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Intestinal B cell defects in common variable immunodeficiency

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SUMMARY

The humoral immune system of the small intestine of 17 patients with common variable immunodeficiency (CVID) was studied by immunohistology using antibodies specific for IgA1,2, IgM, IgG1–4, the J chain and the secretory component (SC). IgA1,2$^+$, IgG2$^+$ and IgM$^+$ lamina propria B cells were totally lacking in 65% (11/17), 41% (7/17) and 18% (3/17) of CVID patients, respectively. One patient exhibited an isolated IgA1 subclass deficiency. The proportion of plasma cells in conventionally stained histological sections of the same intestinal biopsies showed a close correlation with the numbers of IgA$^+$ and IgM$^+$ cells. Considerable numbers of J chain-synthesizing cells were present in all patients with CVID, indicating the presence of early B cells unable to differentiate into immunoglobulin-producing plasma cells. Most of the patients with intestinal IgA and/or IgM defects strongly expressed the SC in their enterocytes, suggesting an immunoglobulin-independent regulation of the SC. Clinically, only CVID patients with intestinal IgA defects developed intestinal infections with *Giardia lamblia*, *Campylobacter jejuni* or *Candida albicans*. The outcome of *in vitro* immunoglobulin synthesis assays with peripheral blood lymphocytes did not predict the presence or absence of the respective isotype-producing B cells in the intestinal lamina propria. Thus, immunohistological examinations of intestinal biopsies are required to determine the extent of mucosal immunodeficiency in CVID patients.

Keywords MALT intestinal immunoglobulins J chain secretory component common variable immunodeficiency

INTRODUCTION

Patients with common variable immunodeficiency (CVID) suffer from idiopathic defects of their antibody production with decreased serum IgG in combination with a decrease of serum IgA and/or IgM [1]. In most patients normal or subnormal levels of circulating B cells are present, but B cell differentiation and antibody secretion are profoundly disturbed [2].

Clinically, patients with CVID develop recurrent bacterial infections, particularly of the respiratory tract. Autoimmune diseases and malignancies of the gastrointestinal tract are relatively frequent [3,4]. Approximately 60% of the patients with CVID show intestinal symptoms like diarrhoea, steatorrhoea, malabsorption and infections with *Giardia lamblia* and other pathogenic microorganisms [5–7]. Histological examination of intestinal biopsies frequently reveals nodular lymphoid hyperplasia and/or villous atrophy [5,7]. It is obvious that most of the intestinal findings and symptoms in patients with CVID are related to defects of the specific mucosa-associated lymphoid tissue (MALT).

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Studies on cryostat sections of gut biopsies from patients with late-onset hypogammaglobulinaemia revealed intestinal IgA$^+$ and IgM$^+$ cells in most patients. Approximately half of the patients produced IgG$^+$ cells in their intestine [8]. In this study we present the immunohistological analysis of mucosal immunoglobulin- and J chain-producing cells and secretory component (SC)-expressing enterocytes in the small intestine of patients with CVID, and compare the results with a control group. Immunohistology with commercially available immunoglobulin MoAbs using the avidin–biotin complex (ABC) method was achieved on formalin-fixed and paraffin-embedded material, assuring good preservation of tissue structures and clear identification of immunoglobulin-positive cells [9]. In particular, our interest was focused on the following questions: (i) are there differences in the relative numbers of intestinal IgA1,2-, IgM- and IgG1–4-synthesizing cells of patients with CVID *versus* controls? (ii) do the levels of serum immunoglobulin correlate with the relative numbers of the respective immunoglobulin-producing cells in the intestine of the patients? (iii) is the J chain synthesis defective? (iv) are there abnormalities in the expression of the SC?

Recently published results [10] of *in vitro* immunoglobulin synthesis of stimulated blood lymphocytes from the same CVID

patients were compared with our *in situ* findings on intestinal immunoglobulin-positive cells.

PATIENTS AND METHODS

Patients

Small intestinal biopsy specimen of 10 female and seven male patients with CVID were available for immunohistological evaluation. The age of the patients ranged from 18 to 82 years, with a mean of 42 years. Hypogammaglobulinaemia was detected analysing the three major immunoglobulin classes. Primary lymphoma, plasmocytoma or other diseases with secondary hypogammaglobulinaemia were excluded. Although the patients received gammaglobulin replacement therapy intravenously, their IgG level in the serum remained below the normal range.

Biopsies with normal histology and without pathologically increased inflammation from the small intestine of 17 female and 15 male patients (mean age 51 years) served as controls. Care was taken that patients with immunological disorders or chronic inflammatory bowel diseases were excluded.

The intestinal biopsies of CVID patients and controls used in the present study were taken for diagnostic purposes as part of the patients' clinical work-up. Informed consent was obtained from each patient and control person.

Microbiological examinations

Giardia lamblia infection was detected microscopically in native preparations of the bile and duodenal juice. *Helicobacter jejuni* enteritis and intestinal candidiasis were diagnosed microbiologically.

Immunohistological methods

Biopsy specimens of CVID patients and controls were routinely fixed in 4% formalin and embedded in paraffin. The immunohistological identification of cytoplasmic immunoglobulin, J chain and SC was done on sections cut at 4 μm using the ABC method [11].

Endogenous peroxidase of the deparaffinized sections was blocked with 0·87% hydrogen peroxide (Merck, Darmstadt, Germany) in 68% methanol. In order to unmask antigen-binding sites, enzymatic digestion with 0·05% proteinase K (Sigma, Deisenhofen, Germany) was performed. Addition of 0·1% bovine serum albumin (BSA; Serva, Heidelberg, Germany) in 10 ml PBS served to avoid non-specific binding of antibodies.

The following primary antibodies and dilutions were used: anti-IgA1 (1:200; Sigma, clone A1-18), anti-IgA2 (1:200; Boehringer, Mannheim, Germany; clone 2E2), anti-IgM (1:100; Dako, Glostrup, Denmark; clone R1/69), anti-IgG1–4 (1:500 to 1:1000; Sigma, clones 8c/6-39, HP-6014, HP-6050 and HP-6025), anti-J chain (1:50; Nordic, Tilbury, The Netherlands) and anti-SC (1:1000; Sigma, clone GA-1). With the exception of anti-J chain, MoAbs were applied. The preparations were treated with biotinylized second antibodies and the ABC reagent from the Vectastain ABC-Kit (Camon, Wiesbaden, Germany). Finally, the sections were counterstained with haematoxylin and covered with glycerine gelatine.

The optimal dilutions of the primary antibodies indicated above were previously determined on formalin-fixed sections of tonsils and intestinal biopsies.

The immunohistological preparations were evaluated semi-quantitatively and the relative numbers of immunoglobulin- or J chain-positive cells were determined in the population of mononuclear cells of the stratum proprium. Two hundred cells were analysed in the villous and crypt region each. Staining intensity of the SC in enterocytes was determined on a three-stage scale.

Estimation of intestinal plasma cells

The proportion of plasma cells in the intestinal lamina propria was determined semiquantitatively in conventional histological preparations stained with haematoxylin and eosin.

Determination of the serum immunoglobulin levels

Serum IgG, IgA and IgM concentrations were determined by nephelometry (Behring AG, Marburg, Germany) according to the manufacturer's recommendations.

Immunoglobulin synthesis in vitro

Stimulated peripheral blood mononuclear cells (PBMC) of 14 CVID patients were tested in parallel by Rump *et al.* [10] for their ability to synthesize IgG and IgM *in vitro*. The method of the stimulation assay and the results have been described in detail [10]. Briefly, the cells were stimulated with pokeweed mitogen (PWM) and/or *Staphylococcus aureus* Cowan I with or without addition of IL-2. The results of the immunoglobulin synthesis *in vitro* and the percentages of intestinal immunoglobulin-positive cells *in situ* were compared.

Statistical analysis

The two sample rank test of Mann and Whitney was used as a two-tailed test to compare the values of CVID patients and controls.

RESULTS

Clinically, all 17 patients showed symptoms and findings characteristic of CVID (Table 1). Serum IgG was below the normal range of controls in all patients, whereas IgA and IgM levels were low in 14 and 11 patients, respectively. A concomitant decrease of all three immunoglobulin classes was found in the serum of 10 patients. All patients suffered from recurrent respiratory tract infections, six showed symptoms of autoimmunity, and one patient died from Hodgkin's disease after an 11-year history of CVID. Gastrointestinal findings included diarrhoea ($n=12$) and malabsorption ($n=1$). Microbiologically, infections with *G. lamblia* ($n=3$), *Campylobacter jejuni* ($n=2$) and *Candida albicans* ($n=2$) were discovered. Morphologically, villous atrophy and nodular lymphoid hyperplasia (NLH) were found in four and seven patients, respectively.

Immunohistological analysis of the small intestine revealed total IgA deficiency in most of the patients as the most conspicuous finding (Fig. 1). This deficiency included both IgA1 and IgA2 subclasses, with the exception of patient 16 showing an isolated IgA1 defect, whereas $IgA2^+$ cells were within the normal range (6·5%). All 12 patients with lack of IgA^+ cells in the small intestine had low serum IgA levels. Conversely, the three patients with normal serum IgA levels exhibited a normal range of IgA1- and IgA2-producing cells in their intestinal mucosa. Interestingly, two patients with low serum IgA had normal percentages of IgA^+ intestinal B cells (Fig. 2a).

Intestinal IgM defects could be detected in 5/17 patients, with a total lack of IgM^+ cells in three cases and percentages of IgM-synthesizing cells below the control range ($< 1·5\%$) in two

Table 1. Clinical symptoms and findings of patients with common variable immunodeficiency (CVID)

Patient no.*	Age	Sex	Defect of serum immunoglobulin†			Non-intestinal findings‡	Gastrointestinal symptoms and findings					
			G	A	M		Infections	Diarrhoea	Malabsorption	NLH	Villous atrophy	Atrophic gastritis
1	32	M	+	+	+	Te, Sm	G.l	+		+		+
2	33	F	+	+	+	Sm		+				
3	27	F	+	+	N	R.d, R.a		+				
4	63	F	+	+	+	Sm, H, H.z						
5	30	M	+	+	+	Con, Sm, H.a	G.l	+		+		
6	46	F	+	+	N	U.tr.i, Vag		+			+	
7	48	M	+	+	+	Sm	G.l, Cand	+			+	
8	64	F	+	N	+							
9	32	M	+	+	N	Sm, H.a, P.a, H.d		+	+		+	+
10	60	F	+	+	+	Sm, P.a		+		+		+
11	29	F	+	+	+	Sm, Te, H.g, H.z		+				
12	82	M	+	N	N							
13	34	M	+	N	N					+		
14	34	M	+	+	+	Con, Pyo	Camp	+		+		+
15	18	F	+	+	N	Fur	Cand	+		+		
16	28	F	+	+	+	U.tr.i, Pyo, Con					+	
17	55	F	+	+	+	Sm	Camp	+		+		

* Patients 12 and 13 with deficiency of serum IgG were included in the present study although they had no defects of serum IgA and IgM. They presented recurrent infections of the respiratory tract, typical for CVID patients.

† Defect of serum immunoglobulin, +; no defect of serum immunoglobulin, N; normal ranges of serum immunoglobulins: 8–18 g/*l* for IgG, 0·9–4·5 g/*l* for IgA, and 0·6–2·8 g/*l* for IgM.

‡ All patients had infections of the respiratory tract, including tonsillitis, sinusitis and mastoiditis.

Camp, *Campylobacter jejuni*; Cand, *Candida albicans*; Con, conjunctivitis; Fur, furunculosis; G.l, *Giardia lamblia*; H, hepatitis; H.a, haemolytic anaemia; H.d, Hodgkin's disease; H.g, herpes genitalis; H.z, herpes zoster; P. a, pernicious anaemia; Pyo, pyodermatitis; R.a, rheumatoid arthritis; R.d, Raynaud's disease; Sm, splenomegaly; Te, tonsillectomy; U.tr.i, infections of the urinary tract; Vag, vaginitis.

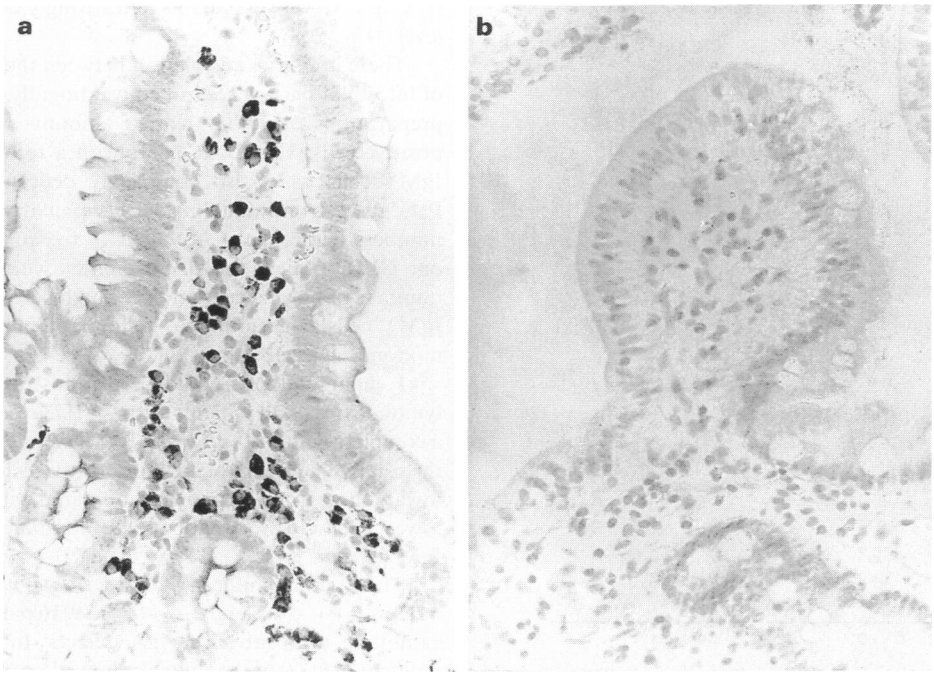

Fig. 1. Immunohistological demonstration of IgA1 in the mucosa of the small intestine. (a) Numerous IgA1$^+$ cells in a control biopsy. (b) Total IgA1 defect in the mucosa of a patient with common variable immunodeficiency (CVID). (× 200.)

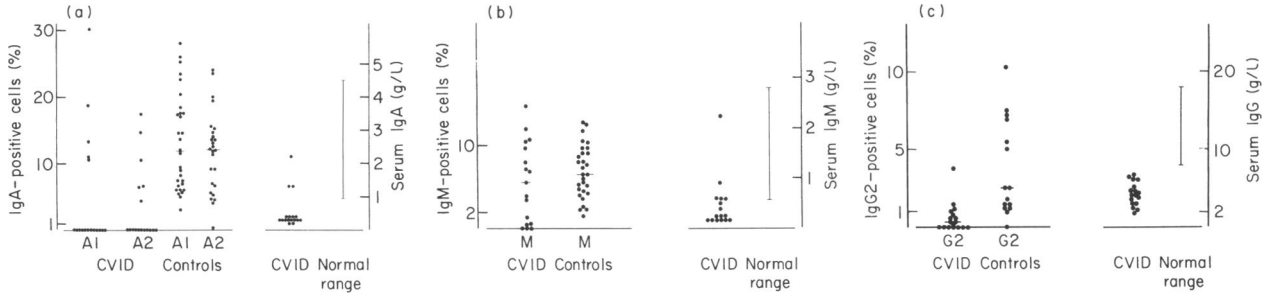

Fig. 2. Percentage of immunoglobulin-positive cells in the small intestine and serum immunoglobulin of common variable immunodeficiency (CVID) patients and controls. (a) IgA. Highly significant differences of the relative numbers of $IgA1^+$ and $IgA2^+$ cells of CVID patients *versus* controls ($P<0.001$). (b) IgM. No significant difference of the relative numbers of IgM-positive cells in both groups. (c) IgG2. Significant differences of the relative numbers of $IgG2^+$ cells of CVID patients *versus* controls (IgG2 cells in the crypt region $P<0.001$, and in the villous region $P<0.05$).

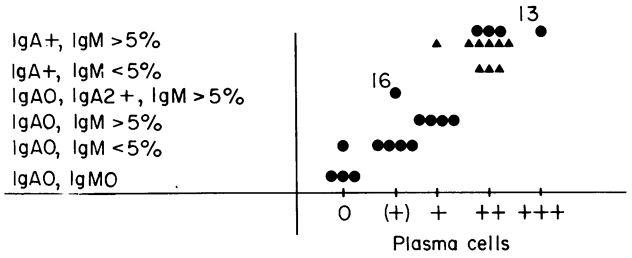

Fig. 3. Defects of IgA^+ and IgM^+ cells in correlation with the numbers of plasma cells in conventionally stained histological preparations of the small intestine. 0, No plasma cells (0%); (+), plasma cells extremely rare (<1%); +, decreased (1–5%); ++, normal (5–30%); +++, increased (>30%) numbers of plasma cells. IgA0, Lack of IgA^+ cells; IgM0, lack of IgM^+ cells. Isolated IgA1 defect was observed in patient no. 16, and increased numbers of IgA^+ and IgM^+ cells totalling 60% of the intestinal mononuclear cells in patient no. 13. ●, Common variable immunodeficiency (CVID); ▲, controls.

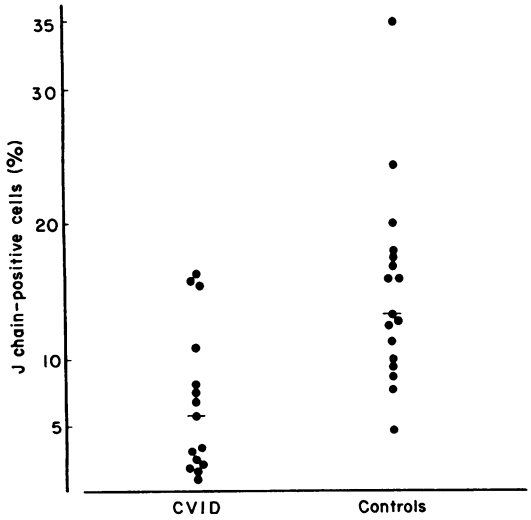

Fig. 4. Percentage of J chain-positive cells in the small intestine of patients with common variable immunodeficiency (CVID) and controls. Significant difference (villous region $P<0.01$; crypt region $P<0.05$).

cases. All five patients had low serum IgM levels. On the other hand, in six additional patients with low serum IgM concentrations significant numbers of intestinal IgM^+ cells could be detected. All CVID patients with normal serum IgM (6/17) exhibited a normal range of IgM^+ cells in their intestine (Fig. 2b).

In the normal intestinal mucosa IgG-synthesizing cells are present in low numbers, most of them lying in the crypt region of the stratum proprium. Immunohistological staining for $IgG1^+$ cells proved to be insufficient, since the IgG1 MoAb failed to yield unequivocal *in situ* signals. In contrast, the antibodies against IgG2, 3 and 4 allowed a clear-cut identification of positive cells. Due to the very low numbers of $IgG3^+$ and $IgG4^+$ cells in control and patient biopsies (<1%), only the results of $IgG2^+$ B cell counts are presented here. In control biopsies $IgG2^+$ cell counts ranged between 0% and 10% with a median of 2·5%, whereas CVID patients exhibited a median of 0·3% (range 0–3·9%). Seven out of 17 CVID patients and one out of 15 controls showed a total lack of $IgG2^+$ cells in the intestinal biopsies. All CVID patients had strongly decreased serum IgG levels (Fig. 2c).

There is a strict correlation between the estimated numbers of intestinal plasma cells in conventionally stained histological preparations and the relative amount of immunoglobulin-positive cells (Fig. 3). Patients with a total lack of IgA^+ and IgM^+ cells were also completely deficient of plasma cells. Patients with intestinal IgA defects regularly showed plasma cell numbers below the ++ values of the controls. Interestingly, one CVID patient (no. 13) presented with increased (+++) numbers of plasma cells and very high percentages of IgA^+ and IgM^+ cells, amounting to a total of 60% of the intestinal mononuclear cells.

J chain synthesis could be demonstrated in the intestinal lymphocytes of all patients with CVID, even in those cases with a complete defect of IgA^+ and IgM^+ cells (Fig. 4). Nevertheless, the relative numbers of J chain-positive cells were significantly lower in the group of patients compared with controls, with the lowest values in those patients with combined intestinal IgA and IgM defect.

The staining intensity of the SC in the enterocytes was moderate (++) to high (+++) in 9/16 patients, seven of them exhibiting total intestinal IgA defects. In contrast, the great majority (15/17) of the controls showed no (0) or only a weak (+ to +/++) immunohistological staining of the SC, only 2/17 controls demonstrating a moderate to high staining intensity.

Table 2. Defects of intestinal immunoglobulin-synthesizing cells of common variable immunodeficiency (CVID) patients in relation to J chain synthesis, expression of the secretory component (SC) and gastrointestinal findings

Category	No. of patients	Intestinal immune system*				No. of patients with gastrointestinal infections			No. of patients with NLH	No. of patients with gastrointestinal symptoms and findings
		IgA	IgM	J chain	Secretory component	G.1	Camp	Cand		
CVID I	5	—	—	3 (2–6)	2·0 (2–3)	2	2	1	3	5
CVID II	6	—	6 (2–12)	4 (1–16)	1·5 (0–3)	1		1	3	5
CVID III	6	21 (7–40)	10 (6–15)	11 (7–16)	1·0 (0–3)				1	4

* Median value of the relative number (%) of positive cells (IgA1-2, IgM, J chain) and of the staining intensity of the secretory component. Camp, *Campylobacter jejuni*; Cand, *Candida albicans*; G.1, *Giardia lamblia*; NLH, nodular lymphoid hyperplasia.

Table 3. Comparison of *in situ* cytoplasmic immunoglobulin synthesis of intestinal B cells and immunoglobulin secretion *in vitro* of stimulated blood lymphocytes* of common variable immunodeficiency (CVID) patients

Patient no.	Intestinal IgG2-positive cells†	IgG secretion of blood lymphocytes‡	Intestinal IgM-positive cells§	IgM secretion of blood lymphocytes‡
1	+	−	−	+
2	−	−	−	+
3	+	−	+	−
4	+	+	+	+
5	−	−	+	−
6	+	−	+	−
7	+	−	−	−
8	+	NT	+	NT
9	+	NT	+	NT
10	−	−	+	−
11	−	−	+	+
12	+	NT	+	NT
13	+	+	+	+
14	−	−	−	−
15	−	+	+	+
16	−	+	+	+
17	+	−	−	−

* The lymphocytes were stimulated with pokeweed mitogen and/or *Staphylococcus aureus* Cowan I suspension with or without addition of IL-2 as indicated by Rump *et al.* [10].

† +, Immunoglobulin-positive cells present; −, immunoglobulin-positive cells absent.

‡ +, Immunoglobulin secretion; −, no immunoglobulin secretion.

§ +, IgM-positive cells $\geq 1{\cdot}5\%$; −, IgM-positive cells $< 1{\cdot}5\%$.

NT, Not tested.

Table 2 summarizes the main results of the immunohistological evaluation, classifying the patients into three categories of their intestinal humoral immunodeficiency: I, patients with defective IgA and IgM synthesis; II, patients with normal IgM^+ but with lack of IgA^+ cells; and III, patients exhibiting sufficient IgA and IgM synthesis. Intestinal infections with *G. lamblia*, *Camp. jejuni* and *C. albicans* occurred only in groups I and II of intestinal immunodeficiency. Patients without gastrointestinal symptoms were all classified in groups II and III. Nodular lymphoid hyperplasia occurred in all three stages of intestinal immunodeficiency, but with a preponderance in groups I and II.

The results of *in vitro* immunoglobulin synthesis assays with blood lymphocytes do not correlate with the frequency of $IgG2^+$ or IgM^+ cells in the intestine (Table 3). Defects of intestinal $IgG2^+$ and IgM^+ cells corresponded with the absence of IgG and IgM secretion of blood lymphocytes in no more than 5/14 and 3/14 patients, respectively. On the other hand, presence of intestinal $IgG2^+$ and IgM^+ cells correlated with a positive *in vitro* secretion of the respective isotype in 2/14 and 5/14 cases.

DISCUSSION

With the introduction of the ABC method and the use of MoAbs, immunohistology on formalin-fixed and paraffin-embedded tissues has been greatly improved [9]. The results of

the plasma cell determinations in conventionally stained intestinal preparations can be regarded as a control for the numbers of IgA^+ and IgM^+ cells detected by immunohistology (Fig. 3). There is, indeed, an excellent correlation between the presence or absence of IgA^+ and IgM^+ cells and the estimated numbers of plasma cells, confirming the reliability of our immunohistological technique.

Total defects of intestinal IgA^+, IgM^+ and $IgG2^+$ B cells were observed in 65%, 18% and 41% of our patients with CVID, respectively. In general, subnormal numbers or a total lack of intestinal immunoglobulin-positive cells invariably correspond to subnormal serum concentrations of the respective isotypes. On the other hand, subnormal serum immunoglobulin values did not always correlate with defects of the respective immunoglobulin-synthesizing cells in the small intestine. Thus, low serum immunoglobulin and normal intestinal B cell counts were observed for IgA in two patients, IgM in six patients and IgG2 in 10 patients. Indeed, there is evidence that serum immunoglobulin and secretory immunoglobulin from the intestine represent different and possibly independent compartments of the immune system. As to the intestinal IgA and IgM, these immunoglobulin classes are secreted as dimers and pentamers, respectively, bound to the SC [12–14], whereas serum IgA is mainly monomeric and shows a predominance of the IgA1 subclass compared with IgA in secretions [15,16]. Immunoglobulin synthesis assays *in vitro* using peripheral blood lymphocytes were carried out to determine the degree of immunodeficiency in CVID patients [10,17]. By comparing the results of the IgG and IgM stimulation assays and the capacity of intestinal B cells to produce cytoplasmic immunoglobulin, we were unable to detect a correlation (Table 3). Thus, *in vitro* stimulation results of blood lymphocytes seem to be of no predictive value for the extent of intestinal immunodeficiency in CVID. Therefore, immunohistological examinations of intestinal biopsies are necessary to determine the deficiency pattern of this immunologic compartment.

Considering the IgA and IgM defects of the intestine, three stages of intestinal immunodeficiency can be distinguished: I, total IgA and IgM defect ($n=5$); II, IgA defect but presence of IgM-synthesizing cells ($n=6$); and III, simultaneous IgA and IgM synthesis ($n=6$). Stage I and stage II patients are highly susceptible to intestinal infections and other intestinal manifestations of the CVID syndrome. Indeed, all patients with pathogenic intestinal organisms belonged to stages I or II of intestinal immunodeficiency, whereas the only three patients without gastrointestinal symptoms belonged to stages II and III (Table 2). Nodular lymphoid hyperplasia, obviously representing a response to gut antigens [18], occurred in all stages of intestinal immunodeficiency, with a clear preponderance in stages I and II. J chain synthesis was present in lamina propria cells of all CVID patients irrespective of the intestinal immunodeficiency grading. The lowest number of J chain-positive cells was found in patients exhibiting a total IgA and IgM defect. The onset of J chain synthesis is an early event in B cell differentiation, starting before cytoplasmic immunoglobulin production [12,14,19]. Thus, our *in situ* finding of J chain synthesis without concomitant IgA and IgM production indicates defects of B cell differentiation and immunoglobulin secretion, although considerable numbers of B cells are present. These data confirm earlier results of Saiki *et al.* [20] describing differentiation defects of blood lymphocytes in CVID.

Surprisingly, CVID patients show a high staining intensity of their enterocytes for SC in those cases lacking IgA- and IgM-secreting cells in the lamina propria. Similar results have been shown in patients with selective IgA deficiency [16,21]. Obviously, the SC represents a constitutive component of enterocytes with additional functions that remain to be elucidated [22].

The pathogenesis of the defective immunoglobulin synthesis is unknown in most cases of CVID. Primary disturbances of terminal B cell differentiation and immunoglobulin secretion have been assumed to be present in CVID [20]. On the other hand, there is increasing evidence that a considerable number of patients with CVID exhibit T cell defects and abnormalities in lymphokine production that disturb normal B cell differentiation and regulation. In particular, decrease of $CD4^+$ cells and/or an increased number of activated $CD8^+$ cells might be the cause of disturbed B cell function in CVID [23–25]. In many cases of CVID a defective IL-2 function was detected [10,26]. In addition, macrophage abnormalities leading to disturbed T cell activation might be present as well [27].

Additional *in situ* studies including T cell-specific monoclonals are required to examine disturbed T and B cell interactions in the local immune response systems of patients with CVID. One of the few *in situ* studies of the intestinal immune system revealed high numbers of intestinal $CD8^+$ cells in the stratum proprium and in the follicular regions of CVID patients with nodular lymphoid hyperplasia, indicating increased $CD8^+$ suppressor cell activity as a possible cause of the inhibition of B cell differentiation in the intestine [28].

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REFERENCES

1 Primary immunodeficiency diseases. Report of a WHO sponsored meeting. Immunodeficiency Rev 1989; **1**:173–205.

2 Saxon A, Giorgi JV, Sherr EH, Kagan JM. Failure of B cells in common variable immunodeficiency to transit from proliferation to differentiation is associated with altered B cell surface-molecule display. J Allergy Clin Immunol 1989; **84**:44–55.

3 Kinlen LJ, Webster ADB, Bird AG, Haile R, Peto J, Soothill JF, Thompson RA. Prospective study of cancer in patients with hypogammaglobulinaemia. Lancet 1985; **I**:263–6.

4 Cunningham-Rundles C. Clinical and immunologic analyses of 103 patients with common variable immunodeficiency. J Clin Immunol 1989; **9**:22–33.

5 Hermans PE, Diaz-Buxo JA, Stobo JD. Idiopathic late-onset immunoglobulin deficiency. Clinical observations in 50 patients. Am J Med 1976; **61**:221–37.

6 Peter HH, Simon C. Zelluläre und humorale Immundefizienz. In: Simon C, ed. Klinische Pädiatrie. Ein Lehrbuch der Kinderheilkunde, 5th edn. Stuttgart: Schattauer, 1986:589–622.

7 Bästlein C, Burlefinger R, Holzberg E, Voeth C, Garbrecht M, Ottenjann R. Common variable immunodeficiency syndrome and nodular lymphoid hyperplasia in the small intestine. Endoscopy 1988; **20**:272–5.

8 Broom BC, de la Concha EG, Webster ADB, Loewi G, Asherson GL. Dichotomy between immunoglobulin synthesis by cells in gut and blood of patients with hypogammaglobulinaemia. Lancet 1975; **II**:253–6.

9 Norton AJ, Isaacson PG. Detailed phenotypic analysis of B-cell lymphoma using a panel of antibodies reactive in routinely fixed wax-embedded tissue. Am J Pathol 1987; **128**:225–40.
10 Rump JA, Jahreis A, Schlesier M, Dräger R, Melchers I, Peter HH. Possible role of IL-2 deficiency for hypogammaglobulinaemia in patients with common variable immunodeficiency. Clin Exp Immunol 1992; **89**:204–10.
11 Naish SJ, ed. Handbuch immunchemischer Färbemethoden II. Dako Corporation, 1989.
12 Brandtzaeg P, Korsrud FR. Significance of different J chain profiles in human tissues: generation of IgA and IgM with binding site for secretory component is related to the J chain expressing capacity of the total local immunocyte population, including IgG and IgD producing cells, and depends on the clinical state of the tissue. Clin Exp Immunol 1984; **58**:709–18.
13 Ahnen DJ, Brown WR, Kloppel TM. Secretory component: the polymeric immunoglobulin receptor. What's in it for the gastroenterologist and hepatologist? Gastroenterology 1985; **89**:667–82.
14 Koshland ME. The coming of age of the immunoglobulin J chain. Ann Rev Immunol 1985; **3**:425–53.
15 Mestecky J, Russell MW. Subclasses in other immunoglobulins. IgA subclasses. Monogr Allergy 1986; **19**:277–301.
16 Underdown BJ, Schiff JM. Immunoglobulin A: strategic defence initiative at the mucosal surface. Ann Rev Immunol 1986; **4**:389–417.
17 Bryant A, Calver NC, Toubi E, Webster ADB, Farrant J. Classification of patients with common variable immunodeficiency by B cell secretion of IgM and IgG in response to anti-IgM and interleukin-2. Clin Immunol Immunopathol 1990; **56**:239–48.
18 Nagura H, Kohler PF, Brown WR. Immunocytochemical characterization of the lymphocytes in nodular lymphoid hyperplasia of the bowel. Lab Invest 1979; **40**:66–73.
19 Kelényi G. Intracellular J chains in lymphoproliferative diseases. Virchows Arch (Pathol Anat) 1985; **405**:365–78.
20 Saiki O, Ralph P, Cunningham-Rundles C, Good RA. Three distinct stages of B-cell defects in common varied immunodeficiency. Proc Natl Acad Sci USA 1982; **79**:6008–12.
21 Dura WT, Bernatowska E. Secretory component, alpha-1-antitrypsin and lysozyme in IgA deficient children. An immunohistochemical evaluation of intestinal mucosa. Histopathology 1984; **8**:747–57.
22 Brandtzaeg P, Nilssen DE, Rognum TO, Thrane PS. Ontogeny of the mucosal immune system and IgA deficiency. Gastroenterol Clin North Am 1991; **20**:397–439.
23 Wright JJ, Wagner DK, Blaese RM, Hagengruber C, Waldmann TA, Fleisher TA. Characterization of common variable immunodeficiency: identification of a subset of patients with distinctive immunophenotypic and clinical features. Blood 1990; **76**:2046–51.
24 Lebranchu Y, Thibault G, Degenne D, Bardos P. Abnormalities in $CD4^+$ T lymphocyte subsets in patients with common variable immunodeficiency. Clin Immunol Immunopathol 1991; **61**:83–92.
25 Baumert E, Wolff-Vorbeck G, Schlesier M, Peter HH. Immunophenotypical alterations in a subset of patients with common variable immunodeficiency (CVID). Clin Exp Immunol 1992; **90**:25–30.
26 Sneller MC, Strober W. Abnormalities of lymphokine gene expression in patients with common variable immunodeficiency. J Immunol 1990; **144**:3762–9.
27 Fiedler W, Sykora KW, Welte K *et al.* T-cell activation defect in common variable immunodeficiency: restoration by phorbol myristate acetate (PMA) or allogeneic macrophages. Clin Immunol Immunopathol 1987; **44**:206–18.
28 Van den Brande P, Geboes K, Vantrappen G, van den Eeckhout A, Vertessen S, Stevens EAM, Ceuppens JL. Intestinal nodular lymphoid hyperplasia in patients with common variable immunodeficiency: local accumulation of B and CD8(+) lymphocytes. J Clin Immunol 1988; **8**:296–306.