in the patients and healthy individuals (CD1c). In agreement with previously published data [6] CD1c expression by intraepithelial lymphocytes was occasionally noticed, while CD1d was never detected in this compartment. These findings can reflect differences in the regulation of the expression of group 1 and 2 CD1 molecules.

The presence of CD1d in the duodenal lamina propria of patients with cow's milk hypersensitivity suggests the possible participation of these molecules in the pathogenesis of adverse reactions to food. It is tempting to propose that the elevated

CD1d expression in the lamina propria can predispose certain individuals toward inflammatory responses in the intestine, e.g. owing to the increased presentation of bacterial products mediated by CD1d. This question has to be further addressed with respect to other inflammatory conditions in the gut such as coeliac and Crohn's disease. On the other hand, the presented data have been obtained in a group of rather mature patients (54 years in average) and we still do not know if a similar pattern of CD1d expression exists in paediatric patients where the cow's milk hypersensitivity is a more common clinical condition. However, such a study is difficult to perform, because of the ethical problems involved in obtaining control biopsy material and performing DBPCFC in children, considering the potential risks of this intervention. Nevertheless, the expression of CD1d in patients of different age groups deserves further studies as do factors inducing upregulation of the local expression of CD1d in the gut and the potential value of CD1d as a diagnostic tool in patients with food hypersensitivity.

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