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Subclass composition and J-chain expression of the 'compensatory' gastrointestinal IgG cell population in selective IgA deficiency

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SUMMARY

The subclass distribution of IgG-producing immunocytes was examined by two-colour immunohistochemistry in gastrointestinal mucosa of 14 patients with selective serum IgA deficiency providing the following biopsy material: gastric ($n=1$); jejunal ($n=12$); colonic ($n=1$); and rectal ($n=2$). All except two patients suffered from various infections, and coeliac disease was observed in six of them. Control reference data were based on biopsies from immunologically intact subjects, including histologically normal jejunal ($n=10$) and large bowel ($n=10$) mucosa and stomach mucosa with slight chronic gastritis ($n=8$). The total mucosal population of immunoglobulin-producing cells per 500 μm gut length unit was only slightly decreased in IgA deficiency because of an increased number of IgG (30%) and especially IgM (71%) immunocytes. The IgG1 immunocyte proportion in the proximal gut (median 87%) was higher than that in the comparable controls (gastric 69%, jejunal 66%). A similar trend was seen in the distal gut (69%) compared with controls from the large bowel mucosa (55%). Conversely, IgG2 and IgG3 cell proportions were significantly decreased compared with the respective controls from the proximal gut. The same was true for IgG4, which also was significantly reduced in jejunal mucosa. Paired staining for cytoplasmic J chain and immunoglobulin isotype showed 71% positivity for jejunal IgG-producing cells in IgA deficiency, which was somewhat reduced compared with comparable controls (89%). J chain appeared to be preferentially expressed by IgG1 cells (75%), but was also found in IgG2 (70%), IgG3 (32%) and IgG4 cells (33%). IgM-producing cells showed a J-chain positivity (99%) in IgA deficiency similar to normal (100%). Our results suggested that the block in mucosal B cell differentiation to IgA expression in the proximal gut is mainly located immediately upstream to the CHα1 gene, giving excessive terminal maturation of J-chain-positive IgG1 immunocytes.

Keywords IgG subclass distribution mucosal immunity gastrointestinal mucosa J chain selective IgA deficiency

INTRODUCTION

B cell differentiation is an extremely complex process both in morphological and molecular terms. The mechanisms of isotype switching and the sequence of events involved in activation of resting B lymphocytes to the final differentiation to immunoglobulin-producing immunocytes (blasts and plasma cells), with or without concurrent J-chain expression, are only partly understood [1–3]. The discovery of directed switch recombination and regulated transcription of recombinogenic sequences has clarified the molecular biology of this process [4,5]. Recent focus on regulatory T cells and their cytokines, such as IL-4, IL-5, interferon-gamma (IFN-γ) and transforming growth factor β (TGF-β), has revealed their significance for isotype expression *in vitro* [6,7], and *in vivo* [8]. Studies of B cell deficiencies may contribute to better understanding of these processes.

In previous Scandinavian studies only minor gastrointestinal disorders were noted in generalized B cell deficiency patients on immunoglobulin replacement therapy [9] and in patients with various IgG-subclass deficiencies without such treatment [10–12]. In this study we selected the few available patients with IgA deficiency (IgAD) who suffered from gastrointestinal disorders seriously enough to demand endoscopic investigation. This was in contrast to the ordinary healthy condition of individuals with this type of immunodeficiency [13,14].

The availability of a clinically and serologically well-characterized patient material encouraged us to examine the cytoplasmic immunoglobulin expression patterns of terminally differentiated intestinal B cells in selective IgAD. The specific

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aim of our study was to characterize the 'compensatory' mucosal IgG cell isotype response pattern to obtain information about regulatory mechanisms involved in local immunity. Previous studies from different sites of the alimentary tract suggested that when the terminal differentiation of mucosal B cells is blocked, as in IgAD, the precursors develop locally to IgM- and IgG-producing plasma cells [15–18].

Cytoplasmic J chain present in substantial amounts appears to be a marker of terminally differentiated B cells belonging to newly generated memory clones, such as those homing to mucosal sites [3,19,20]. It was therefore also of interest to examine J-chain expression by the immunocyte population replacing mucosal IgA cells in selective IgAD.

PATIENTS AND METHODS

IgA-deficient patients

Five children (< 15 years) and nine adult patients with serologically and clinically well-characterized IgAD were included in the study (altogether eight females and six males; median age 18 years, range 1·5–56 years). None of them had detectable IgA in serum (< 0·10 g/*l*), and all had total serum IgG and IgM levels within the normal range. IgAD was confirmed in at least two serum samples (not during treatment with drugs like phenytoin, penicillamine or Captopril). Only one child with ulcerative colitis received steroids. None of the patients were receiving immunoglobulin replacement therapy. IgG subclass deficiency was defined as a concentration below the lower limit of the 95 percentile range [14]. All patients with such a deficiency had been excluded.

The patients were referred to our hospital during the period 1976–1991, mainly for endoscopic investigation of moderate gastrointestinal disorders (Table 1). Coeliac disease was diagnosed in six of them, two children and four adults. Good response on gluten-free diet was noted in four of the coeliacs (including both children); after ending 16 months of gluten restriction one child still remained a good responder. A few patients suffered from food intolerance, irritable colon, gastric ulcer, atopic eczema, asthmatic bronchitis or periodic depression. Autoimmune disease was observed in one patient with systemic lupus erythematosus (SLE) and in another with Raynaud's disease. Malignancies like gastrointestinal non-Hodgkin's lymphoma occurred later on in one patient. Only few of the patients suffered from chronic lung disease, except bronchitis, which was quite common. Twelve of the patients suffered from recurrent infections, mostly of the respiratory tract.

Quantification of serum immunoglobulins

Determinations of total IgG and IgM were performed by the Institute of Immunology and Rheumatology (IGRI), The National Hospital, Oslo, Norway, by a turbidimetric assay in relation to a commercial standard (Behringwerke, Marburg-Lahn, Germany). Measurements of IgA and the four IgG subclasses were performed by Allergenlab, Nycomed Pharma AS, Oslo, Norway. IgA was determined by the same method as mentioned above, whereas IgG subclasses were determined by a standard ELISA. Microtitre plates (Nunc, Roskilde, Denmark) were coated with 100 μl/well of the following murine MoAbs (ascites appropriately diluted in distilled water): anti-IgG1 (HP6069, clone 2B6; Dr. O. Mäkelä, Helsinki, Finland); anti-IgG2 (HP6014, clone AC3-AA11; Oxoid, Basingstoke, UK); anti-IgG3 (HP6050, clone CC4-DC10; Oxoid); and anti-IgG4 (HP6025, clone CB2-CC5, ICN, High Wycombe, UK). Both test and standard samples were prepared in phosphate-buffered saline (PBS), pH 7·2, containing 5 g/*l* of bovine serum albumin (BSA) and 0·05% Tween-20; triplicates were incubated for 1 h at 37°C. After being washed three times in isotonic saline with 0·05% Tween-20, the wells were incubated with a peroxidase-conjugated rabbit antibody specific for human IgG (Nycomed Pharma) for 2 h at 37°C. After the final washes, enzyme activity was detected with 2,2′-azino-di-(3-ethylbenzthiozolin sulphonate) (Boehringer, Mannheim, Germany) dissolved at 180 μg/ml in 0·1 M acetate buffer (pH 4) and 8 μl 3% H_2O_2 per 10 ml. The optical density was read at 414 nm after 30 min. Standards were Nycomed Pharma's reference sera titrated against appropriate human monoclonal IgG proteins provided by WHO. Frozen or fresh serum samples were available from all patients except some of the controls.

Biopsy specimens

The following mucosal biopsy material was obtained from the IgAD patients: gastric ($n=1$); jejunal ($n=12$); colonic ($n=1$); and rectal ($n=2$). Small intestinal biopsy specimens were mostly excised from the distal or adjacent duodenum parts of jejunum, the colonic from the proximal ascending segment, and rectal ones from the distal third of the rectum, mainly 5–10 cm up on the posterior wall. One patient was represented by both a jejunal, colonic and rectal biopsy, while one had only a gastric biopsy.

Specimens of jejunal mucosa from two previously reported patients with selective IgAD [18] were, together with three additional biopsies, evaluated for interobserver reproducibility.

Two-colour immunohistochemical staining

The tissue specimens, before ethanol fixation and paraffin embedding, were extracted in cold isotonic PBS (pH 7·5) for 24–48 h to avoid interstitial immunoglobulin staining [21]. At least 10 serial sections from each tissue block were cut at 6 μm, dewaxed and subjected to immunofluorescence staining. The two-colour method used has been described previously, including characteristics of the fluorochrome conjugates [22], the source of IgG-subclass-specific monoclonal antibodies [23], and other details of the staining procedure [24]. Briefly, each of four serial sections was subjected to paired staining for one of the four IgG subclasses and for total IgG by first applying murine MoAb (ascites 1/800) to IgG1 (HP6070, clone 2C7), IgG2 (HP6009, clone GOM2), IgG3 (HP6048, clone CB1-AH7), or IgG4 (HP6011, clone RJ4) and subsequently a mix of FITC-labelled rabbit anti-mouse IgG and rhodamine B sulphonyl chloride (RB200SC)-labelled anti-human IgG (γ-chain specific). Both incubations took place at room temperature for 20 h. Pairs of contrasting rabbit fluorochrome-labelled antibody reagents were used to determine the mucosal IgG-, IgA-, IgM- and IgD-immunocyte class distribution by direct staining as previously described [25]. The specificities of the applied fluorochrome conjugate combinations were as follows: Mouse IgG (FITC) and human γ-chain (RB200SC); human γ-chain (tetramethyl rhodamine isothiocyanate=TRITC) and human α-chain (FITC); human α-chain (TRITC) and human μ-chain (FITC); human μ-chain (TRITC) and human γ-chain (FITC); and human μ-chain (TRITC) and human δ-chain (FITC).

Table 1. Clinical summary of patients with selective IgA deficiency

Patient no.	Sex, age (years)	Biopsy site	Intestinal mucosal inflammation*	Small intestinal villous atrophy	Gastrointestinal disorders previous to endoscopy	Frequent infections	Other diseases
1	M, 1·5	J	Slight	None	Periodic diarrhoea	URI	Growth retardation
2	M, 2	R	Moderate		Periodic diarrhoea	URI, asthmatic bronchitis	Atopic eczema, glomerulonephritis, food intolerance (milk), UC verified later
3	F, 4	J	Moderate	Total	Coeliac disease, GFD earlier	URI	Food intolerance (milk)
4	F, 10	J	Slight	Subtotal	Dyspepsia, coeliac disease, GFD	URI, bronchitis	Growth retardation, eczema
5	F, 10	J, colon, R	Slight	None	Colitis	Asthmatic bronchitis	Psoriasis, depression, UC verified later
6	F, 15	J	None	None	Coeliac disease, GFD	URI, bronchitis	
7	M, 17	J	None	None	Periodic diarrhoea	URI, asthmatic bronchitis	Food intolerance (orange)
8	F, 19	J	None	None	Carbimazole treatment, malabsorption	URI, bronchitis	Hyperthyreoid disease, atopic eczema, Raynaud's disease
9	M, 20	J	None	None	Coeliac disease in early childhood, GFD since	URI, pneumonia	Diabetes mellitus
10	M, 21	Antral mucosa	Severe		Gastric ulcer, operated	Bronchitis	SLE, Calve-Legg-Perthe
11	F, 36	J	Slight	Partial	Coeliac disease	URI, tub. meningitis early childhood, cystitis	
12	F, 37	J	None	None	Steatorrhoea, cholelithiasis	URI, bronchitis	Spondylosis
13	F, 50	J	Slight	Subtotal	Coeliac disease (kidney donor)	None	Epilepsy earlier No treatment last year
14	F, 56	J	Slight	Subtotal	Dypepsia	None	Urolithiasis, malignant gastrointestinal non-Hodgkin's lymphoma verified later

* Based on tissue sections stained with haematoxylin and eosin.

GFD, Gluten-free diet; J, jejunum; R, rectum; URI, upper respiratory tract infections; UC, ulcerative colitis; SLE, systemic lupus erythematosus.

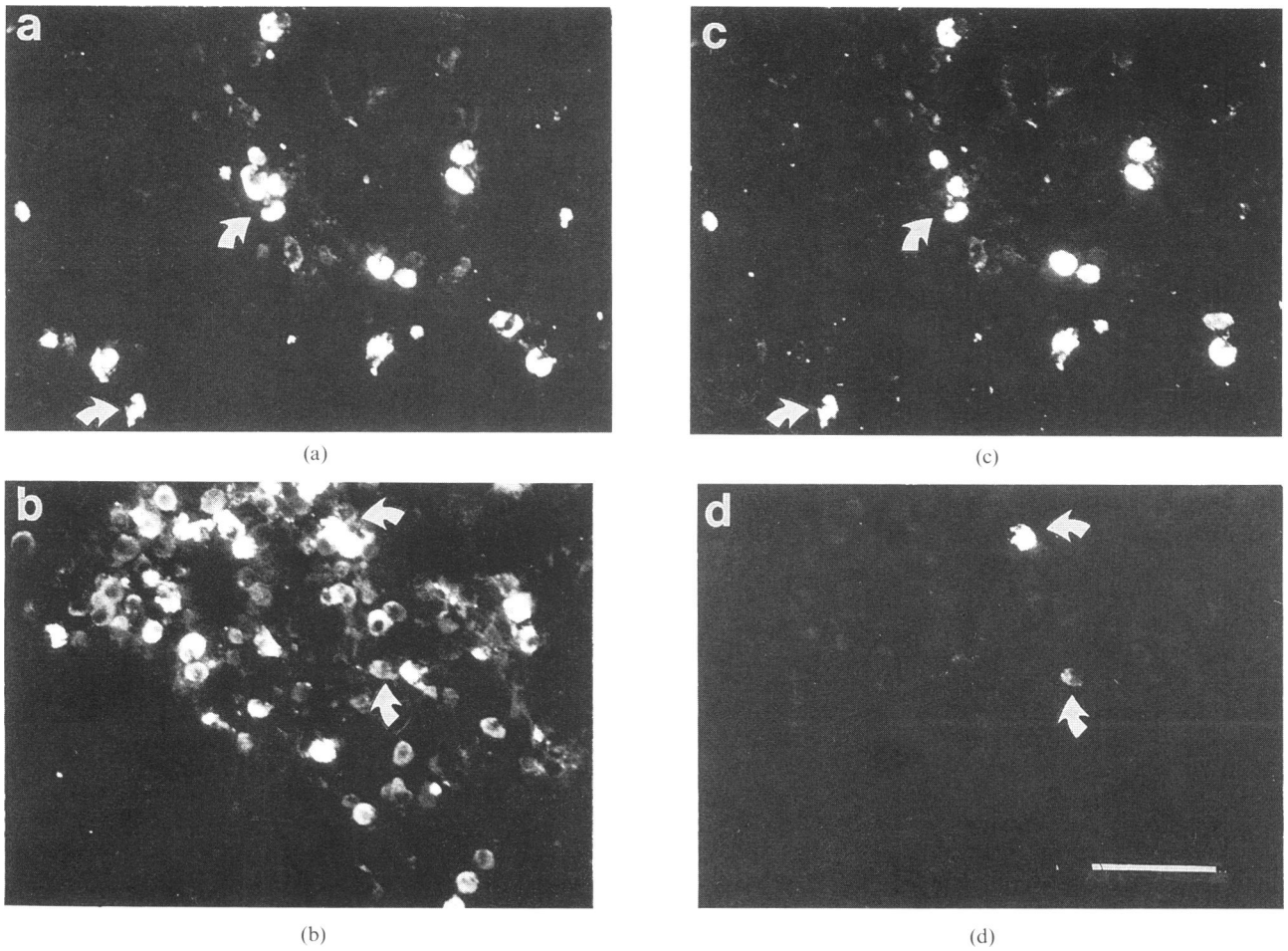

Fig. 1. Two-colour immunofluorescence staining for total IgG (a, b rhodamine) and IgG1 or IgG2 (c, d fluorescein) in non-inflamed jejunal mucosa from female patient with selective IgA deficiency and partial villous atrophy. Note that most (92%) IgG-producing cells are of the IgG1 subclass (example arrowed). In contrast a rather small proportion (6%) IgG2-producing cells (arrowed) were observed in the same patient. Bar = 40 μm.

For paired staining of cytoplasmic immunoglobulin class and J chain, dewaxed serial sections were incubated for 20 h with a mix of FITC-labelled isotype-specific reagent and TRITC-labelled rabbit anti-J chain (0·04 g/*l*) after pre-treatment with 6 M urea (pH 3·2–3·5) for 1 h at 4°C to unmask hidden J-chain determinants [26]. For paired staining of IgG subclass and J chain, we used murine MoAbs as detailed above. The first incubation step (20 h) included TRITC-labelled rabbit anti-J chain (0·04 g/*l*) mixed with one of the appropriately diluted unlabelled MoAbs. After washing a mixture of TRITC-labelled anti-J chain (0·04 g/*l*) and FITC-labelled rabbit anti-mouse IgG was applied for another 20 h [22,27].

Microscopy and cell counting

Fluorescent cells were observed in a Leitz Orthoplan microscope equipped with ×25 and ×40 immersion objectives, an ×10 ocular, and a Ploem-type vertical illuminator with interference filters for selective observation of green (fluorescein) or red (rhodamine) emission. For each tissue specimen a median of 816 cells (lower range 186) showing 'red' (class-specific) cytoplasmic staining were examined for concomitant 'green' (subclass-specific) fluorescence. The proportion of cells containing one of the four subclasses was then calculated in relation to the total number of IgG-producing immunocytes detected in the evaluated area of the same section (Fig. 1). Several series of sections often had to be counted in order to obtain a sufficiently high number of IgG cells. The proportions of green cells (all four subclasses) added on average up to slightly below 100% (98·3%, range 96–101%) for each specimen. The IgM/IgG cell ratio was based on enumerations of 'red' IgM (median 687, lower range 253) and 'green' IgG (median 265, lower range 109) cells in the same sections. Also IgG/IgA- and IgA/IgM-staining combinations were performed to confirm IgAD, and IgD/IgM to enumerate the rare IgD cells.

The absolute number of each immunocyte isotype was based on counts made in a defined 'mucosal tissue unit' constituting a 6-μm thick and 500-μm wide block of tissue, including the mucosa at full height from the muscularis mucosae to the surface epithelium [28]. We used a simplified modification of the original method by photographing the IgG cells in a serial section from each specimen through a ×10/0·45 Leitz immersion objective, including a morphologically acceptable field at each end of the section. The colour slides were subsequently projected at a magnification of ×500 on a screen, and the 500-μm wide unit was defined. The total IgG cell number per unit was determined, and the corresponding number of the other

immunocyte isotypes could be calculated on the basis of the previously estimated isotype ratios in relation to IgG.

Only immunocytes showing distinct (moderate or strong) diffuse cytoplasmic 'red' staining for J chain were considered positive for this marker. For each tissue specimen a median of 192 'green' (subclass-specific) cells (lower range 118) were examined for concomitant 'red' (J chain) fluorescence. All cell counts were performed by the same observer throughout the study.

An additional section from each series was subjected to conventional histological evaluation after staining with haematoxylin and eosin.

Control reference data from mucosal immunocyte isotype distribution

The normal subclass distribution of IgG-producing cells was determined by two-colour immunofluorescence staining as described above in jejunal mucosa of control subjects (five females and five males; median age 22 years, range 1–52 years) with no histological or immunological mucosal abnormalities. Similar data recorded previously by the same method in subjects without known IgG subclass deficiency or IgAD were obtained for normal colonic mucosa ($n=10$, median age 59 years) [29] and gastric mucosa ($n=8$) with slight chronic gastritis [30]. In addition, reference data were available in our laboratory for the distribution of IgA, IgM and IgG immunocytes in normal jejunal ($n=8$, median age 28 years) and normal large bowel ($n=8$, median age 50 years) mucosa quantified on the basis of mucosal tissue units defined as described above [31,32]. The normal J-chain expression by jejunal IgG-producing cells was partly ($n=4$) based on a previous study [20], partly ($n=4$) obtained from the reference material described above.

Statistical analysis

Correlations between the proportions of the four cellular IgG subclasses observed in jejunal mucosa and the IgG subclass levels in serum obtained near the time of biopsy, were determined by Spearman rank correlations (IBM-Statgraphics). Wilcoxon's two-sample rank test (two-tailed) was used to determine the differences between the cellular proportions of the four IgG subclasses, total IgG, IgM and IgM- and IgG-associated J-chain expression in jejunal mucosa and comparable control samples. The inter- and intra-observer reproducibility of fluorescent cell enumerations were well correlated (Kendall $\tau=0{\cdot}80$; $P=0{\cdot}05$; $\tau=1{\cdot}00$, $P<0{\cdot}02$, respectively; $n=5$ in both tests).

RESULTS

Distribution of compensatory IgM and IgG immunocytes

Light-microscopic evaluation, based on staining with haematoxylin and eosin, showed slight to moderate inflammation in jejunal mucosa from seven of 12 IgAD patients, including four with coeliac disease, whereas moderate to severe inflammation was seen in one gastric and one rectal biopsy; the latter patient later turned out to have ulcerative colitis. Two other coeliacs with non-inflamed jejunal mucosa obviously were good responders on gluten-free diet (Table 1).

Most IgAD patients showed, as expected, a compensatory jejunal immunocyte population consisting of the IgM (median 71%) and IgG (median 30%) classes (Fig. 2a). All patients except one had none or very few detectable IgA cells ($<0{\cdot}3\%$) in their jejunal mucosa. This was in agreement with our previous observations [18]. A substantial number of IgA-producing cells was detected in one jejunal specimen, but the proportion (51%) was lower than in normal controls (79%). Also this 4-year-old girl with coeliac disease, food intolerance (milk), and verified serum IgAD had a compensatory increase of IgM (37%) and IgG (12%) cells in her jejunal mucosa compared with normal controls (19% and 4%, respectively). Conversely, the IgG cell proportion was particularly increased (median 70%) in the three distal gut specimens from two very young IgAD patients (Table 1, Fig. 2a) contrasting the predominance of IgM (79%) cells in the jejunal mucosa from one of them. IgD-producing cells (median $<0{\cdot}2\%$) were only observed in about two-thirds of the specimens.

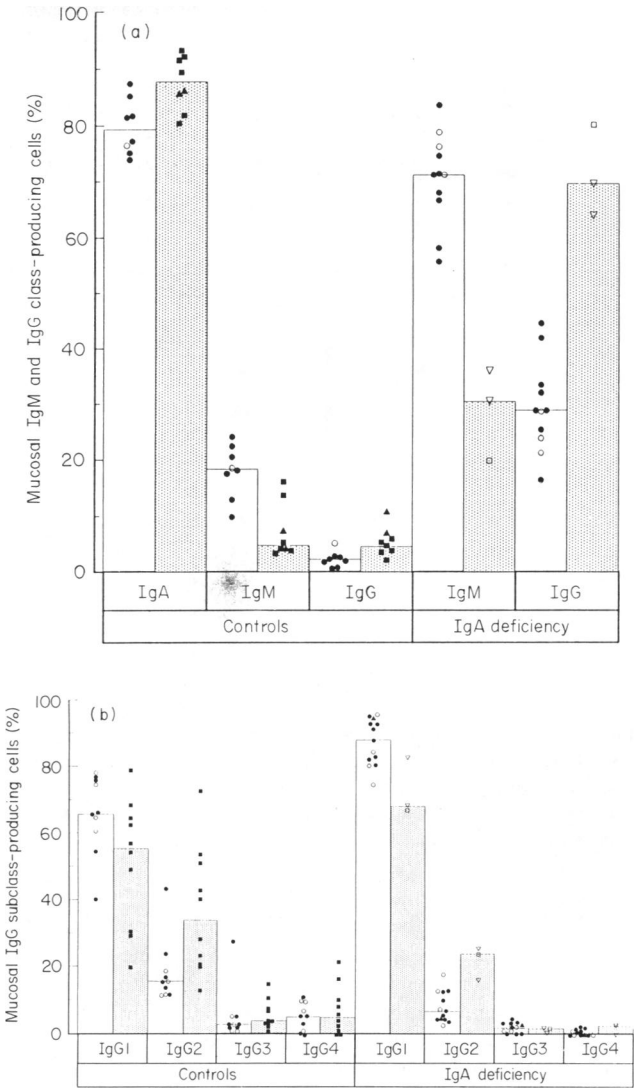

Fig. 2. (a) Median percentage distribution of mucosal IgG and IgM class-producing cells in proximal (□) and distal (▨) gut segments of patients with selective IgA deficiency ($n=12$) compared with normal controls ($n=8$). (b) Median percentage distribution of IgG subclass-producing cells in gastrointestinal mucosa of patients with selective IgAD ($n=14$) compared with immunologically normal control subjects ($n=10$). ▲, Stomach; ○, duodenum/jejunum; ■, colon; ▼, rectum; ○, children < 15 years; ●, adults.

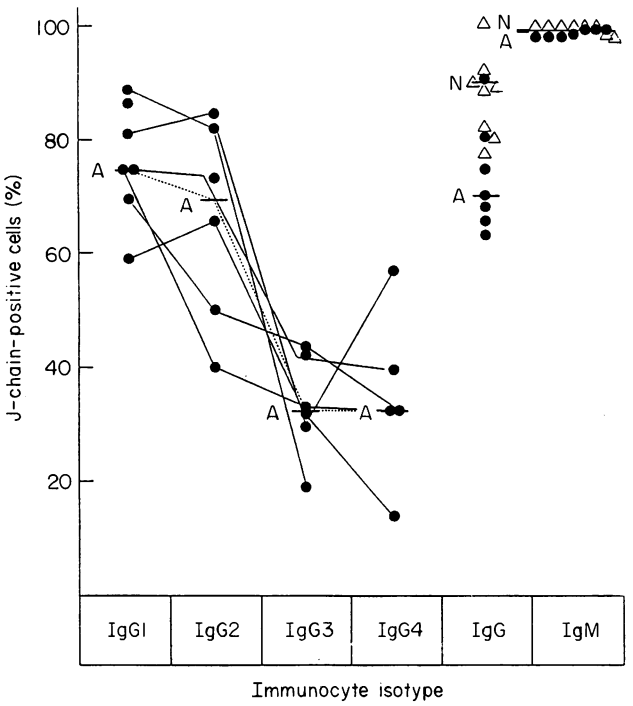

Fig. 3. Median percentage distribution (horizontal bars) of J-chain-positive IgG subclass-, IgG- and IgM-producing cells in proximal small intestinal mucosa of patients with selective IgA deficiency ($n=7$) compared with immunologically normal subjects ($n=8$). A (●), IgA deficiency; N (△), normal controls.

The median number of IgG and IgM immunocytes per jejunal mucosal tissue unit in specimens with none or very few IgA cells ($n=11$) was 112 (observed range 39–176), which was slightly but significantly decreased ($P<0.03$) compared with values found for total immunocyte numbers in controls ($n=8$) containing a normal intestinal IgA cell population (median 134, observed range 86–165). No difference in total jejunal immunocyte numbers was found between adults ($n=8$) and children below 15 years ($n=3$) with IgAD. However, a significant numerical difference ($P<0.03$) was found between jejunal samples with chronic inflammation (median 128, observed range 73–176) and those with normal mucosal histology (median 73, observed range 39–112), although the IgM/IgG cell ratio did not differ ($P=0.09$). In the three large bowel samples, the total immunocyte numbers per defined mucosal tissue unit also tended to be decreased (median 110, observed range 86–302) compared with controls ($n=8$) containing a normal colorectal IgA cell population (median 173, observed range 69–265), but here the age difference (median age 10 years *versus* 50 years in controls) and small number of specimens made comparison difficult.

Distribution of IgG subclass-producing immunocytes

The IgG1 cell proportion in the proximal gut (median 87%) of IgAD patients was higher than that in the comparable controls (gastric 69%, jejunal 66%), significantly so for the jejunal mucosa ($P<0.0002$). The same trend (69%) was seen in the limited number of specimens from the distal gut compared with large bowel control mucosa (55%). Conversely, IgG2 and IgG3 cell proportions were significantly decreased ($P<0.005$ and $P<0.04$ respectively) compared with the comparable controls from the proximal gut. The same was true for the IgG4 ratio (Fig. 2b), which was also significantly reduced ($P<0.005$) in jejunal mucosa (0·3% *versus* 5·6%). None of the jejunal IgG subclass proportions were well correlated with the corresponding serum levels.

J chain expression by compensatory mucosal immunocytes

IgG-producing cells showed 71% J-chain positivity in IgA deficiency, which was slightly but significantly reduced ($P<0.03$) compared with normal small intestinal mucosa (89%). J chain seemed to be preferentially expressed by the compensatory IgG1 cells (75%), but was also found in IgG2 (70%), IgG3 (32%) and IgG4 (33%) cells, although their small numbers made enumerations difficult and less certain. IgM-producing cells showed a median J chain positivity of 99% in IgA deficiency (Fig. 3), virtually the same as in normal jejunal mucosa (100%).

DISCUSSION

This study confirms and extends previous information about the predominating so-called 'compensatory' IgM-producing plasma cell population in gastrointestinal mucosa of patients with selective IgAD [15–18]. In addition, we found a substantial mucosal IgG response, which preferentially included IgG1 cells, both in those with and without coeliac disease. The jejunal IgG1 proportion was thus significantly higher than that recorded in normal controls. The same trend was seen in the limited number of IgAD specimens available from the distal gut. Moreover, the IgG2, IgG3 and IgG4 immunocyte proportions in jejunal mucosa were significantly reduced compared with comparable normal controls. The jejunal IgG2 cell proportion is usually raised in untreated coeliac disease compared with coeliacs on a gluten-free diet or patients with food allergy [33]. Although the striking increase of IgG1 in our patients made comparison difficult, only one of the two patients without gluten restriction appeared to have slightly increased IgG2 proportion. IgD-producing cells were virtually absent in the intestinal mucosa. This was in agreement with previous studies where IgD-producing cells were found predominantly in the upper digestive and respiratory tracts both normally and particularly in IgAD [18,25].

Pentameric IgM containing J chain is, like polymeric IgA (poly-IgA), subjected to external transport via secretory component (SC) or the poly-immunoglobulin receptor [3]. Therefore, in view of the enhanced local synthesis of IgM, most IgAD subjects have raised levels of IgM in saliva [34] and intestinal secretions [35,36]. In contrast to the situation for secretory IgA (SIgA), there is no covalent stabilization of SIgM with SC [37]. This probably contributes to the fact that SIgM is subjected to rapid degradation in the intestinal lumen [38] and hence may exert less efficient surface protection than SIgA in the gut [39]. In addition, rare IgAD patients may produce defective IgM molecules that fail to be externally translocated by the SC-mediated transport mechanism [40].

Determination of the total number of mucosal immunocytes based on an individually defined mucosal tissue unit [28] revealed a slight, although significant, decrease of jejunal immunoglobulin-producing cells in IgAD. Notably, there were considerable overlaps between total immunocyte numbers in

IgAD patients and in controls containing a normal intestinal IgA cell population. This agrees with our previous studies, which also showed normal total numbers of immunoglobulin-producing cells/mm^2 tissue section in parotid and lacrimal glands from patients with selective IgAD [18]. Altogether these results suggest that the migration or 'homing' of B cells to secretory sites, and their local terminal differentiation, are fairly well maintained despite a block in the final pathway to IgA production.

The clinical importance of the mucosal IgM and IgG production in IgAD has been considered in several studies, and IgM compensation appears to be beneficial [13,14,34,35]. Friman *et al.* [41] recently reported that specific IgG production rather than IgM in intestinal mucosa was a striking feature after oral immunization with cholera toxin B subunit/whole cell vaccine in a subgroup with IgAD exhibiting frequent respiratory tract infections. Our finding that the intestinal IgG response in IgAD mainly includes the IgG1 is interesting in view of the phlogistic properties of this subclass [42]. Compensation with SIgM performing immune exclusion may not be sufficient in every IgAD patient to dampen untoward immune reactions in the lamina propria because of penetrating antigens. The clinical outcome often appears to depend on whether or not the non-specific defence mechanisms are functioning adequately [43]. In our study we deliberately, for practical reasons, selected IgAD patients who apparently suffered from an inadequate mucosal defence system; seven of the 12 specimens showed histologically mucosal inflammation. Also, the histological evaluation of gastric and distal gut mucosa showed severe to slight chronic inflammation with an abundance of IgG-producing cells in agreement with the diagnosis of chronic gastritis and inflammatory bowel disease [24,30]. This fact might also explain the slightly reduced J chain expression shown by jejunal IgG cells in our patients compared with normal controls.

Even patients presenting with truly selective serum IgAD often have a few scattered IgA-producing cells in their intestinal mucosa and rare cases have been reported to have fairly normal numbers of such immunocytes [44–46]. However, the jejunal IgM cell population always seems to be aberrantly expanded like that found in mucosa of a young girl in our study. This suggests that a generally defective IgA system occasionally may be topically activated owing to the persistent antigenic and mitogenic load on mucosa-associated lymphoid tissues.

Since most of the IgG cells after terminal maturation in the proximal gut mucosa express J chain, they probably belong to relatively early memory clones derived from gut-associated lymphoid tissue [19,20]. These immunocytes might be considered 'spin-offs' from clones that through CH gene switching (5′→3′) are on their way to poly-IgA expression. This final pathway is blocked in patients with selective IgAD. Our results suggest that the block in differentiation to IgA in the proximal gut is mainly located immediately upstream to the CHα1 gene, giving excessive terminal maturation of IgG1-producing immunocytes. Accordingly, there was no overactivation of the more distally located CHγ2 and CHγ4 genes. This might reflect that vectorial switching is a major regulatory pathway leading to the normally predominant IgA1 immunocyte population reported for the proximal gut [47].

Among the compensatory IgG immunocytes, J chain seems to be preferentially expressed along with IgG1, but it was also found in IgG2, IgG3 and IgG4 cells. We recently observed relatively prominent J chain positivity of IgG2-producing cells in mesenteric lymph nodes, suggesting that these immunocytes are largely generated in Peyer's patches, perhaps as spin-offs during normal differentiation to IgA2 [48]. To our knowledge, only one earlier study attempted to relate human J chain and IgG subclass expression [49]. The results were rather inconclusive because of the small number of tissue specimens or cells evaluated, but examinations of pokeweed mitogen-stimulated peripheral blood lymphoid cells indicated preferential J chain expression associated with IgG2 and IgG4.

Helper factors for IgG1 responses might be upregulated in IgAD. It has been shown that low doses of IL-4 suppress IgG3 secretion in lipopolysaccharide (LPS)-stimulated B cell cultures and promote both membrane expression and secretion of IgG1 [50]. Cytokines such as IL-5 appear to act as cofactors that enhance terminal differentiation to IgA production; mechanisms triggering IL-5 production by T cells are positively influenced by polyclonal activators such as anti-CD3 or recombinant IL-2 [51]. Also, TGF-β synergizes with IL-2 and IL-5 to promote IgA production [52]. Inhibition of such signal substances has to be taken into account in etiological considerations regarding selective IgAD. However, a defect of IL-5 production could not be confirmed in a recent study using *in situ* hybridization on unstimulated lymphocytes from IgAD patients [53].

These immunoregulatory aspects are obviously only part of a complex cellular and molecular interplay normally taking place in the gastrointestinal tract. Regulatory events underlying the striking heterogeneity in B cell differentiation pathways employed within the human mucosal system are still poorly understood [54]. Further studies focused on regional environmental factors together with characterization of accessory cells and cytokines interacting in various inductive lymphoid tissues, might contribute to a better understanding of mucosal immunity. IgAD is one 'experiment of nature' that may aid these efforts.

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