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Antibody-dependent cell-mediated cytotoxicity to β-lactoglobulin-coated cells with sera from children with intolerance of cow's milk protein

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SUMMARY

The capacity of serum antibodies against β-lactoglobulin to mediate antibody-dependent cell-mediated cytotoxicity (ADCC) was analysed in sera from children with cow's milk protein intolerance (CMPI). The children with CMPI were divided into three groups according to clinical features: delayed-onset CMPI with gastrointestinal symptoms ($n=8$); immediate-onset CMPI with gastrointestinal and skin symptoms ($n=8$); and immediate-onset CMPI with skin symptoms only ($n=8$). The CMPI groups were compared with children with untreated ($n=9$) or treated ($n=8$) coeliac disease and a control group ($n=22$). Sera from the children were examined for cytotoxic effects using lymphocytes from healthy adults as effector cells and radiolabelled β-lactoglobulin-coated erythrocytes from the same donor as target cells. In addition, IgG and IgA serum antibodies against β-lactoglobulin were determined with ELISA. Sera from children with CMPI and gastrointestinal symptomatology showed a significantly increased capacity to induce ADCC reactivity as compared with controls. This increased capacity was seen in sera from those with immediate as well as delayed onset of the gastrointestinal symptoms. In contrast, sera from children who had an immediate-onset CMPI with only skin symptoms mediated no such increase in ADCC reactivity. Moreover, children with coeliac disease with a few exceptions, demonstrated low ADCC reactivity, despite the fact that they had high levels of antibodies against β-lactoglobulin. ADCC may be an immunopathogenic mechanism in certain cases of CMPI with gastrointestinal symptoms.

Keywords cow's milk protein intolerance antibody-dependent cell-mediated cytotoxicity coeliac disease beta-lactoglobulin antibodies children

INTRODUCTION

Cow's milk protein intolerance (CMPI) may be defined as adverse reactions to cow's milk proteins connected with immunological reactions against milk proteins (Walker-Smith, 1988). Intolerance to cow's milk proteins includes a variety of symptoms involving the skin, the respiratory or the gastrointestinal tracts. In addition, the time of onset of symptoms after the ingestion of milk varies. Most likely more than one pathogenic immune mechanism is involved in CMPI and the predominant mechanism may vary with the type of clinical manifestation. IgE-mediated reactions are well-established mechanisms operating in CMPI but can not explain all clinical manifestations. When gastrointestinal symptoms predominate in CMPI, they are often connected with mucosal changes similar to those in coeliac disease, although less extensive (Kuitunen *et al.*, 1975; Hill *et al.*, 1979; McCalla *et al.*, 1980). Animal experiments have shown that IgE-mediated reactions alone are not likely to be responsible for these changes in the structure of the small intestinal mucosa (Ferguson, 1980). Further, positive skin-prick tests and elevated serum IgE antibodies to cow's milk proteins are seldom found in CMPI with symptoms primarily of a gastrointestinal nature (Danneus & Johansson, 1979; Firer, Hoskings & Hill, 1987).

Antibody-dependent cell-mediated cytotoxicity (ADCC) is an immune mechanism where antibodies directed against antigens on the cell membrane of the target cell interact with effector cells via their Fc portions. ADCC may participate in immunopathological phenomena as well as in host defence in the gastrointestinal tract. Previous studies have indicated that ADCC could be a pathogenic mechanism in inflammatory bowel disease (Shorter, McGill & Bahn, 1984; Das, Kadano & Fleischner, 1984). The aim of this study was to evaluate the possible role of an ADCC reaction in the pathogenesis of CMPI, focussing on the ADCC-mediating capacity of serum antibodies against β-lactoglobulin. Sera from children with CMPI were examined for cytotoxic potency and compared with sera from children with coeliac disease and a control group.

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Table 1. Details of presenting symptoms, dietary history and laboratory tests in 24 children with CMPI grouped according to predominant reaction

No.	Sex	Age at introduction of cow's milk (months)	Age at onset of symptoms (months)	Onset of reaction in relation to ingestion of cow's milk	Presenting symptoms	Skin-prick test to milk	RAST to milk	Age at examination (months)	ADCC (%)
1	F	1	2	delayed	D, V, C, FTT	—	Negative	3	56·90
2	F	1	2·5	delayed	D, V, C, FTT	Positive	Negative	4	7·37
3	F	1	4·5	delayed	D, V, FTT	Negative	Negative	33	7·03
4	F	1·5	2·5	delayed	C, FTT	—	Negative	3	12·97
5	F	2	5	delayed	D, V, FTT	—	Negative	6	11·80
6	F	3	10	delayed	D, FTT	—	Negative	12	3·91
7	M	0·5	3	delayed	D, V, FTT	Negative	Negative	6	5·86
8	M	3	4	delayed	D, FTT	Negative	Negative	11	8·00
9	F	0·5	2	immediate	D, C, U,	Negative	Negative	6	0·75
10	F	2	2	immediate	V, U	Positive	Positive	14	16·88
11	F	5	5	immediate	D, V, C, U	Negative	Negative	10	21·25
12	F	5·5	5·5	immediate	V, U	Negative	Positive	6	17·89
13	M	3	3	immediate	D, V, C, U	—	Negative	12	19·26
14	M	4	4·5	immediate	D, V, R	—	Positive	5	24·25
15	M	5·5	5·5	immediate	V, R	Positive	Negative	7	13·67
16	M	6	6	immediate	D, V, C, U	—	Positive	6	22·21
17	F	1	2	immediate	U	Negative	Negative	10	3·69
18	F	6	6	immediate	U	Negative	Negative	6	1·29
19	F	6	8	immediate	U	Positive	Negative	8	2·43
20	F	6	9	immediate	U	Positive	Positive	9	0
21	M	1	1·5	immediate	R	Positive	Negative	7	0·54
22	M	2·5	3	immediate	U	Negative	Positive	4	5·05
23	M	5	5	immediate	U	Positive	Positive	9	3·55
24	M	5·5	5·5	immediate	U	Positive	Negative	8	0·16

Presenting symptoms: V, vomiting; D, diarrhoea; C, colic; U, urticaria; R, rash; FTT, failure to thrive.

SUBJECTS AND METHODS

Patients

Twenty-four sera of CMPI patients were examined for ADCC-mediating efficacy. For comparison, groups of children with coeliac disease and a control group were included in the study. The diagnosis of CMPI was based on the clinical history, clinical improvement with a cow's-milk-free diet and at least one positive provocation test. The diagnosis of coeliac disease was based on the criteria of European Society for Paediatric Gastroenterology and Nutrition (ESPGAN) (Meeuwisse, 1970).

The CMPI children, illustrated as to clinical features in Table 1, were divided in three groups according to the type of symptoms and the time of their appearance after the ingestion of milk. Immediate reactions occurred within 2 h and delayed reactions after 2 h and onwards, usually a few days after the first exposure to milk proteins.

The children included in the study were grouped as follows:

Delayed-onset CMPI with gastrointestinal symptoms. Eight children (six girls and two boys) aged 3–33 months (median age 6·0 months) showed gastrointestinal symptoms and failure to thrive. Six of the eight patients were examined with peroral jejunal biopsy showing subtotal or total villous atrophy.

Immediate-onset CMPI with gastrointestinal and skin symptoms. Eight children (four girls and four boys) aged 5–14 months (median 6·5 months) had a rapid onset of gastrointestinal symptoms such as diarrhoea and vomiting, sometimes associated with abdominal pain. All had cutaneous symptoms, urticaria or rash.

Immediate-onset CMPI with skin symptoms only. Eight children (four girls and four boys) aged 4–10 months (median age 6·5 months) had urticarial skin eruptions appearing rapidly after cow's milk exposure, but there were no gastrointestinal symptoms.

Untreated coeliac disease. Nine children (seven girls and two boys) aged 8–24 months (median age 12 months) were on a gluten-containing diet and jejunal biopsy showed villous atrophy.

Treated coeliac disease. Eight children (six girls and two boys) aged 15–78 months (median age 27·5 months) who had been on a gluten-free diet for 3–40 months (median 15 months) and had undergone a jejunal biopsy demonstrating a normal or just slightly altered villous structure.

Controls. Twenty-two children (13 girls and nine boys) aged 1–48 months (median age 19·5 months) were on a cow's-milk-containing diet. Ten children had been examined because of various conditions unrelated to CMPI, mainly minor viral infections. The remaining 16 children had been followed due to asymptomatic bacteriuria, but at the time for serum sampling they were abacteriuric (Wettergren, Jodal & Jonasson, 1985).

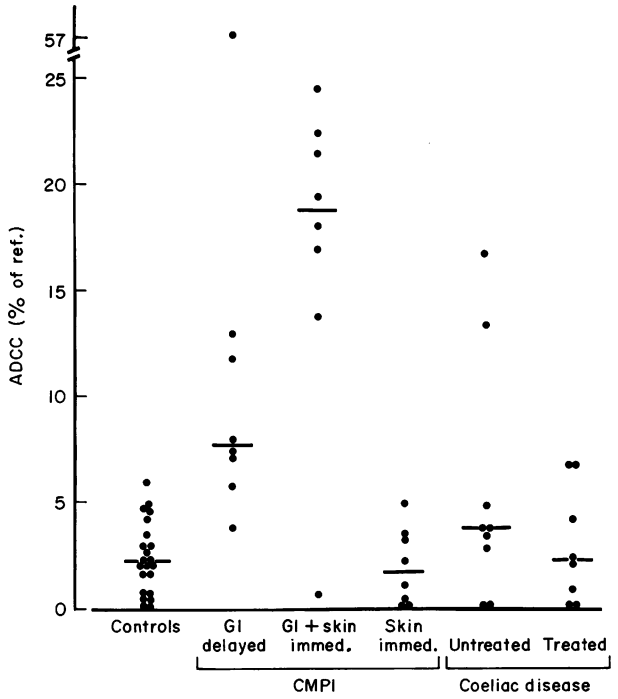

Fig. 1. ADCC reactivity of the sera in the diagnostic groups: controls, delayed-onset CMPI with gastrointestinal (GI) symptoms, immediate-onset CMPI with GI and skin symptoms, immediate-onset CMPI with skin symptoms only, untreated coeliac disease and treated coeliac disease. Medians are indicated.

The ADCC assay

Target cells. Papainized human erythrocytes from adult healthy donors were used as target cells in the ADCC assay (Urbaniak, 1976; Yust, Frisch & Goldsher, 1980). The papain treatment improved the specific cytotoxic lysis of the target cells. Heparinized blood was drawn and the cells were washed three times in normal saline (0·85% NaCl). One volume of erythrocytes was mixed with nine volumes of 1% papain solution (30·000 USP U/mg; Merck, Darmstadt, Germany) and incubated for 30 min at 37°C, followed by washings. β-lactoglobulin A and B (0·1 ml) (Sigma Chemical, St Louis, MO) in normal saline (0·5 mg/ml) was mixed with 0·1 ml of packed papain erythrocytes and thereafter 0·1 ml of chromium chloride solution ($CrCl_3 \times 6\ H_2O$) at 0·01% w/v in saline was added dropwise (Goding, 1976). The mixture was incubated at 30°C for 45 min and the erythrocytes were washed twice in saline and once in phosphate-buffered saline (PBS) pH 7·2. The erythrocytes were resuspended in 4 ml of tissue culture medium (RPMI 1640, supplemented with 5% fetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 20 mM HEPES buffer; Flow Labs, Ayrshire, UK). One-tenth of a millilitre of the suspension was mixed with 0·05 ml of $Na_2{}^{51}CrO_4$ 10 mCi/ml (Amersham International, Amersham, UK) and incubated for 2 h at 37°C. After two washings in Eagle's minimum essential medium (MEM) (Flow Labs) with 2·5% FCS, the cells were suspended to 2×10^6 cells/ml.

Sera. The serum samples from the children with CMPI were obtained in connection with positive provocation. A commercial rabbit anti-β-lactoglobulin serum (Nordic Immunology, Tilburg, The Netherlands) was used as a reference. The sera, stored at −20°C until use, were inactivated at 56°C for 30 min and absorbed with washed erythrocytes for 60 min at 37°C.

Effector cells. Lymphocytes were obtained from the blood of adult healthy donors. One-hundred millilitres of heparinized blood were centrifuged for 15 min at 400–500 *g* to remove the plasma and most of the platelets. The blood cells were suspended in Eagle's MEM to the initial volume, 1/3 volume of 3% gelatine (Kemi-Intressen, Sundyberg, Sweden) dissolved in Eagle's MEM was added, and the erythrocytes were sedimented for 30 min at 37°C.

Phagocytic cells in the supernatant were removed by a magnet after incubation with carbonyl iron powder (grade SF; General Anilin and Film Co, New York, USA) during slow rotation for 30 min at 37°C. The remaining cell suspension was centrifuged on Lymphoprep (1·077 g/ml; Nyegaard, Oslo, Norway) for 20 min at 400–500 *g* (Perlmann *et al.*, 1976). Mononuclear leucocytes from the interphase were collected and washed three times with Eagle's MEM containing 2·5 FCS. The cells were then suspended in tissue culture medium and incubated for 2 h at 37°C to eliminate any antibodies adsorbed to the cell membranes. The viability of the cells was determined using 0·01% trypan blue.

Test procedure. The cytotoxicity assay was set up basically as described previously (Hagberg, Ahlstedt & Hanson, 1982) in duplicate wells in sterile microtitre plates with 50 μl of ^{51}Cr-labelled β-lactoglobulin-coated papainized erythrocytes mixed with 50 μl of a 1×10^7/ml lymphocyte suspension and 25 μl of serum (10-fold dilution steps, starting with 1/10). The plates were incubated for 18 h at 37°C and released chromium in 75 μl of the supernatants was detected in a gamma counter. Controls in which the lymphocytes were replaced by untreated erythrocytes as well as controls in which serum was replaced by tissue culture medium were included. In addition, controls with uncoated target cells were performed.

The percentage of specific cytotoxicity (C) was calculated using the formula:

$$C = \frac{125}{75} \times \frac{\text{ct/min for } ^{51}\text{Cr target cells (serum)} - \text{ct/min for } ^{51}\text{Cr target cells (medium)}}{\frac{\text{ct/min for 500 } \mu\text{l } ^{51}\text{Cr target cells}}{10}} \times 100\%$$

The ADCC values in the individual patient sera (diluted 1/10) were expressed as percentage of the reference serum (diluted 1/100).

ELISA

An ELISA for the determination of serum antibodies against β-lactoglobulin was performed as previously described (Ahlstedt *et al.*, 1978; Mellander *et al.*, 1986). Equal quantities of β-lactoglobulin A and B (Sigma) at 5 μg/ml were used for coating of microtitre plates (Dynatech, Alexandria, VA). Rabbit anti-human IgA, IgG (Dakopatts, Copenhagen, Denmark) and secretory component (Seward Labs, London, UK) were conjugated to alkaline phosphatase detection of antibodies against β-lactoglobulin.

Radioallergosorbent test (RAST)

Milk-specific IgE antibodies were determined at the time of serum sampling with the Phadebas RAST kit (Pharmacia

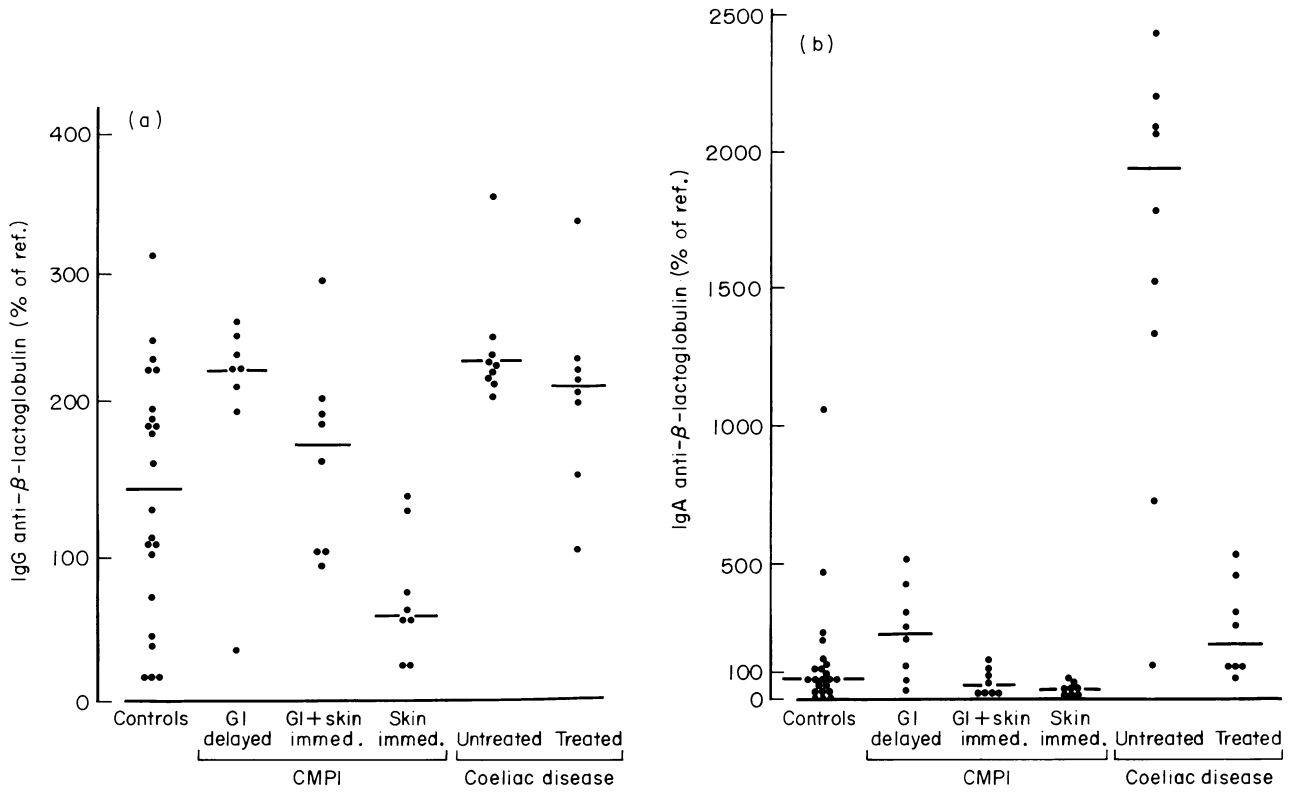

Fig. 2. ELISA IgG (a) and IgA (b) serum antibody levels against β-lactoglobulin in the different patient groups. Medians are indicated.

Diagnostics, Uppsala, Sweden). Positive RAST was defined according to the RAST score 1–4.

Skin-prick test

The skin-prick tests using skimmed milk were carried out at the time of serum sampling. The skin weal diameter was measured after 15 min and compared with the weal elicited by 10 mg/ml histamine. Any weal diameter exceeding 1/4 of the size of the histamine weal was regarded as positive.

Statistical analysis

Statistical comparison of the different patient groups was carried out by the Mann–Whitney *U*-test. Correlations were assessed using Spearman's correlation coefficient.

RESULTS

ADCC assay with patient sera

Sera from CMPI patients with gastrointestinal symptoms showed significantly increased ADCC reactivity as compared with controls ($P < 0.01$) (Fig. 1). This increased capacity to mediate an ADCC reaction was seen both in cases of immediate-onset and delayed-onset CMPI with gastrointestinal symptoms. In contrast, children with immediate-onset CMPI with skin symptoms only had ADCC values in the same range as the controls.

The CMPI children with gastrointestinal symptoms, whether of delayed or immediate type, had significantly increased ADCC reactivity as compared with untreated coeliac disease ($P < 0.05$) as well as treated coeliac disease ($P < 0.01$). Although two patients with untreated coeliac disease had high ADCC levels, the groups with coeliac disease did not differ significantly from the controls.

There was no significant correlation ($P > 0.05$) between ADCC reactivity and the age of the children in the different patient groups (data not shown).

ELISA IgG and IgA serum antibody levels against β-lactoglobulin

Serum IgG antibody levels against β-lactoglobulin showed a considerable overlap between the different diagnostic groups (Fig. 2a). Increased levels of IgG antibodies against β-lactoglobulin were found in the group of children with delayed-onset CMPI and gastrointestinal symptoms as compared with controls ($P < 0.05$). High levels of IgG antibodies against β-lactoglobulin were also found in the coeliac disease patients, especially in those with untreated coeliac disease, who had significantly increased levels compared with controls ($P < 0.01$).

Increased levels of IgA antibodies against β-lactoglobulin were found in children with delayed-onset CMPI with gastrointestinal symptoms, compared with controls ($P < 0.05$) (Fig. 2b). The highest levels of IgA antibodies to β-lactoglobulin were demonstrated in the untreated coeliac disease group ($P < 0.001$), whereas patients with treated coeliac disease had levels of IgA antibodies in the same range as delayed-onset CMPI with gastrointestinal symptoms. Antibodies carrying secretory component and directed against β-lactoglobulin were also determined in sera from CMPI children with gastrointestinal symptoms and increased ADCC reactivity. However, the levels were very low in all sera analysed (data not shown).

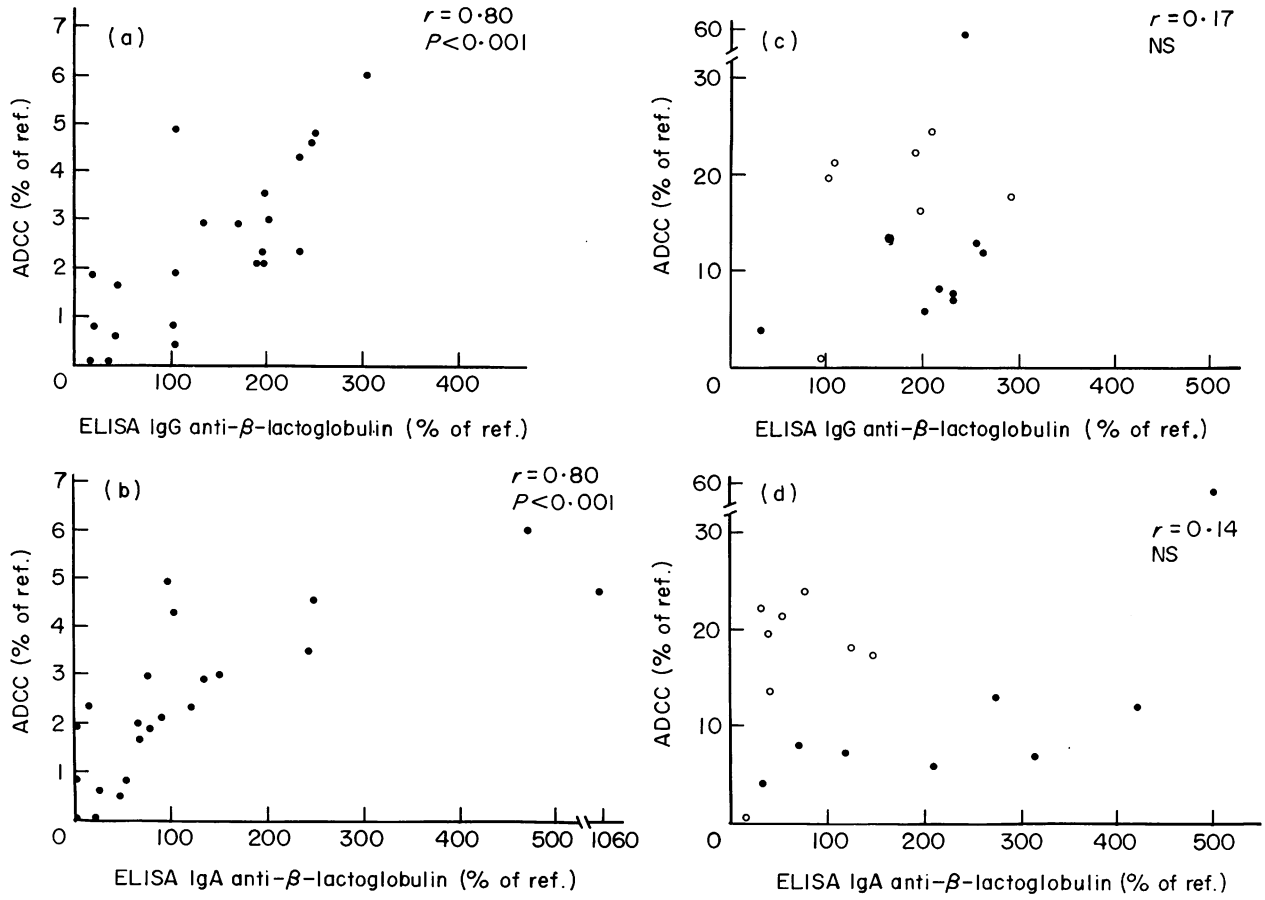

Fig. 3. Correlation between ADCC reactivity and ELISA IgG (a, c) and IgA (b, d) serum antibody levels in controls (a, b) and the CMPI patients with gastrointestinal symptoms combined in one group (c, d) (●, delayed-onset CMPI with gastrointestinal symptoms; ○, immediate onset CMPI with gastrointestinal and skin symptoms).

Correlation between ADCC reactivity and ELISA serum antibody levels against β-lactoglobulin

In sera from the controls significant correlations were demonstrated between the ADCC reactivity and IgG as well as IgA serum antibody levels determined with ELISA (Fig. 3a). In contrast, no clear relation was found between the ADCC reactivity and ELISA antibody levels in sera of the CMPI patients with gastrointestinal symptoms and increased capacity to mediate an ADCC reaction (Fig. 3b).

In both controls and the CMPI patients with gastrointestinal symptoms, a significant correlation between serum IgG and IgA antibody levels was found ($P < 0{\cdot}001$) (data not shown).

DISCUSSION

Sera from children with CMPI and gastrointestinal symptoms of immediate or delayed onset were found to have an increased capacity to induce ADCC, compared with controls and CMPI patients with skin symptoms only. In view of this finding, the possibility of an ADCC mechanism in the gastrointestinal mucosa participating in the development of certain forms of CMPI may be considered.

Interestingly, sera from the children with coeliac disease induced a low ADCC reactivity, despite their high levels of IgG and IgA antibodies against β-lactoglobulin. Thus, there is a discrepancy between the antibody levels estimated by ELISA and the functional capacity to mediate an ADCC reaction. This might be explained by different characteristics of the antibodies, e.g. in the ability to bind to Fc receptors on the effector cells or to antigen epitopes. Such functional divergences might be due to differences in the IgG subclass distribution between the patient groups. IgG1 and IgG3 have been proposed to be the main operative subclasses in IgG-mediated human ADCC systems (Anderson & Looney, 1986). Furthermore, the subclass profile of serum antibodies to dietary antigens may not only vary with the antigen, but also with the clinical disorder, e.g. in CMPI and coeliac disease (Husby *et al.*, 1986; Kemeney *et al.*, 1986; Shakib *et al.*, 1986).

No clear relation was seen between ADCC reactivity and antibody levels against β-lactoglobulin in sera from CMPI children with gastrointestinal symptoms and increased ADCC-mediating capacity. The lack of correlation may be explained by the fact that ADCC-mediating antibodies compose a small fraction, the change of which is not reflected in the specific antibody titres. A similar phenomenon was described in a study of ADCC in HIV infection (Ljunggren *et al.*, 1988).

Although most ADCC systems are IgG dependent, mediation via serum IgA antibodies also has been observed in human systems (Tagliabue *et al.*, 1985). Further, an ADCC reaction against enteric bacteria mediated by secretory IgA and intrae-

pithelial lymphocytes has been reported in mice (Taliabue *et al.*, 1983). Thus, an ADCC reaction via IgA antibodies against β-lactoglobulin, particularly of the secretory type might take place in the gut mucosa. However, secretory IgA antibodies against β-lactoglobulin in the sera of the CMPI children were only present at low levels, but such secretory IgA antibodies with ADCC-mediating capacity may well exist locally in the gut.

An increase of immunocytes producing IgA, IgM and IgG antibodies (Savilahti, 1973; Stern, Dietrich & Muller, 1982), as well as an increased density of intraepithelial lymphocytes (Walker-Smith *et al.*, 1978) is seen in cow's milk-induced enteropathy. The occurrence of high levels of serum antibodies to dietary proteins probably reflects an increased permeability to antigens due to mucosal changes. Such mucosal alterations might also include an increased exudation of antibodies from the circulation into the lumen of the gut. Thus, the conditions for an ADCC reaction taking place in the intestinal mucosa might be present. For example, in cases of CMPI an epithelial cell absorbing food antigens such as β-lactoglobulin could become the target cell and a lymphocyte in the intestinal mucosa the effector cell via antibodies present in the area and directed to, e.g. β-lactoglobulin.

Considering our results, we speculate whether an ADCC reaction could contribute to the enteropathy including epithelial cell damage, which is present in delayed-onset CMPI with gastrointestinal symptoms (Kuitunen, Kosnai & Savilahti, 1982; Stern *et al.*, 1982). In the group of children with immediate-onset CMPI and gastrointestinal symptoms the implication of ADCC as a pathogenic mechanism is more open to debate. However, minor mucosal changes are occasionally found even in this group of subjects with CMPI (Hill *et al.*, 1979; Iyngkaran *et al.*, 1978) and theoretically an ADCC reaction might co-exist with other, e.g. IgE-mediated reactions.

It is likely that various specific humoral and cellular immune mechanisms participate in CMPI, and that more than one type of immunological reaction may operate within the mucosa at one time. Our results give rise to the suggestion that an ADCC mechanism might be of a pathogenic importance in cases of CMPI with gastrointestinal symptomatology.

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REFERENCES

AHLSTEDT, S., CARLSSON, B., HANSON, L.Å., KAIJSER, B., MATTSBY-BALTZER, I. & SOHL-KERLUND, A. (1978) Application of the ELISA for determination of immunoglobulin class-specific *Escherichia coli* antibodies. *Scand. J. Immunol.* **8,** (Suppl. 7), 119.

ANDERSSON, C.L. & LOONEY, R.J. (1986) Human leukocyte IgG Fc receptors. *Immunol. Today*, **7,** 264.

DANNEUS, A. & JOHANSSON, S.G.O. (1979) A follow up study of infants with adverse reactions to cow's milk. Serum IgE, skin test reactions and RAST in relation to clinical course. *Acta paediatr. Scand.* **68,** 377.

DAS, K.M., KADANO, Y. & FLEISCHNER, G.M. (1984) Antibody dependent cell-mediated cytotoxicity in serum samples from patients with ulcerative colitis. *Am. J. Med.* **77,** 791.

FERGUSON, A. (1980) Pathogenesis and mechanisms in the gastrointestinal tract. In *Proceedings of the First Fisons Food Allergy Workshop* p. 28 Medicine Publishing Foundation, Oxford.

FIRER, M.A., HOSKINGS, C.S. & HILL, D.J. (1987) Humoral immune response to cow's milk in children with cow's milk allergy. *Int. Arch. Allergy appl. Immunol.* **84,** 173.

GODING, J.W. (1976) The chromic chloride method of coupling antigens to erythrocytes; definition of some important parameters. *J. immunol. Methods*, **10,** 61.

HAGBERG, M., AHLSTEDT, S. & HANSON, L. (1982) Antibody dependent cell-mediated cytotoxicity against *Escherichia coli* O antigen. *Eur. J. clin. Microbiol. infect. Dis.* **1,** 59.

HILL, D.J., DAVIDSON, G.P., CAMERON, D.J.S. & BARNES, G.L. (1979) The spectrum of cow's milk allergy in childhood; clinical gastroenterological and immunological studies. *Acta paediatr. Scand.* **68,** 847.

HUSBY, S., FOGED N., OXELIUS, V.-A. & SVEHAG S.-E (1986) Serum IgG subclass antibodies to gliadin and other dietary antigens in children with coeliac disease. *Clin. exp. Immunol.* **64,** 526.

IYNGKARAN, N., ROBINSON, M.J., PRATHAP, K., SUMITHRAN, E., YADAV, M. (1978) Cow's milk protein-sensitive enteropathy. (Combined clinical and histological criteria for diagnosis). *Arch. Dis. Child.* **53,** 20.

KEMENY, D.M., URBANEK, R., AMLOT, P.L., CICLITIRA, P.J., RICHARDS, D. & LESSOF, M.H. (1986) Subclass of IgG in allergic disease. I. IgG subclass antibodies in immediate and non-immediate food allergy. *Clin. Allergy*, **16,** 571.

KUITUNEN, P., VISAKORPI, J.K., SAVILAHTI, E. & PELKONEN, P. (1975) Malabsorption syndrome with cow's milk intolerance. *Arch. Dis. Child.* **50,** 351.

KUITUNEN, K., KOSNAI, I. & SAVILAHTI, E. (1982) Morphometric study of the jejunal mucosa in various childhood enteropathies with special reference to intraepithelial lymphocytes. *J. pediatr. Gastroenterol. Nutr.* **1,** 525.

LJUNGGREN, K., BROLIDEN, P.-A., MORFELDT-MÅNSSON, L., JONDAL, M. & WAHREN, B. (1988) IgG subclass response to HIV in relation to antibody-dependent cellular cytotoxicity at different clinical stages. *Clin. exp. Immunol.* **73,** 343.

MCCALLA, R., SAVILAHTI, E., PERKKI, M., KUITUNEN, P. & BACKMAN, A. (1980) Morphology of the jejunum in children with eczema due to food allergy. *Allergy*, **35,** 563.

MEEUWISSE, G.N. (1970) Diagnostic criteria in coeliac disease. *Acta paediatr. Scand.* **59,** 461.

MELLANDER, L., BJÖRKANDER, J., CARLSSON, B. & HANSON, L.Å. (1986) Secretory antibodies in IgA deficient and immunosuppressed individuals. *J. clin. Immunol.* **6,** 284.

PERLMANN, H., PERLMANN, P., PAPE, R. & HALLDEN, G. (1976) Purification, fractionation and assay of antibody-dependent lymphocytic effector cells (K-cells) in human blood. *Scand. J. Immunol.* **5,** (Suppl. 5), 57.

SAVILAHTI, E. (1973) Immunochemical study of the malabsorption syndrome with cow's milk intolerance. *Gut*, **14,** 491.

SHAKIB, F., MORROW BROWN, H., PHELPS, A. & REDHEAD, R. (1986) Study of IgG subclass antibodies in patients with milk intolerance. *Clin. Allergy*, **16,** 451.

SHORTER, R.G., MCGILL, D.B. & BAHN, R.C. (1984) The cytotoxicity of mononuclear cells for autologous colonic epithelial cells in colonic diseases. *Gastroenterology*, **86,** 13.

STERN, M., DIETRICH, R. & MULLER, J. (1982) Small intestinal mucosa in coeliac disease and cow's milk protein intolerance; morphometric and immunofluorescent studies. *Eur. J. Pediatr.* **139,** 101.

TAGLIABUE, A., NENCIONI, L., VILLA, L., KEREN, D.F., LOWELL, G.H. & BORASCHI, D. (1983) Antibody-dependent cell-mediated antibacterial activity of intestinal lymphocytes with secretory IgA. *Nature*, **306,** 184.

TAGLIABUE, A., VILLA, L., BORASCHI, D., PERI, G., DE GORI, V. & NENCIONE, L. (1985) Natural antibacterial activity against *Salmonella typhi* by human $T4^+$ lymphocytes armed with IgA antibodies. *J. Immunol.* **135,** 4178.

URBANIAK, S.J. (1976) Lymphoid cell dependent (K-cell) lysis of human erythrocytes sensitized with rhesus allo-antibodies. *Br. J. Haematol.* **33,** 409.

WALKER-SMITH, J., HARRISON, M., KILBY, A., PHILLIPS, A. & FRANCE, N. (1978) Cow's milk sensitive enteropathy. *Arch. Dis. Child.* **53,** 375.

WALKER-SMITH, J.W. (1988) *Diseases of the Small Intestine in Childhood* 3rd edn. Butterworth, London.

WETTERGREN, B., JODAL, U. & JONASSON, G. (1985) Epidemiology of bacteriuria during the first year of life. *Acta paediatr. Scand.* **74,** 925.

YUST, I., FISCH, B. & GOLDSHER, N. (1980) Antibody-dependent cell-mediated cytotoxicity against human red blood cells; correlation of effector cell type with enzymatic alteration of the target cell surface. *Eur. J. Immunol.* **10,** 127.