Clin. exp. Immunol. (1986) **67**, 626–636

The clinical condition of IgA-deficient patients is related to the proportion of IgD- and IgM-producing cells in their nasal mucosa

P. BRANDTZAEG, G. KARLSSON,* G. HANSSON,† B. PETRUSON,* J. BJÖRKANDER‡ & L. Å. HANSON§ *Laboratory for Immunohistochemistry and Immunopathology, Institute of Pathology, University of Oslo, The National Hospital, Rikshospitalet, Oslo, Norway, * Department of ENT, Sahlgren's Hospital, University of Göteborg, † Department of Pathology, Östra Hospital, Göteborg, ‡ Asthma and Allergy Research Centre, Sahlgren's Hospital, University of Göteborg, § Department of Clinical Immunology, University of Göteborg, Göteborg, Sweden*

(Accepted for publication 13 August 1986)

SUMMARY

Nasal biopsy specimens from 15 adult patients with selective IgA deficiency but normal IgG-subclass levels were examined by immunohistochemistry for the presence of immunocytes producing various Ig isotypes. The mucosal samples were completely IgA-deficient except in two cases where 0·9% and 8·4% IgA cells were found, respectively (normal, 69·8%). Numerous IgG- (mainly IgG1-) producing cells were present in 10 samples; in five of these there were additional IgM- but virtually no IgD-producing cells, whereas in the other five a marked dominance of the IgD over the IgM isotype was seen. The latter category of patients had more upper airways infections (recurrent acute rhinosinusitis, otitis media, and tonsillitis) than the former, who had no recurrent upper respiratory tract infections except one patient with recurrent acute rhinosinusitis. The five remaining samples, which contained very few Ig-producing cells, were derived from patients with even more frequent infections than those showing IgD predominance. Our results indicate that IgM acts as a compensatory secretory Ig in the upper respiratory tract of some IgA-deficient subjects. However, immunoregulatory events favouring local IgD responses apparently do not support mucosal defence satisfactorily, either because local production of IgM is hampered or because IgD (which is not a secretory Ig) blocks complement-dependent reactions mediated by IgG and IgM antibodies within the mucosa.

Keywords immunoglobulin-producing cells IgD IgM IgA deficiency nasal mucosa

INTRODUCTION

Optimal mucosal surface protection in the respiratory tract depends on acquired specific immunity afforded by the secretory immune system (Brandtzaeg, 1984). The secretory immunoglobulin IgA and IgM antibodies function by aggregating and neutralizing antigens. In nasal mucosa Ig production is mediated by two immunocyte populations more or less separate: one in the glandular areas normally dominated by IgA cells and another in the stroma beneath the surface epithelium often dominated by IgG cells (Brandtzaeg, 1985a). Both populations include IgM- and IgD-producing cells in quite low numbers, although there normally are relatively more IgD

Correspondence: Professor Per Brandtzaeg, LIIPAT, Rikshospitalet, 0027 Oslo 1, Norway.

immunocytes in the glandular areas of nasal mucosa than in any other secretory tissue site (Brandtzaeg *et al.*, 1979; Brandtzaeg, 1983a).

It has been postulated that increased susceptibility to infections of the upper airways as seen in some patients with selective IgA deficiency may be explained by local compensation with IgD instead of IgM and hence decreased surface protection (Brandtzaeg, 1984). IgD does not combine with the epithelial secretory component (SC) and, therefore, should not be considered a true secretory Ig (Brandtzaeg, 1977). Conversely, patients who compensate for the lack of IgA with a raised number of IgM-producing cells, as in the gut, might be expected to preserve their mucosal protection better because IgM is actively secreted by an SC-dependent transport mechanism (Brandtzaeg, 1985b).

The aim of the present investigation was to study the IgM and IgD immunocyte populations in nasal mucosa of patients with selective IgA deficiency and to relate the isotype distribution to the history of infections in the upper airways. IgG-producing cells were also studied and characterized with regard to subclass distribution.

MATERIAL AND METHODS

Patients. Fifteen subjects (ten women and five men; mean age, 36·5 years and range, 17–64 years) of a group of 18 clinically well-characterized IgA-deficient patients (Karlsson *et al.*, 1985a) were included in this study. They were referred to hospital mainly because of repeated infections of the respiratory tract; a few had inflammatory bowel disease or rheumatoid arthritis. Informed consent was obtained from all patients and the study was approved by the Ethic's Committee of the University of Göteborg.

Clinical examinations. All patients were subjected to a general ear, nose and throat (ENT) examination complemented by a questionnaire, an interview, and checking of earlier ENT records. Furthermore, fibre-endoscopic examination of the nasal cavities was performed in all patients and unilateral sinoscopy of a maxillary sinus in nine patients. Sinus roentgenogram or ultrasound examination of the paranasal sinuses was also carried out as well as rhinomanometry as described previously (Karlsson *et al.*, 1985a).

Recurrent rhinosinusitis was defined as more than one purulent infection per year confirmed by sinus roentgenogram or sinus lavage. A diagnosis of recurent acute otitis media required a red, swollen and bulging eardrum and/or purulent discharge from the ear, at least twice a year and confirmed by a physician. Recurrent acute tonsillitis was defined as a sore throat combined with fever ($>38°C$) for more than 3 days; the patient had had to be seen by a physician who recommended treatment with antibiotics because of that diagnosis at least twice a year.

Serum Ig quantifications. All Ig determinations except IgE were made by single radial immunodiffusion according to Mancini, Carbonara & Heremans (1965) using anti-IgA and anti-IgM (Hyland Laboratories, Costa Mesa, California, USA), anti-IgG and anti-IgD (Behringwerke AG, Marburg-Lahn, West Germany), and the Behring standards. IgD was measured with reference to the British Research Standard 67/37 which was determined to contain 1·76 μg IgD/U by comparison with a purified IgD myeloma protein (Brandtzaeg, Surjan & Berdal, 1978). IgE was quantified with Phadebas IgE PRIST (Pharmacia AB, Uppsala, Sweden). IgG subclasses were measured by an electroimmunoassay (Oxelius, 1978).

Cell-mediated immunity. An index for cell-mediated immunity was calculated from results of testing *in vitro* as described elsewhere (Karlsson *et al.*, 1985b). Briefly, the index was based on lymphocyte stimulation with concanavalin A and phytohaemagglutinin performed in parallel with four normal controls. The index obtained for each patient appears in Table 1.

Immunohistochemistry. Biopsy specimens were excised from the lower edge of the inferior turbinate at least 4 cm from the nasal tip. In nine patients an additional mucosal sample was obtained during sinoscopy from one of the maxillary sinuses. The specimens were immediately placed in ice-cold isotonic phosphate-buffered saline (PBS, pH 7·2) and further processed by washing in PBS for 48 h at 4°C, fixed in ethanol and embedded in paraffin (Brandtzaeg, 1974). Serial sections were cut at 6 μm.

Table 1. Serum concentrations of IgG and IgG subclasses, IgM, IgA, IgD (all in g/l), IgE (kU/l), and index for cell-mediated immunity (CMI) in 15 patients with selective IgA deficiency (normal ranges in brackets)

Patient no.	IgG (7–17)	IgG1 (4·22–12·92)	IgG2 (1·17–7·47)	IgG3 (0·41–1·28)	IgG4 (0–2·91)	IgM (0·5–1·7)	IgA (0·5–3·5)	IgD (<0.002–0·350)	IgE (<5–115)	CMI (0·73–1·27)
1	13·9	10·54	4·83	1·02	0	1·7	0	<0.002	110	1·05
2	15·2	8·50	3·16	0·88	0·18	1·0	0	<0·002	<5	0·65
3	18·7	7·48	4·33	0·79	0	1·1	0	0·174	<5	1·45
4	13·3	11·02	5·16	1·06	0	2·2	0	<0·002	<5	1·05
5	10·0	8·84	5·13	1·97	0·54	2·9	0	<0·002	<5	1·40
6	23·0	18·60	4·83	1·58	1·27	0·8	0	0·410	9	1·20
7	15·5	12·92	3·66	1·39	0·32	0·7	0	0·118	17	1·00
8	10·7	9·18	2·73	0·64	0	2·2	0	<0·002	<5	0·90
9	15·0	8·84	3·23	1·06	0·32	1·0	0	<0·002	110	1·40
11	13·3	10·54	3·20	0·44	0·44	0·9	0	0·004	<5	1.40
12	10·5	6·19	2·33	0·45	0	1·9	<0·05	<0·002	<5	0·80
13	12·9	11·22	1·33	0·58	0	2·7	0	<0·002	<5	1·30
14	14·2	9·52	4·32	0·61	0·94	1·1	0	<0·002	65	1·00
15	12·7	10·88	5·00	0·77	ND	0·6	0	0·058	20	0·90
16	29·0	13·26	6·66	0·91	ND	2·8	0	0·216	<5	0·75

ND, Not done.

Paired immunofluorescence staining was performed with rabbit IgG fluorochrome reagents specific for the five human Ig classes and a sheep conjugate specific for SC; these reagents were prepared in our laboratory and applied for 20 h in various combinations at concentrations reported elsewhere (Brandtzaeg, 1981). For detection of IgG subclasses, the sections were first incubated with a murine monoclonal antibody (ascites 1:800) for 20 h, and then with a mixture of fluorescein-labelled rabbit anti-mouse IgG and rhodamine-labelled anti-human IgG (Brandtzaeg *et al.*, 1986). The IgG-subclass specific monoclonal antibodies were selected on the basis of an international collaborative study (Jefferis *et al.*, 1985) and comprised anti-IgG1 (clone 2C7), anti-IgG2 (clone GOM2), anti-IgG3 (clone CB1-AH7), and anti-IgG4 (clone RJ4). IgA subclasses were demonstrated by a similar paired staining procedure as described previously (Kett *et al.*, 1986).

Sections of specimens with only small numbers of Ig-producing cells were stained with monoclonal antibodies to leucocyte common antigen (LCA) and B cells obtained from Dakopatts (Copenhagen, Denmark); the reagents were applied for 20 h at 1:20 and visualized with a biotin–avidin system (Brandtzaeg & Rognum, 1983).

Microscopy. Sections were evaluated in a Leitz Orthoplan fluorescence microscope with a Ploem-type epi-illuminator without knowledge of the immunological or clinical condition of the patients. Ig-producing immunocytes with distinct cytoplasmic staining were counted. Their isotype distribution was determined with two conjugate combinations (rhodamine-labelled anti-IgM + fluorescein-labelled anti-IgG; fluorescein-labelled anti-IgM + rhodamine-labelled anti-IgD) and the counts for each specimen were based on 100–2000 positive cells (median, 363). The subclass distribution of IgG-producing immunocytes was based on evaluation of 136–1640 cells per specimen (median, 599).

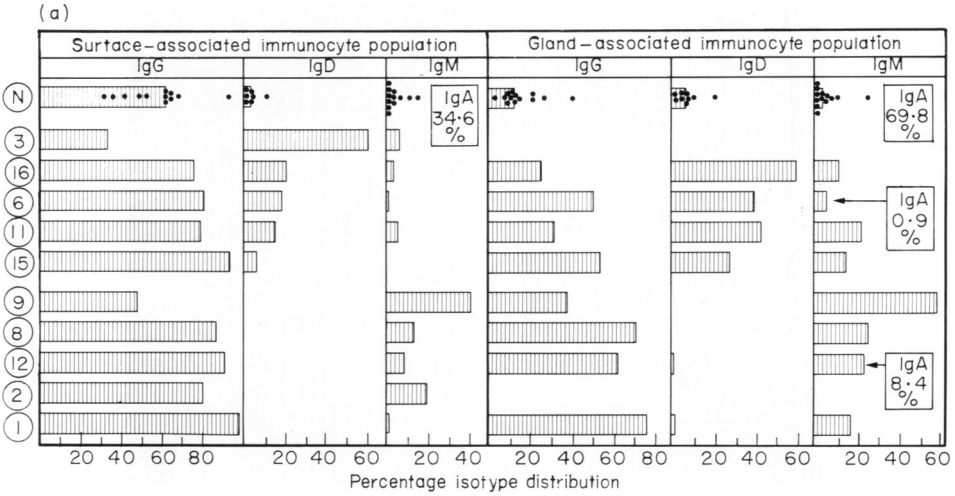
Control material. Control specimens of nasal mucosa were obtained from 13 subjects who apparently were immunologically intact; the isotype distribution of Ig-producing immunocytes in this material has been reported previously (Brandtzaeg, 1985a).

Statistical analysis. Data are presented as medians and statistical comparisons were based on the non-parametric Mann-Whitney test (one-tailed).

RESULTS

General immunohistochemical observations. Five of the nine sinus specimens and 14 of the 15 nasal specimens were satisfactory for immunohistochemistry. Both sinus and nasal mucosa could be evaluated for five subjects and the results were comparable. Immunocyte counts (Fig. 1a) were generally performed on nasal mucosa except for patient No. 3 (quality of sinus specimen was better) and No. 9 (unacceptable nasal specimen).

(a)



(b)

Pt No.	Age (yrs)	Sex	Response category	Rec. rh.sin.	Rec. AOM	Rec. ac.tons.	Tonsillectomy	Rec. LRTI
4	48	♀	Poor local Ig production	■		■	■	
5	41	♀		■		■	■	
7	52	♂			■			■
13	21	♀		■	■			
14	17	♂		■	■		■	■
3	29	♀	Prominent local IgD production	■				■
16	22	♀		■	■		■	
6	59	♂				■		
11	30	♀		■				
15	42	♂						
9	26	♂	Prominent local IgM production					
8	64	♀						
12	25	♀						
2	41	♀						
1	30	♀		■				

Fig. 1. (a) Percentage distribution of IgG-, IgD- and IgM-producing mucosal immunocytes in control subjects with a normal immune system (N) and in 10 patients with IgA deficiency (each patient's No. encircled on the left). The percentages of IgA immunocytes for the control material and for two of the IgA-deficient patients (Nos 6 and 12) are indicated in boxes on the right. All specimens were from nasal mucosa except in Nos 3 and 9 in whom paranasal sinus mucosa was examined. Surface- and gland-associated immunocyte populations were enumerated separately in all except Nos 3 and 2; the former specimen lacked a gland-associated population and in the latter the two populations could not be distinguished. (b) Clinical findings in the respiratory tract of 15 adult patients with selective IgA deficiency categorized according to local Ig production as observed by immunohistochemistry. For comments on patient No. 15, see Results. Rec. rh. = recurrent rhinosinusitis, Rec. AOM = recurrent acute otitis media, Rec. ac. tons. = recurrent acute tonsillitis, Rec. LRTI = recurrent lower respiratory tract infections.

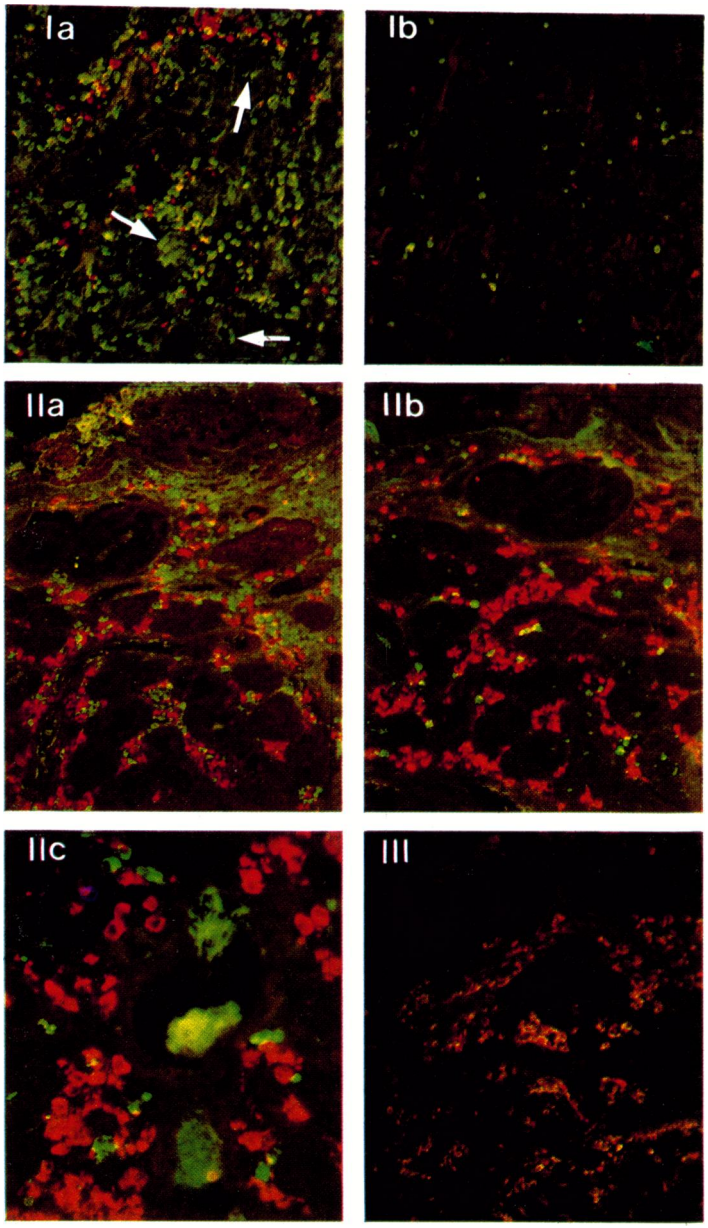


Fig. 2. I. Immunofluorescence staining for IgG, IgA, IgM, and IgD in control specimen of nasal mucosa. Two comparable fields in glandular area are illustrated. (a) Paired staining for IgG (red) and IgA (green) shows marked predominance of immunocytes producing the latter isotype and selective uptake of IgA in acinar and ductal epithelium (arrows). Note that most IgG-producing cells are located at the top of the field which is close to the subepithelial stroma. (b) Paired staining for IgM (red) and IgD (green) in adjacent section. Note that only rare cells normally produce the former isotype in nasal mucosa. (Double-exposed colour slides: × 88.) **II.** Immunoflurescence staining for IgG, IgM, and IgD in nasal mucosa of an IgA-deficient patient (No. 6). Surface epithelium is at the top; some of the luminal cells show Ig uptake. (a) Paired staining for IgD (red) and IgG (green) shows preponderance of immunocytes producing the latter isotype in the superficial stroma whereas IgD-producing cells predominate in the glandular area, particularly in the deeper parts. (b) Paired staining for IgD (red) and IgM (green) in comparable field from adjacent section. Note striking predominance of IgD- over IgM-producing cells. (c) Larger magnification of field from glandular area of latter section; note selective accumulation of IgM in glandular lumen despite predominance of juxtaposed IgD-producing cells. (Double-exposed colour slides: a, b × 88, c × 108.) **III.** Paired immunofluorescence staining for IgG (red) and IgG1 (green) in nasal mucosa of an IgA-deficient patient (No. 6). Surface epithelium showing some IgG-uptake is at the top. Note that most IgG-producing cells are of this subclass as indicated by the yellow (mixed) colours. The purely red immunocytes represent other subclasses. (Double-exposed colour slide: × 88.)

IgA-producing cells were found in low numbers in two specimens (Nos 6 and 12) whereas the remainder completely lacked such immunocytes (Fig. 1a). A normal distribution of SC was found in epithelial elements of all specimens. One specimen (No. 6) contained a few IgE-producing immunocytes, and IgE-bearing mast cells were seen in three (Nos 1, 9, and 15).

A satisfactory number of IgG-, IgM-, and IgD-producing cells ($\geqslant 100$) was available for quantification of cytoplasmic isotype distribution by paired immunofluorscence staining in ten specimens. It was usually possible to count immunocytes both in the stroma beneath the surface epithelium and in the glandular area deeper in the mucosa. Most of the Ig-producing cells were generally found in the latter, the median ratio between immunocyte counts available for the two populations being 1·6 (range, 0·7–4·6). As the cells were unevenly distributed in both locations, no attempt was made to determine the actual immunocyte density; but it appeared to be quite comparable to that seen in control specimens containing IgA-producing cells (Fig. 2 I and II a, b). No gland-associated immunocyte population was available for one of the included sinus specimens (No. 3; Fig. 1a); and in one nasal specimen it was not possible to distinguish the two populations so all immunocytes were categorized as surface-associated (No. 2; Fig. 1a).

In these 10 specimens IgG-producing cells generally predominated in both the surface-associated and the gland-associated immunocyte population, particularly in the former (Figs. 1a, and 2IIa). In five specimens (Nos 3, 16, 6, 11, and 15) there was a predominance of IgD over IgM immunocytes, whereas in the other five (Nos 9, 8, 12, 2, and 1) IgD-producing cells were virtually lacking. In the remaining material paired staining resulted in a total of only 8–60 Ig-producing cells per specimen in four (Nos 4, 5, 7 and 14) and none in one (No. 13) which, however, lacked the gland-associated stroma.

On the basis of these immunohistochemical findings the material was categorized into three groups: (1) Prominent local IgD production ('D group'); (2) prominent local IgM production ('M group'); and (3) poor local Ig production.

Prominent local IgD production. This was found in 'D group' Nos 3, 16, 6, 11 and 15 (Fig 1a). The gland-associated immunocyte population was composed of 42% IgG, 43% IgD, and 14% IgM cells (Fig. 2IIa, b). These percentages were significantly higher than the control values of 14% IgG ($P<0·01$), 8·0% IgD ($P<0·001$) and 2·2% IgM ($P<0·03$) cells mainly because IgA cells were lacking but, compared with IgG, there was a disproportionate shift (1·8–2·2 times) towards IgD and IgM production.

The surface-associated immunocyte population was composed of 79% IgG, 19% IgD, and 3·8% IgM cells (Fig. 2 IIa, b). Again these median percentages were higher than the control values (62% IgG, 3·3% IgD, and 0·6% IgM cells) although significantly so only for IgD ($P<0·003$), and there was a shift (4·4–5·0 times) towards IgD and IgM production compared with IgG.

Epithelial staining for IgM, mainly apically in the surface epithelium, was seen in three specimens (Nos 6, 11 and 15) as sign of SC-mediated external transport. Also, when secretory material was preserved in duct lumens, there was selective staining for IgM despite a striking dominance of IgD- over IgM-producing cells in the adjacent immunocyte population (Fig. 2IIc).

Prominent local IgM production. This was found in 'M group' Nos 9, 8, 12, 2, and 1 (Fig. 1a). The gland-associated immunocyte population was composed of significantly more IgG (69%, $P<0·001$) and IgM (26%, $P<0·01$) cells but fewer IgD cells (1%, $P<0·01$) than in the controls. Compared with the 'D group' the proportion of IgM cells was significantly higher ($P<0·03$) and that of IgD cells lower ($P<0·01$).

The surface-associated immunocyte population was likewise composed of significantly more IgG (86%, $P<0·03$) and IgM (14%, $P<0·01$) cells but fewer IgD cells (none detected) than in the controls. The percentage of IgM cells was significantly higher ($P<0·02$) than in the 'D group'. Signs of SC-mediated IgM transport were seen in the surface epithelium of all specimens in the 'M group'.

Subclass distribution of Ig-producing cells. Since there was no apparent difference in subclass distribution between surface- and gland-associated IgG-producing cells (Fig. 2III), the two immunocyte populations were considered collectively (Fig. 3). Most of the IgG cells were of the IgG1 subclass with overlapping results in controls and IgA-deficient specimens. However, the proportion of IgG1 cells was significantly higher ($P<0·03$) in the 'D group' than in the 'M group'. Moreover, there were more IgG2 cells in the IgA-deficient specimens ($P<0·05$), especially in the 'M

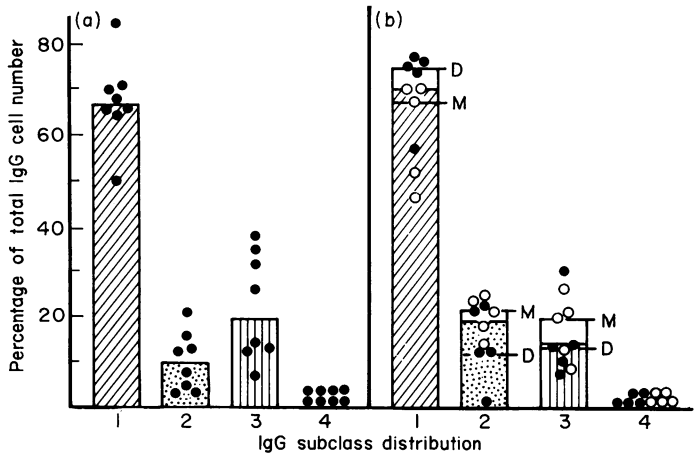


Fig. 3. Distribution of mucosal immunocytes producing the four IgG subclasses in percentage of the total number of IgG-producing cells. (a) Control specimens of nasal mucosa. (b) Specimens from Iga-deficient patients. Hatched or dotted columns represent medians for all specimens. Medians for specimens with pronounced local IgD production (D: closed symbols) or IgM production (M: open symbols) are indicated by horizontal lines.

group' ($P < 0{\cdot}01$). The mucosa of one patient in the 'D group' (No. 3) lacked IgG2 cells, but his serum contained a normal level of this subclass (arrowed in Fig. 4).

In the specimen (No. 12) that contained 8·4% IgA-producing cells, 93% of them were of the IgA1 subclass, which is well within the normal range (Kett *et al.*, 1986). The subclass distribution of the rare IgA cells (0·9%) found in specimen No. 6 could not be determined.

Poor local Ig production. This was found in Nos 4, 5, 7, 13 and 14. The scanty immunocytes found in these specimens showed the following tentative isotype distribution: No. 4, 75% IgG and 25% IgM cells; No. 5, 81% IgG and 19% IgM cells; No. 7, 75% IgD and 25% IgM cells; and No. 14, 48% IgG, 39% IgD and 13% IgM cells (No. 13 contained no Ig-producing cells). Despite the low number of immunocytes, staining with monoclonal antibodies revealed scattered lymphocytes positive for LCA and B cell marker in all specimens but one (No. 4) which had a large accumulation of such cells. This showed that the specimens were adequately preserved for immunohistochemistry. Also, they appeared histologically similar to the other specimens by haematoxylin and eosin staining.

Systemic immunity. Serum IgA could not be detected in any patient except one (No. 12) in whom traces ($< 0{\cdot}05$ g/l) of IgA were found. No other deficiency was revealed except some undetectable IgG4 and IgD levels which, however, were also seen in the control material (Table 1). The indices for cell-mediated immunity were within the normal range (Table 1).

A comparison of the various class and subclass levels in the 'D group' and 'M group' is shown in Fig. 4. The former patients tended to have higher IgG concentrations than the latter, particularly of the IgG2 subclass. This was the reverse of the situation in the respiratory mucosa with regard to IgG2-producing cells (Fig. 3). With regard to IgD, the 'D group' had strikingly higher serum levels than the 'M group', although all values except one were within the normal range (Fig. 4). The only additional patient with a relatively high IgD concentration (Table 1, No. 7) belonged to the group with poor local Ig-production; IgD ws the dominating immunocyte isotype in his nasal mucosa.

Clinical findings and relation to local Ig production. Eight patients had recurrent rhinosinusitis, three had recurrent acute otitis media, and three had recurrent acute tonsillitis. Tonsillectomy had been performed in four patients. Recurrent lower respiratory tract infections (more than five episodes of pneumonia verified by chest roentgenogram) had occurred in three patients.

The relation between clinical and immunohistochemical findings appears in Fig. 1b. In the 'M group' the patient (No. 1) with the poorest IgM response (Fig. 1a) had had recurrent rhinosinusitis

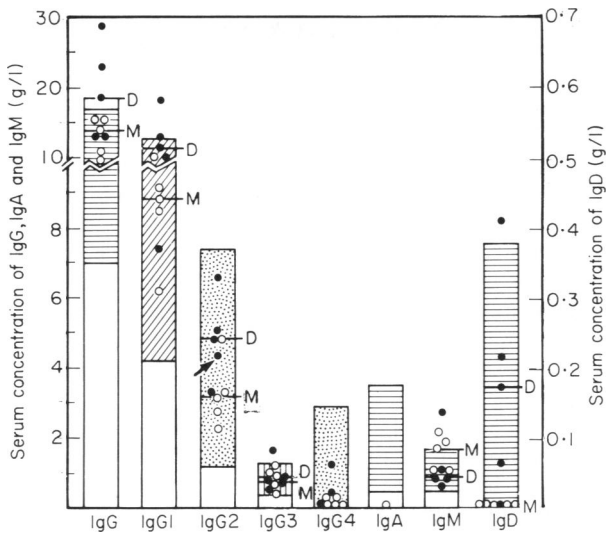


Fig. 4. Serum concentrations of IgG (and IgG subclasses), IgA, IgM, and IgD in IgA-deficient patients with a prominent local production of IgD (D, closed symbols) or IgM (M, open symbols). Hatched and dotted areas of the columns represent the normal ranges, and the medians of the two groups of patients are indicated by horizontal lines. Arrow indicates the IgG2 level of a patient (No. 3) who apparently lacked mucosal immunocytes producing this subclass.

whereas the others had not had recurrent upper respiratory tract infections. All patients in the 'D group' had recurrent upper airways infections. However, we could not verify that the one with the poorest IgD response (No. 15, Fig. 1a) had had recurrent rhinosinusitis. Nevertheless, he had complained of 'common colds' at least 10 times every year over the last 15 years, whereas none of the other fourteen patients reported more than five 'common colds' per year. He had pus in his nose at the time of investigation and pneumococci could be isolated from his nasopharynx. Also the five patients with poor local production of all Ig isotypes had all suffered from recurrent infections of the upper respiratory tract; three of the four tonsillectomies had been performed in this group.

DISCUSSION

Our results indicate that IgA-deficient patients who compensate with local production of IgG and IgM have better protection of their respiratory mucosa than those with additional marked IgD production. Raised levels of IgM have been demonstrated in nasal secretions of patients with selective IgA deficiency (Plebani *et al.*, 1983; Mellander *et al.*, 1986). Clinical observations have suggested that such compensatory IgM may act as protective antibody in a 'first line' of defence (Arnold *et al.*, 1977; Mellander *et al.*, 1986). Ogra *et al.* (1974), moreover, observed specific IgM antibodies in nasopharyngeal secretions of some IgA-deficient subjects after intranasal application of inactivated poliovaccine. Nevertheless, the antibody most consistently found was IgG, which might have been serum-derived as well as locally produced. There is evidence both from animal experiments (Fazekas de St Groth, Donnelly & Graham, 1951) and in immunodeficiency patients (Karlsson *et al.*, 1986) that leakage of IgG through respiratory surface epithelium may be of protective value.

Why then should additional marked local production of IgD be associated with poor resistance to infection? IgD may function as antibody (Sewell *et al.*, 1978); but since it is apparently unable to activate complement and other amplification systems (Spiegelberg, 1974), it may have blocking antibody activities like IgA. Some reports have suggested that there is a facilitated transfer of IgD

into external secretions in both man (Keller *et al.*, 1985) and rat (Steele & Leslie, 1985), but immunohistochemical observations (Brandtzaeg *et al.*, 1979; Fig. 2IIc) do not support this view. Moreover, the epithelial Ig receptor protein (SC) shows affinity neither for purified serum IgD (Brandtzaeg, 1977) nor for intracellular IgD (Brandtzaeg, 1983b). Plebani *et al.* (1983) were, in fact, unable to find raised levels of IgD in nasal secretions or saliva from IgA-deficient subjects.

The putative blocking function of IgD may take place both within the mucosa and in the circulation. Heddle, Kwitko & Shearman (1980) identified a group of IgA-deficient subjects with particularly low serum titres of IgM antibodies (detected by passive haemagglutination) to *Escherichia coli*. These subjects might be similar to our 'D group' in whom we found raised levels of circulating IgD (Fig. 4), most likely of mucosal origin. If IgD antibodies in this group were able to block opsonizing and bacteriolytic effects of IgM (and perhaps IgG) antibodies, local defence would be compromized. There was no other apparent explanation for the tendency to infections in subjects of the 'D group' as their IgG-subclass production and cell-mediated immunity seemed to be within normal limits.

The possibility that prominent local IgD production is a marker of an inadequate compensatory IgM response in the respiratory mucosa requires further attention. This could not be evaluated satisfactorily in our study because we were unable to quantify the total number of local immunocytes. When we first reported that a prominent IgD response is often seen in nasal, lacrimal and salivary glands (in contrast to the gut) of IgA-deficient subjects, this observation was taken to reflect heterogeneity of the secretory immune system in terms of B cell precursors (Brandtzaeg *et al.*, 1979). The local IgG- and IgD-producing cells show prominent J-chain expression (Brandtzaeg & Korsrud, 1984) and are therefore thought to reflect 'spin-off' from immature B cell clones which, through sequential switching of phenotype, are on their way to IgA differentiation (Brandtzaeg, 1985b). When this pathway is blocked, as in IgA deficiency, B cells destined for the nasal, lacrimal and salivary glands may to a large extent be forced to terminate locally with IgG and IgD production. This process does not depend on inflammation since it was also observed in normal glandular tissues (Brandtzaeg *et al.*, 1979)

Heterogeneity of the human secretory immune system may be explained by seeding of the intestinal mucosa with precursors originating mainly from gut-associated lymphoid tissue (GALT), such as Peyer's patches, whereas glands of the upper aero-alimentary tract usually receive most of their B cells from bronchus-associated lymphoid tissue (BALT) and tonsils. This theory is based on the subclass profile of IgA-producing cells in various secretory tissues (Kett *et al.*, 1986) and on the fact that tonsils contain much more IgD-producing cells (Brandtzaeg *et al.*, 1978; Korsrud & Brandtzaeg, 1980) than do GALT (Bjerke & Brandtzaeg, 1986). While precursors from GALT may mainly develop into IgA2-producing cells by direct switching from IgM expression, secretory immunity of the upper aero-alimentary tract probably depends more on differentiation to IgA1 production through sequential switching of B cells according to the order of the heavy chain constant genes (Flanagan & Rabbits, 1982), that is: IgM→IgD→IgG3→IgG1→IgA1. The increased proportion of IgG1- along with IgD-producing cells in the nasal mucosa of our 'D group' of IgA-deficient patients is in keeping with this hypothesis. Conversely, seeding of the respiratory mucosa with B cells mainly derived from GALT may explain the finding in our 'M group'. Thus, we can speculate that when secretory immunity depends on compensatory antibodies, the former category of subjects are clinically worse off than the latter in whom a blocked IgA differentiation pathway gives rise to substantial development of gland-associated IgM immunocytes in respiratory mucosa.

In conclusion, our results imply that tonsils and BALT are important precursor sources for B cells homing to respiratory mucosae in IgA deficiency, but it may be an advantage that these cells have mainly originated in GALT, or at least exhibit properties of GALT-derived B cells. We cannot offer any explanation for the poor nasal Ig production observed in the five patients who showed the highest frequency of infections in the upper respiratory tract. Three of them might have belonged to the same category as our 'D group', but removal of their tonsils (Fig. 1b) could have compromised seeding of B cells to their respiratory mucosa. This would be keeping with the classical report of Ogra (1971) on strikingly reduced IgA-antibody titres to poliovirus in nasopharyngeal secretions of children after tonsillectomy and adenoidectomy. In our experience nasal biopsy usually provides

representative mucosal samples, and it was not possible to distinguish the three categories of our study by conventional histological examination. We therefore suggest that our results reflect individual immunoregulatory differences.

The authors express their sincere thanks to V.A. Oxelius MD for the quantification of the serum IgG-subclasses. This study was supported by the Norwegian Cancer Society, the Norwegian Research Council for Science and the Humanities, Anders Jahre's Fund, the Medical Faculty of the University of Göteborg, the Göteborg Medical Society, and the Hesselman Foundation.

REFERENCES

ARNOLD R.R., COLE, M.F., PRINCE, S. & MCGHEE, J.R. (1977) Secretory IgM antibodies to *Streptococcus mutans* in subjects with selective IgA deficiency. *Clin. Immunol. Immunopathol.* **8,** 475.

BJERKE, K. & BRANDTZAEG, P. (1986) Immunoglobulin- and J chain-producing cells associated with lymphoid follicles in the human appendix, colon and ileum, including Peyer's patches. *Clin. exp. Immunol.* **64,** 432.

BRANDTZAEG, P. (1974) Mucosal and glandular distribution of immunoglobulin components. Immunohistochemistry with a cold ethanol-fixation technique. *Immunology* **26,** 1101.

BRANDTZAEG, P. (1977) Human secretory component. VI. Immunoglobulin binding properties. *Immunochemistry* **14,** 179.

BRANDTZAEG, P. (1981) Prolonged incubation time in immunohistochemistry: effects on fluorescence staining of immunoglobulins and epithelial components in ethanol- and formaldehyde-fixed paraffin-embedded tissues. *J. Histochem. Cytochem.* **29,** 1302.

BRANDTZAEG, P. (1983a) The secretory immune system of lactating mammary glands compared with other exocrine organs. *Ann. NY Acad. Sci.* **409,** 353.

BRANDTZAEG, P. (1983b) Immunohistochemical characterization of intracellular J-chain and binding site for secretory component (SC) in human immunoglobulin (Ig)-producing cells. *Molec. Immunol.* **23,** 941.

BRANDTZAEG, P. (1984) Immune functions of human nasal mucosa and tonsils in health and disease. In *Immunology of the Lung and Upper Respiratory Tract* (ed. by J. Bienenstock) p.28. McGraw-Hill, New York.

BRANDTZAEG, P. (1985a) Cells producing immunoglobulins and other immune factors in the human nasal mucosa. *Protides biol. Fluids.* **32,** 363.

BRANDTZAEG, P. (1985b) Role of J chain and secretory component in receptor-mediated glandular and hepatic transport of immunoglobulins in man *Scand. J. Immunol.* **22,** 111.

BRANDTZAEG, P. & KORSRUD, F.R. (1984) 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.* **58,** 709.

BRANDTZAEG, P. & ROGNUM, T.O. (1983) Evaluation of tissue preparation methods and paired immunofluorescence staining for immunocytochemistry of lymphomas. *Histochem. J.* **15,** 655.

BRANDTZAEG, P., GJERULDSEN, S.T., KORSRUD, F., BAKLIEN, K., BERDAL, P. & EK, J. (1979) The human secretory immune system shows striking heterogeneity with regard to J chain positive immunocytes. *J. Immunol.* **122,** 503.

BRANDTZAEG, P., KETT, K., ROGNUM, T.O., SÖDERSTRÖM, R., BJÖRKANDER, J., SÖDERSTRÖM, T., PETRUSON, B. & HANSON, L.Å. (1986) The distribution of mucosal IgA and IgG subclass-producing immunocytes and alterations in various disorders. *Monogr. Allergy.* **20,** 179.

BRANDTZAEG, P., SURJAN, L. & BERDAL, P. (1978) Immunoglobulin systems of human tonsils. I. Control subjects of various ages: quantification of Ig-producing cells, tonsillar morphometry, and serum Ig-concentrations. *Clin exp. Immunol.* **31,** 367.

FAZEKAS DE ST. GROTH, S., DONNELLEY, M. & GRAHAM, D.M. (1951) Studies in experimental immunology of influenza. VIII. Pathotopic adjuvants. *Aust. J. exp. Biol. med. Sci.* **29,** 323.

FLANAGAN, J.G. & RABBITS, T.H. (1982) Arrangement of human immunoglobulin heavy chain constant region genes implies evolutionary duplication of a segment containing γ, ε and α genes. *Nature* **300,** 709.

HEDDLE, R.J., KWITKO, A.O. & SHEARMAN, D.J.C. (1980) Specific IgM and IgG antibodies in IgA deficiency. *Clin. exp. Immunol.* **41,** 453.

JEFFERIS, R., REIMER, C.B., SKVARIL, F., DE LANGE, G., LING, N.R., LOWE, J. WALKER, M.R., PHILLIPS, D.J., ALOISIO, C.H., WELLS, T.W., VAERMAN, J.P., MAGNUSON, C.G., KUBAGAWA, H., COOPER, M., VARTDAL, F., VANDVIK, B., HAAIJMAN, J.J, MÄKELA, O., SARNESTO, A., LANDO, Z., GERGELY, J., RAJNAVÖLGYI, E., LASZLO, G., RADL, J. & MOLINARO, G.A. (1985) Evaluation of monoclonal antibodies having specificity for human IgG subclasses: results of an IUIS/WHO collaborative study. *Immun. Letters* **10,** 223.

KARLSSON, G., PETRUSON, B., BJÖRKANDER, J. & HANSON, LÅ. (1985a) Infections of the nose and paranasal sinuses in adult patients with immunodeficiency. *Arch. Otolaryngol.* **111,** 290.

KARLSSON, G, HANSSON, H.-A., PETRUSON, B., BJÖRKANDER, J. & HANSON, LÅ. (1985b) Goblet cell number in the nasal mucosa relates to cell-mediated immunity in patients with antibody deficiency syndromes. *Int. Archs Allergy appl. Immun* **78,** 86.

KARLSSON, G., BRANDTZAEG, P., HANSSON, G., PETRUSON, B., BJÖRKANDER, J. & HANSON L.Å.

(1987) Humoral immunity in nasal mucosa of patients with common variable immunodeficiency. *J. clin. Immunol.*, **7**, 29.

KELLER, A.M., HEINER, D.C., MYERS, A.S. & REISINGER, D.M. (1985) IgD in human colostrum. *Pediatr. Res.* **19**, 122.

KETT, K., BRANDTZAEG, P., RADL, J. & HAAIJMAN, J.J. (1986) Different subclass-distribution of IgA-producing cells in human lymphoid organs and various secretory tissues. *J. Immunol.* **136**, 3631.

KORSRUD, F.R. & BRANDTZAEG, P. (1980) Immune systems of human nasopharyngeal and palatine tonsils: histomorphometry of lymphoid components and quantification of immunoglobulin-producing cells in health and disease. *Clin. exp. Immunol.* **39**, 361.

MANCINI, G., CARBONARA, A.O. & HEREMANS, J.F. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry* **2**, 235.

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

OGRA, P.L. (1971) Effect of tonsillectomy and adenoidectomy on nasopharyngeal antibody response to poliovirus. *N. Engl. J. Med* **284**, 59.

OGRA, P.L., COPPOLA, P.R., MACGILLIVRAY, M.H. & DZIERBA, J.L. (1974) Mechanisms of mucosal immunity to viral infections in γ A immunoglobulin-deficiency syndromes. *Proc. Soc. exp. Biol. Med.* **145**, 811.

OXELIUS, V.A. (1978) Crossed immunoelectrophoresis and electroimmunoassay of human IgG subclasses. *Acta pathol. microbiol. scand. (C)* **86**, 109.

PLEBANI, A., MIRA, E., MEVIO, E., MONAFO, V., NOTARANGELO, L.D., AVANZINI, A. & UGAZIO, A.G. (1983) IgM and IgD concentrations in the serum and secretions of children with selective IgA deficiency. *Clin. exp. Immunol.* **53**, 689.

SEWELL, H.F., CHAMBERS, L. MAXWELL V., MATTHEWS, J.B. & JEFFRIS, R. (1978) The natural antibody response to *E. coli* includes antibodies of the IgD class. *Clin. exp. Immunol.* **31**, 104.

SPEIGELBERG, H.L. (1974) Biological activities of immunoglobulins of different classes and subclasses. *Adv. Immunol.* **19**, 259.

STEEL, M.G. & LESLIE, G.A. (1985) Immunoglobulin D in rat serum, saliva and milk. *Immunology* **55**, 571.