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Quantitative immunohistochemistry of immunoglobulin- and J-chain-producing cells in human parotid and submandibular salivary glands

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Summary. Ig-producing cells were quantified by paired immunohistochemical staining in saline-extracted and paraffin-embedded normal tissue specimens from human parotid (ten) and submandibular (seven) salivary glands. The density of such cells (number/mm^2 of 6 μm thick tissue section) was significantly higher in the submandibular than in the parotid gland ($P < 0{\cdot}005$), but the Ig-class distribution was fairly similar. The mean percentage class ratios for IgG, IgA, IgM and IgD cells in the parotid were 4·5:86·5:5·9:3·1, and in the submandibular gland 3·7:86·9:7·9:1·6. In the parotid gland of a patient with selective IgA deficiency the same class ratios were 27:0:20:53. Thus, the IgA cells were especially replaced by IgD cells. In normal glands most of the IgA (80–93%), IgM (99–100%) and IgD cells (81–95%) were J-chain-positive; this was likewise true for a substantial proportion of the IgG cells (32–46%). Of additional interest was the finding that in the IgA-deficient parotid gland, 99% of the numerous IgD cells and 86% of the increased number of IgG cells contained cytoplasmic J chain. IgE-producing cells were virtually absent from the IgA-deficient as well as from the normal salivary glands.

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INTRODUCTION

The immunoglobulin (Ig)-producing cell systems and the secretory component (SC)-dependent Ig transport in the intestinal tract have been well characterized (Brandtzaeg & Baklien, 1976; 1977), whereas similar information about more inaccessible secretory sites, such as the major salivary glands, is insufficient. Subsequent to the first demonstration of IgA- and SC-producing cells in the human parotid gland (Tomasi, Tan, Solomon & Prendergast, 1965), the production of IgG, IgA, IgM and free SC was shown in tissue cultures of human major salivary glands (Hurlimann & Zuber, 1968). More recently the presence of small numbers of IgA-producing cells in the human parotid gland was verified by Kraus & Mestecky (1971). In addition, they found some scattered IgM cells, but no IgG cells. Mogi (1975) likewise in a study of human submandibular gland tissue found few and scattered interstitial IgA-producing cells and none or very few IgG cells, while he failed to demonstrate IgM and IgE cells. No quantitative report on the Ig-producing cell populations of human salivary glands, however, has been published.

There has recently been an increasing interest in the production of immunoglobulins in the salivary glands since animal experiments have indicated the possibility of enhancing secretory immunity to dental caries by vaccination (Michalek, McGhee, Mestecky, Arnold & Bozzo, 1976). More basic information about the Ig-producing cells systems of the human salivary glands is therefore needed. Moreover, normal refer-

ence data on these cells are required as a baseline for future studies of alterations of local Ig production associated with neoplastic lesions (Witz, 1977) and other diseases of the salivary glands. We therefore quantified immunohistochemically the Ig-producing cells present in normal parotid and submandibular glands and further characterized these cells by their J-chain content. The parotid gland of a patient with selective IgA deficiency was included as an important control.

MATERIALS AND METHODS

Salivary gland specimens

Histologically normal specimens were obtained from the parotid gland, well away from the lesion, in seven women and three men (mean age 48·7, range 26–73 years) undergoing surgery for benign tumours, and from the submandibular gland in four women and three men (mean age 46·8, range 25–64 years). Four of the latter patients were operated on because of benign non-inflammatory conditions, two because of tumours of the submandibular gland, and one because of malignant tumour of the thyroid gland. All of these patients were immunologically intact. In addition, a 53 year old man (B.L.) with selective IgA deficiency, allergic rhinitis, and nasal polyposis volunteered for a parotid-gland biopsy (histologically normal). More information about this patient can be found elsewhere (Brandtzaeg, 1971; Brandtzaeg, Gjeruldsen, Korsrud, Baklien, Berdal & Ek, 1979).

The tissue specimens were placed directly in ice-chilled PBS (0·01 M phosphate buffer, pH 7·5, 0·15 M NaCl) and brought to the laboratory within 1 h. The specimens were further subdivided into pieces of about 3 × 3 mm and then extracted in cold PBS for 48 h before ethanol fixation and paraffin embedding (Brandtzaeg, 1974a).

Immunohistochemistry

Serial sections were cut at 6 μm. One section was stained with haematoxylin and eosin for morphological evaluation. The adjacent sections were used for paired immunohistochemical staining; they were incubated with various pairs of rabbit IgG–fluorochrome conjugates monospecific for human Ig heavy chains. The preparation and characterization of these fluorescein isothiocyanate (FITC) or tetramethylrhodamine isothiocyanate (MRITC) conjugates, and details of the paired immunohistochemical staining procedure, have been described elsewhere (Brandtzaeg, 1973a,b; 1976b). For evaluation of the J-chain content of the different immunocyte classes, a J-chain specific rhodamine conjugate was combined with the various heavy-chain specific fluorescein conjugates (Brandtzaeg, 1976b). Exposure of hidden J-chain determinants in the cytoplasma was achieved by pre-treatment (denaturation) of the tissue sections in 6 M urea, pH 3·2, for 1 h at 4° (Brandtzaeg, 1976b,c).

Enumeration of Ig-producing cells

Fluorescing cells were counted in a Leitz Orthoplan microscope equipped with an Osram HBO 200 W lamp for rhodamine excitation, an XBO 150 W lamp for fluorescein excitation, a Leitz × 25 immersion objective, and a × 10 ocular. A Ploem-type vertical illuminator with interference filters was used for selective evaluation of green and red fluorescence. Cell enumerations were made in tissue units as defined by the outer frame of an ocular grid (Leitz code No. 519902) representing a tissue section area of 0·08 mm^2. The counting was carried out in a systematic manner throughout each section. All cells with a discernible nucleus, and cell-like bodies (diameter of 8–12 μm) with a pure green or red cytoplasmatic colour, were counted. To obtain a representative mean cell number for IgA immunocytes, it was generally found satisfactory to evaluate twenty such tissue units (Fig. 1); in

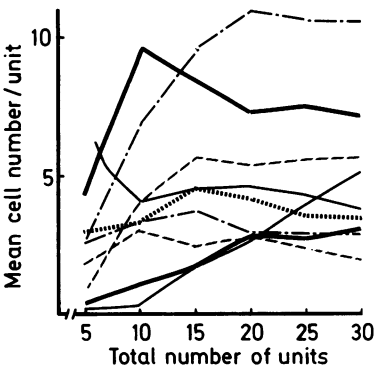

Fig 1. Enumerations of IgA immunocytes in five randomly selected salivary glands. Counts per tissue unit (0·08 mm^2 section area) in two different specimens from each of four glands are indicated by lines of identical quality. The accumulative means show that the data generally are representative for each specimen after evaluation of about twenty tissue units. The paired counts illustrate that a pronounced intraglandular variation usually becomes apparent even after evaluation of less than ten units.

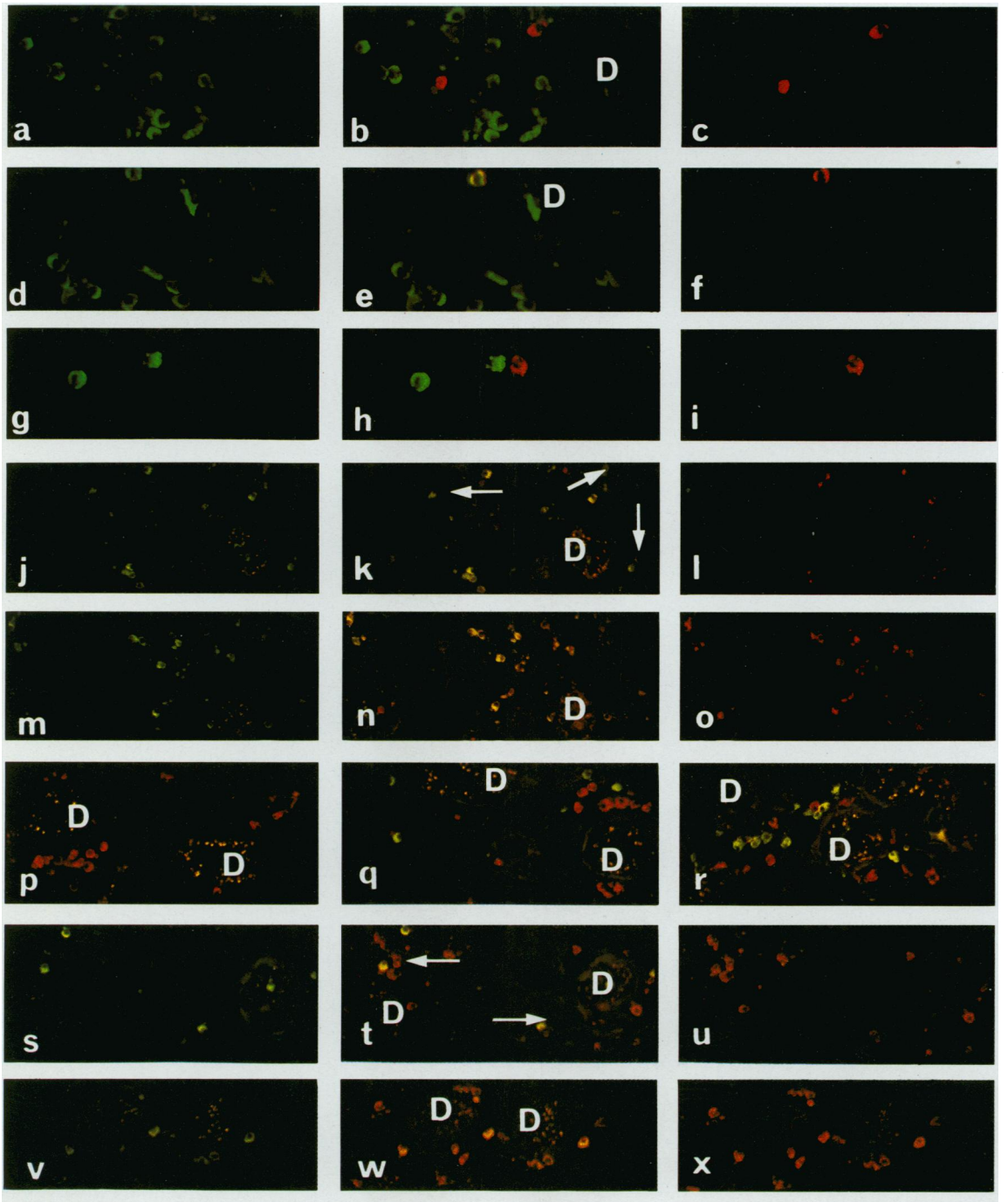

Figure 2.

(*facing p. 131*)

practice at least thirty units were included for each immunocyte class in each specimen.

One immunocyte class was always counted with reference to another by paired staining. Thus, green IgA cells were recorded together with red IgG cells; in an adjacent section red IgM cells were recorded together with green IgD cells, and the two latter cell classes were re-evaluated in a third serial section with reversed colours to enhance the reliability of their counts. Tissue sections from five parotid and five submandibular specimens were moreover screened for IgE-producing cells with both a fluorescein and a rhodamine conjugate (Baklien & Brandtzaeg, 1975).

Evaluation of cytoplasmic J chain

Immunocytes of each heavy-chain class (except IgE) were evaluated for concomitant content of J chain by paired staining. A non-denatured and an adjacent urea-denatured tissue section were used for each immunocyte class, including together 11–93 IgG, 18–94 IgM, 8–19 IgD, and 215–345 IgA cells. The intensity of the J-chain staining was graded from negative or negligible through moderate (+) to bright (++), taking as a reference the overall impression of the J-chain-positive cells in the actual tissue section. Three specimens of the parotid and three of the submandibular gland were included in these experiments, each immunocyte class being considered together for each gland type.

Data analyses

The density of each immunocyte class was expressed as mean cell number/mm^2 of 6 μm thick tissue section, based on at least thirty tissue units for IgG and IgA cells, and on at least sixty units for IgM and IgD cells in each case. The percentage class distribution of the cells was likewise calculated for each case, thus giving the same statistical weight in the mean figure. Due to the scarcity of IgE-producing cells, these were not included in the calculations. Differences were tested by non-parametric (Wilcoxon) statistical analyses. The data were based on enumerations performed by the same observer. Duplicate counts of IgA cells in ten different specimens with an interval of 2 weeks, produced a correlation coefficient (r) of 0·874 ($P < 0·001$) and an average coefficient of variation (CV) of $12·6 \pm 10·7$ (mean $\pm$ SD). Counts performed by another observer in the same tissue sections showed an r of 0·755 ($P < 0·01$) and a CV of $13·9 \pm 10·4$ compared with the mean counts of the first observer.

RESULTS

Ig-producing cells

All classes of Ig-producing cells, except for IgE, were regularly present in both glands. Duplicate sections of five parotid and five submandibular specimens were screened for IgE immunocytes, but in only one of the ten cases was a single such cell found. A clear-cut differentiation between the other classes was shown by the use of selective filters after paired staining with various combinations of the conjugates (Fig. 2a–i); an occasional double-producer was seen especially expressing both IgA and IgG (Fig. 2d–f), but such cells were not found in every section.

Figure 2. (a–o) Paired immunohistochemical staining for cytoplasmic Ig class and J chain in salivary-gland immunocytes as demonstrated in saline-extracted normal tissue specimens. Fluorescein (green) and rhodamine (red) fluorescence were in (a–i) selectively recorded through the Leitz secondary barrier filters AL525 and K590, respectively, whereas in (j–x) no secondary filter was used. (a–c) Paired staining of IgA (green) and IgG (red) cells in field adjacent to striated duct (D) in normal submandibular gland; note in double-exposed picture (b) class restriction to individual cells as shown by pure colours. (d–f) Similar field contained an IgA–IgG double producer as shown by yellow colour in double exposure (e) with staining conditions as in (a–c). (g–i) Paired staining for IgM (green) and IgG (red) in same gland showed scattered cells with pure colour in double exposure (h), evidencing class restriction. (j–l) After paired staining for IgA (green) and J chain (red) in normal parotid gland all IgA cells, except for three negative ones (arrows), were faintly stained for J chain as shown by yellow tint in double exposure (k); two cells with pure red colour belong to another Ig class, and yellow autofluorescent granules in striated duct (D) show mixed colour. (m–o) Corresponding field in adjacent tissue section treated with acid urea; note intensification of red J-chain colour in (o) and mixed yellow colour in double exposure (n) with same staining and photographic conditions as in (j–l). (p–x) Paired immunohistochemical staining for cytoplasmic Ig class and J chain in a saline-extracted histologically normal parotid tissue specimen from a patient with selective IgA deficiency; yellow autofluorescent granules were abundant in striated ducts (D). (p–r) Double exposures of red staining for IgD combined with green staining for IgA (p), IgG (q) or IgM (r); note complete absence of IgA cells and large number of IgD cells adjacent to striated ducts. (s–u), Paired staining for IgG (green) and J chain (red) shows that two IgG cells (arrows) contain abundant J chain and appear yellow in double exposure (t); the purely red cells belong to the IgD or IgM class. (v–x) Paired staining for IgD (green) and J chain (red) in adjacent tissue section treated with acid urea; all IgD cells in this field contained abundant J chain and appear yellow in double exposure (w). Urea treatment did not intensify J-chain staining of IgD cells, neither of IgG and IgM cells which show pure red colour in (w). Urea treatment had some adverse effect on the green IgD fluorescence in (v). Magnification: × 255 (a–i); × 160 (j–x).

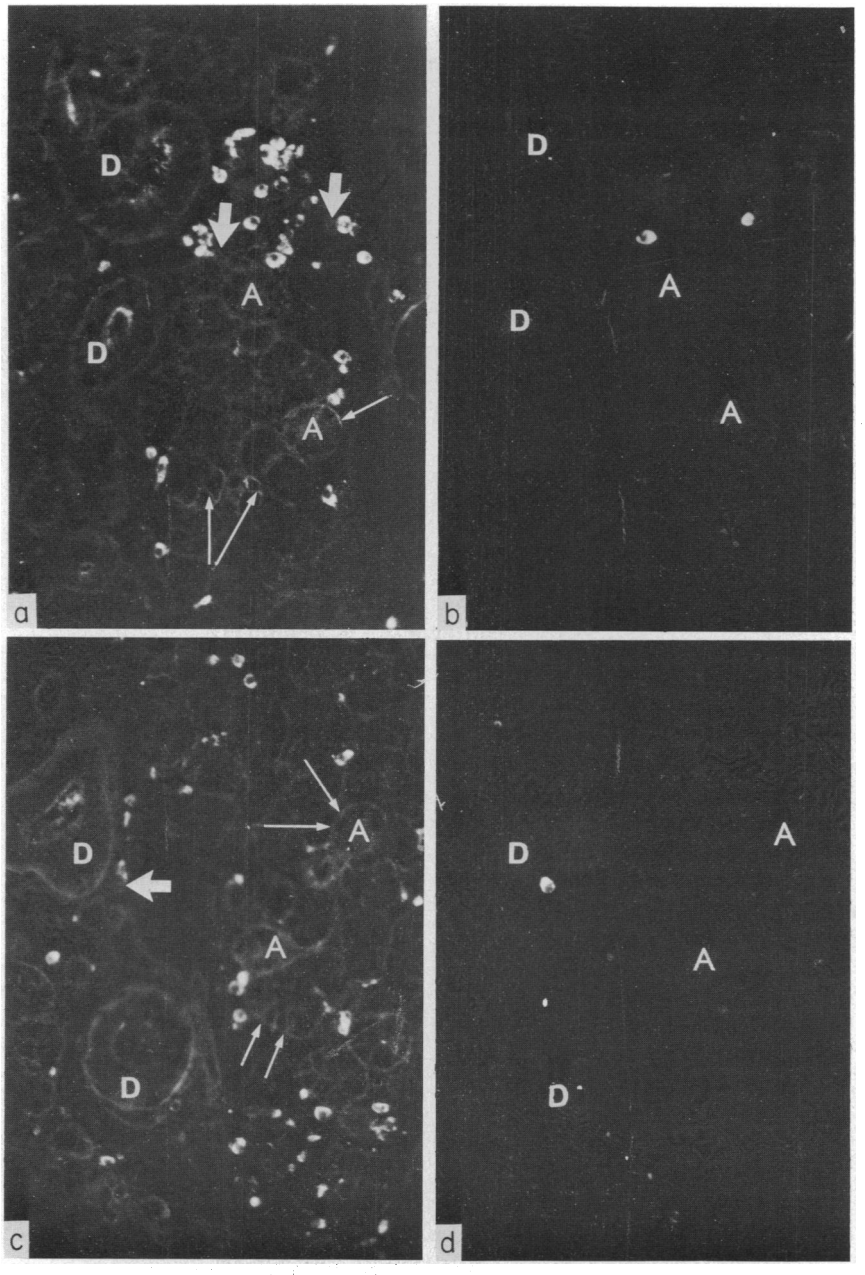

Figure 3. (a,b) Paired immunohistochemical staining for IgA (a) and IgM (b) in a section of saline-extracted normal submandibular gland specimen. Note predominance of IgA-producing cells scattered between acini (A) and clustered adjacent to striated ducts (D); large arrows indicate the locations of the two IgM-producing cells among the relatively numerous IgA immunocytes. Note also retention of IgA along basal and lateral epithelial cell membranes (small arrows) and luminal rim of IgA in striated ducts. (c,d) Adjacent section stained for IgA (c) and IgG (d). Large arrow indicates the location of the single IgG-producing cell among the relatively numerous IgA immunocytes. Note again epithelial retention of IgA in contrast to the virtually complete removal of IgG from epithelial cell membranes. Magnification: × 165.

Immunocytes were scattered in the stroma between the acini (Fig. 3). In addition, clusters of IgA cells along with a few IgM cells were present adjacent to many of the striated ducts (Fig. 3a and b). The distribution of immunocytes conformed to that of pyroninophilic cells in sections stained with methyl green-pyronin (Chen & Chang, 1963). The various duct segments could be identified in serial sections stained with haematoxylin and eosin. Moreover, striated ducts were often directly identifiable in the immunohistochemical preparations due to their cytoplasmic content of yellow autofluorescent lipofuscin granules (Fig. 2j–x), as described before (Kraus & Mestecky, 1971; Buchner & David, 1978).

Owing to the tissue extraction procedure used (Brandtzaeg, 1974a), the Ig-producing cells were revealed against a background that was relatively unstained. There was, however, some retention of IgG and especially of IgA along acinar and ductal basement membrane zones (Fig. 3). Moreover, IgA was selectively present along the lateral aspects of the epithelial cells as well as in the apical part of their cytoplasm (Fig. 3a and c). A luminal rim of IgA was especially notable in the striated ducts (Fig. 3a and c).

The IgA class of immunocytes showed a striking predominance (87%) in both types of gland; their density (number of cells/mm^2 section area) was significantly higher ($P < 0{\cdot}005$) in the submandibular than in the parotid (Table 1). Also the density and percentage of IgM cells tended to be higher in the submandibular (7·9%) than in the parotid gland (5·9%), whereas the reverse was true for IgD cells (3·1% *v.* 1·6%). These differences, however, were not statistically significant since there were wide individual variations (Table 1). Thus, two parotid specimens apparently lacked IgD cells. The number of IgG-producing cells fell, on the average, between the IgM and IgD classes and tended to be somewhat higher in the submandibular than in the parotid, although the IgG-cell percentages were quite similar in the two glands (3·7% *v.* 4·5%).

In five of the subjects, the parotid cell enumerations were based on two tissue specimens from the same gland (Fig. 1 and Table 2). The intra-individual variations in cell density were pronounced, although not as large as the variations in the whole material. Conversely, the percentage class ratios of the immunocytes seemed to be a much better individual characteristic (Table 2).

The total number of Ig-producing cells/mm^2 parotid tissue section in the IgA-deficient patient was somewhat below the normal mean (43·8 compared to 68·3), but was well within the cell density range observed for the ten controls (14·8–132·8). As reported before (Brandtzaeg, Fjellanger & Gjeruldsen, 1968), there was a complete lack of IgA cells (Fig. 2p) and a replacement with IgG- and IgM-producing cells in his glandular tissue (Figs 2q,r and 4a,b). In addition, the present study revealed a striking predominance of IgD-producing cells, amounting to a density about

Table 1. Immunoglobulin-producing cells in normal parotid and submandibular salivary glands (mean ± SD and observed range)

	Parotid gland ($n=10$)		Submandibular gland ($n=7$)	
Immunocyte class	Cell density*	Class percentage	Cell density*	Class percentage
IgG	2·9 ± 1·8 (0·8 – 7·2)	4·5 ± 2·0	5·8 ± 4·9 (2·4 – 14·4)	3·7 ± 1·9
IgA	58·4 ± 26·1† (12·0 – 108·4)	86·5 ± 7·4	122·9 ± 54·7† (86·8 – 239·2)	86·9 ± 4·1
IgM	4·1 ± 4·1 (0·2 – 11·6)	5·9 ± 5·2	10·0 ± 4·4 (4·4 ± 18·4)	7·9 ± 4·5
IgD	2·9 ± 4·1 (0 – 10·4)	3·1 ± 3·6	2·2 ± 2·2 (0·2 – 5·4)	1·6 ± 1·7
All classes	68·3 ± 33·3† (14·8 – 132·8)	100	141·3 ± 60·1† (99·4 – 270·0)	100

* Number of cells/mm^2 of 6 μm thick tissue section.
† Significantly different ($P < 0{\cdot}005$), Wilcoxon test.

Table 2. Comparison of immunocyte density and percentage class distribution (%) between two specimens (I and II) taken from the same parotid gland in five cases

	Immunocyte class							
	IgG		IgA		IgM		IgD	
Case	I	II	I	II	I	II	I	II
1	2·4 (3·3%)	1·2 (4·5%)	68·4 (93·2%)	24·0 (90·9%)	1·8 (2·4%)	0·8 (3·0%)	0·8 (1·1%)	0·4 (1·5%)
2	2·8 (6·9%)	2·8 (3·1%)	36·8 (91·1%)	85·6 (94·3%)	0·8 (2·0%)	2·4 (2·6%)	0	0
3	0·8 (2·2%)	3·6 (3·7%)	34·0 (91·9%)	87·2 (89·0%)	2·0 (5·4%)	4·6 (4·7%)	0·2 (0·5%)	0·2 (0·5%)
4	1·6 (2·2%)	2·8 (4·8%)	62·0 (87·1%)	47·2 (81·7%)	2·6 (3·7%)	2·2 (3·8%)	5·0 (7·0%)	5·6 (9·7%)
5	2·0 (3·8%)	3·2 (4·5%)	100·4 (88·4%)	76·0 (89·2%)	11·0 (9·7%)	5·8 (6·8%)	0·2 (0·2%)	0·2 (0·2%)
Observed range for whole material:								
Cell density	0·8–7·2		12·0–108·4		0·2–11·6		0–10·4	
Class percentage	1·3–8·1%		74·7–96·8%		0·4–17·9%		0–8·5%	

seven times higher than the normal mean (Table 3, Figs 2p–r and 4d).

J-chain-producing cells

There was no apparent difference between the parotid and the submandibular gland with regard to J-chain synthesis (Table 4). Most of the IgA cells (80–93%) contained cytoplasmic J chain, as revealed when the tissue section had been denatured in acid urea (Fig. 2m–o). Such treatment increased the positivity percentage and also the staining intensity of the positive cells (Table 4, Fig. 2j–o), indicating unmasking of hidden J-chain determinants (Brandtzaeg, 1976d).

Most IgM cells gave J-chain staining without denaturation in urea, and all of them turned out to be positive after such treatment (Table 4). Most IgD cells (81–95%) and up to 47% of the IgG cells were J-chain positive; an effect of urea on their staining was not apparent, taking the small number of such cells into account (Table 4). These results were confirmed on the relatively larger number of IgD, IgG and IgM cells present in the parotid specimen of the IgA-deficient patient (Fig. 2s–x, Table 5). The only difference was a marked increase in the percentage (86%) of J-chain positive IgG cells.

Table 3. Immunoglobulin-producing cells in the parotid gland of a patient with selective IgA deficiency

Immunocyte class	Cell density*	Class percentage
IgG	11·7	26·7
IgA	0	0
IgM	8·8	20·1
IgD	23·3	53·2
Total number	43·8	100

*Number of cells/mm^2 of 6 μm thick tissue section.

DISCUSSION

This study confirms on a quantitative basis the predominance of IgA-producing cells in the parotid (Tomasi *et al.*, 1965; Bradtzaeg *et al.*, 1968; Kraus & Mestecky, 1971) and submandibular salivary gland (Rossen, Morgan, Hsu, Butler & Rose, 1968; Mogi, 1975). These results are in accordance with similar findings in other secretory sites of man (Crabbé & Heremans, 1966; Brandtzaeg & Baklien, 1976; Gjeruldsen & Brandtzaeg, 1978). In addition, we demonstrated the regular presence of IgG- and IgM-producing cells, which only inconsistently have been shown to be present in the salivary glands before (Kraus & Mesteky, 1971; Mogi, 1975) Moreover, in contrast to the intestinal mucosa (Brandtzaeg & Baklien, 1976), but similarly to the lacrimal and nasal glands (Brandtzaeg *et al.*, 1979), the salivary glands were normally found to contain a substantial proportion of IgD-producing cells. Conversely, IgE-producing cells were virtually absent from the salivary glands, as is also the

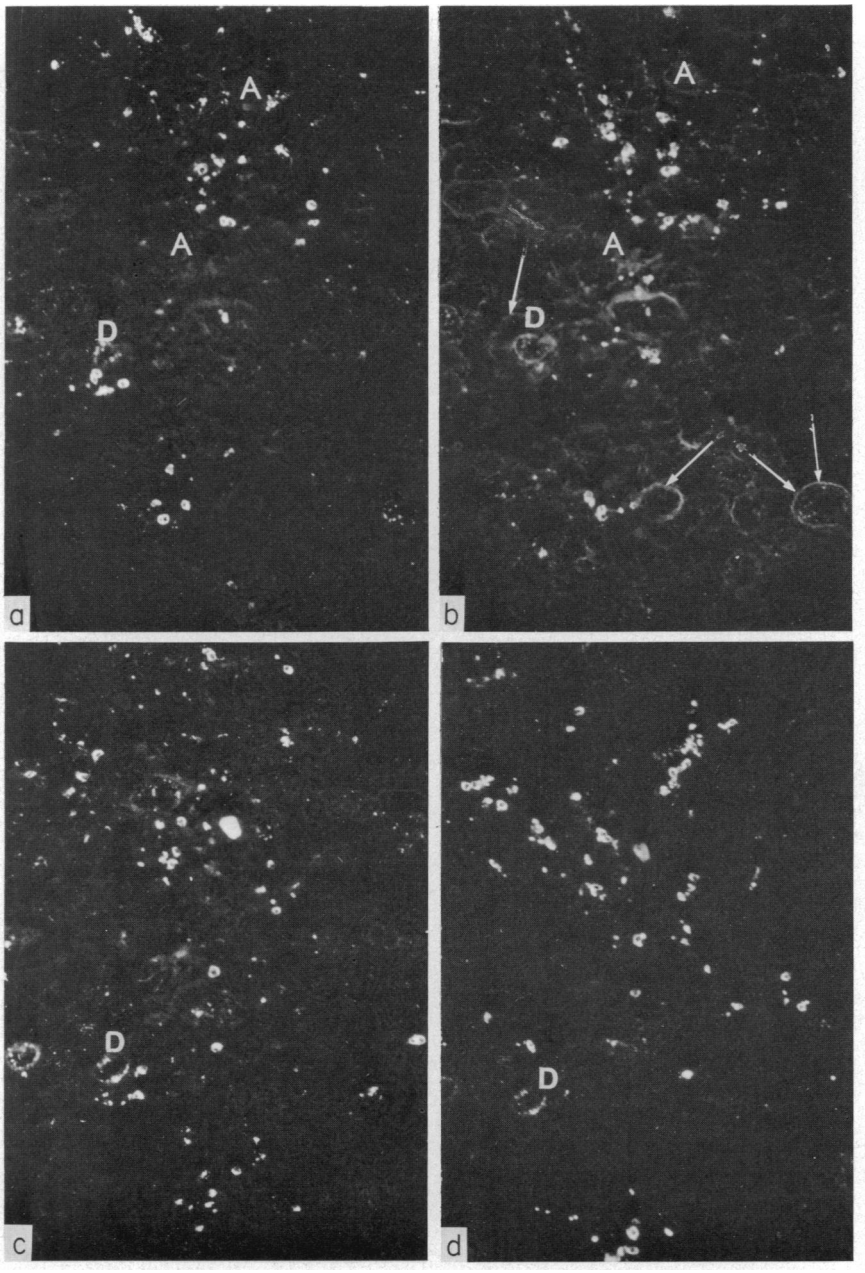

Figure 4. (a,b) Paired immunohistochemical staining for IgM (a) and IgG (b) in a section of saline-extracted histologically normal parotid gland specimen from a patient with selective IgA deficiency. There was a fairly similar number of IgM- and IgG-producing cells scattered between acini (A) and clustered adjacent to striated ducts (D), which in this gland contained abundant yellow autofluorescent granules. There was some retention of IgG along epithelial basement membrane zones (arrows), probably ascribed to extravascular distribution of relatively high concentrations of serum IgG in this patient. (c,d) Adjacent section stained for IgM (c) and IgD (d). Note predominance of IgD-producing cells, which along with the increased number of IgM (a,c) and IgG (b) immunocytes show a distribution mimicking that of IgA immunocytes in salivary glands from immunologically intact individuals. Magnification × 105.

Table 4. Percentage J-chain positivity of various immunocyte classes in normal human parotid and submandibular salivary glands

Immunocyte class	Before urea treatment			After urea treatment		
		Positive			Positive	
	Negative	+	++	Negative	+	++
Parotid gland						
IgG (31)*	61·3	38·7		(17)* 64·7	35·3	
		38·7	0		35·3	0
IgA (353)	45·6	54·4		(375) 7·5	92·5	
		53·8	0·6		76·0	16·5
IgM (39)	20·5	79·9		(26) 0	100	
		74·4	5·1		96·2	3·8
IgD (19)	5·3	94·8		(13) 7·7	92·3	
		63·2	31·6		61·5	30·8
Submandibular gland						
IgG (108)	67·6	32·4		(114) 53·3	46·5	
		29·6	2·8		43·9	2·6
IgA (403)	48·4	51·6		(507) 19·7	80·3	
		50·1	1·5		72·8	7·5
IgM (101)	6·9	93·1		(92) 1·1	98·9	
		83·2	9·9		84·8	14·1
IgD (22)	13·6	86·4		(21) 19·0	81·0	
		59·1	27·3		52·4	28·6

* Number of cells evaluated by paired staining.

Table 5. Percentage J-chain positivity of various immunocyte classes in the parotid gland of a patient with selective IgA deficiency

Immunocyte class	Before urea treatment			After urea treatment		
		Positive			Positive	
	Negative	+	+	Negative	+	++
IgG (40)*	13·1	86·9		(35)* 15·4	84·6	
		66·1	20·8		29·8	54·8
IgM (51)	10·9	89·1		(39) 0	100	
		23·8	65·3		0	100
IgD (86)	0	100		(104) 3·2	96·8	
		4·1	95·9		0	96·8

* Number of cells evaluated by paired staining.

case for other normal secretory sites (Brandtzaeg & Baklien, 1976).

The density of Ig-producing cells was considerably lower in the salivary glands than in other secretory sites studied by us. Conversion of tissue unit data obtained for normal colon mucosa (Baklien & Brandtzaeg, 1975) gives a mean immunocyte number of 572/mm^2 section area, or 1307/mm^2 of lamina propria (Rognum, Brandtzaeg, Baklien & Hognestad, 1979). The average immunocyte density in normal lacrimal glands is 566 cells/mm^2 section area (Gjeruldsen & Brandtzaeg, 1978). Thus, the overall immunocyte den-

sity in the submandibular is only about 25% of that in the lacrimal gland, and in the parotid only 12%. It therefore appears that the density of Ig-producing cells may be related to the duct length between the gland and the adjacent mucosal surface—the parotid being the most remote gland. This explanation is conceivable if topical antigen influences local retention and perhaps proliferation of B cells after their entering the glandular site, as has been suggested for the intestinal lamina propria (Husband, Monié & Gowans, 1977; Pierce & Sack, 1977; Cebra, Gearhart, Robertson & Tseng, 1978).

The immunocyte class ratios are fairly similar for the two salivary glands (Table 1), both showing an IgA-cell proportion (87%) near that found in the colon (90%) (Baklien & Brandtzaeg, 1975) and somewhat higher than that of the jejunum (79%), lacrimal (77%) and nasal (67%) glands (Brandtzaeg *et al.*, 1979). The proportion of IgM cells (6–8%) is similar to that of colonic, nasal and lacrimal glands, but lower than that of jejunal glands (18%). Of special interest is the finding of a substantial proportion of IgD-producing cells in the submandibular (1·6%) and even more so in the parotid gland (3·1%). This is in contrast to the intestinal mucosa where only rare such cells are encountered, normally amounting to much less than 1% of the total immunocyte population (Brandtzaeg & Baklien, 1976). In the nasal and lacrimal glands, on the other hand, the proportion of IgD cells is normally about 9% (Brandtzaeg *et al.*, 1979).

The attraction of B cells to the parotid gland and their local maturation to Ig-producing cells was quantitatively unaffected in the patient with selective IgA deficiency, as his parotid density of immunocytes (43·8 cells/mm^2 section area) was well within the normal range (14·8–132·8 cells/mm^2). His parotid IgA immunocytes had especially been replaced by IgD-producing cells (53%), along with an approximately equal number of IgG (27%) and IgM (20%) cells. As discussed in more detail elsewhere (Brandtzaeg *et al.*, 1979), this replacement contrasts with that of mainly IgM, and to a lesser extent IgG cells, in the gastrointestinal mucosa of patients with a complete and selective IgA deficiency. It accords, on the other hand, with the 60–80% predominance of IgD cells seen in the lacrimal and nasal glands of such patients (Brandtzaeg *et al.*, 1979).

The observed heterogeneity among glandular sites with regard to their immunocyte populations has led us to postulate two new aspects of the secretory immune system (Brandtzaeg *et al.*, 1979). First, precommitment to IgA synthesis cannot be a requirement for B-cell blasts to home to and settle in glandular sites. That also most gland-associated IgD cells, and likewise a substantial proportion of the local IgG-producing immunocytes, are derived from circulating B cells in an early phase of clonal differentiation, was indicated by their J-chain positivity that amounted to 94·8% and 46·5% respectively, which is similar to that of circulating blasts of these classes (Brandtzaeg, 1976c). This conclusion gained strong support from the positive J-chain staining seen in the relatively numerous IgD (97–100% positive) and IgG (84·6–86·9% positive) cells of the parotid gland lacking IgA immunocytes. Since J chain is only secreted from IgA and IgM cells in which it combines with the Ig product, cytoplasmic accumulation of free J chain may result in a negative feedback leading to repression of its synthesis in IgD and IgG cells (Brandtzaeg, 1976d). Such a mechanism may explain the absence of J chain in most IgG-producing cells in sites of chronic inflammation and extrafollicularly in lymphoid organs where B cells probably belong to mature memory clones (Brandtzaeg 1976d; Brandtzaeg & Tolo, 1977).

Our second conclusion is that B-cell blasts homing to secretory sites of the upper aero-digestive tract (nasal, lacrimal and salivary glands) may partly be recruited from another precursor population than those homing to the gastro-intestinal mucosa. Alternatively, the local gland-associated immunocyte differentiation may follow different maturational patterns in the upper and lower regions of the body. The first possibility is of interest in relation to experiments carried out to stimulate synthesis of specific salivary IgA antibodies (Mestecky, McGhee, Michalek, Arnold, Crago & Babb, 1978). These studies have indicated that after deposition of bacterial vaccines in the intestinal lumen, stimulated B cells migrate from Peyer's patches to the salivary glands and mature locally to IgA-producing cells. It would in addition be of great interest to know if exposure of the tonsils to a selected antigen will give rise to cells producing the corresponding antibody in the salivary glands. B cells derived from clonal expansion processes in the palatine (Brandtzaeg, Surjan & Berdal, 1978) and nasopharyngeal tonsils (Crabbé & Heremans, 1967; Korsrud & Brandtzaeg, 1979) show a substantial potency for IgD production. A similar potency shown by B cells settled in secretory sites of the upper aero-digestive system hints that the tonsils may contribute to the immunocyte precursor pool of these sites. A rational

artificial stimulation of secretory immunity in the prevention of oral diseases will certainly depend on further understanding of the mechanisms underlying B-cell homing to salivary glands.

In a study with intravenous administration of labelled proteins, Strober, Blase & Waldmann (1970) showed that only about 4% of the salivary IgA was derived from serum. Since their study was based on analyses of whole saliva, that small fraction had probably entered the oral cavity through extraglandular sites (Brandtzaeg, Fjellanger & Gjeruldsen, 1970). Subsequently an SC-dependent glandular transport mechanism selective for dimeric IgA has been well established (Brandtzaeg & Baklien, 1977; Brandtzaeg, 1978; Crago, Pringe, Kulhavy & Mestecky, 1978), and cytoplasmic transfer of IgA through both acinar and ductal epithelium was apparent in our immunohistochemical studies (Fig. 3). Since SC shows non-covalent specific affinity only for Ig polymers that contain J chain (Brandtzaeg, 1976a), it is functionally important that most gland-associated IgA- and IgM-producing cells were found to be J-chain positive. The SC-binding site of IgA is formed intracellularly (Brandtzaeg, 1973c; 1976d) in accordance with the partial concealment of cytoplasmic J-chain determinants of IgA-producing cells (Table 4, Fig. 2j–o). A J-chain dependent SC-binding site (Eskeland & Brandtzaeg, 1974) is likewise found in the gland-associated IgM immunocytes (Brandtzaeg, 1974b). In these cells, however, there is an excess of free J chain (Mather & Koshland, 1977), which agrees with the cytoplasmic J-chain fluorescence shown by most cells of the IgM class even without prior urea denaturation (Tables 4 and 5).

It has recently been established that also IgM can be regarded as a true SC-dependent secretory immunoglobulin (Brandtzaeg, 1976d), and the parotid secretion of our IgA-deficient patient contained about thirteen times more IgM than normal (Brandtzaeg, 1971). Despite the relatively large number of J-chain-positive IgD- and IgG-producing cells in his parotid gland (Table 3), the IgG concentration in his parotid secretion was increased only six times and IgD was hardly detectable. These immunoglobulins do not combine with J chain, and accordingly there was no definite concealment of cytoplasmic J chain determinants in immunocytes of these classes (Tables 4 and 5). Moreover, these immunoglobulins show no affinity for SC (Brandtzaeg, 1977). This fact most likely explains their lack of active external transport through glandular epithelium. The increased IgG concentration in the parotid secretion of our IgA-deficient patient could be explained by a raised serum concentration of this immunoglobulin, giving rise to an enhanced passive external IgG 'leakage' (Brandtzaeg, 1971).

Several attempts have been made to relate the salivary gland secretion of IgA to various diseases. The balance of evidence indicates that susceptibility to dental caries is associated with a low output of salivary IgA (Örstavik & Brandtzaeg, 1975; Challacombe, 1976; Everhart, Klapper, Carter & Moss, 1977). *Streptococcus mutans* is considered of great importance in the etiology of dental caries, and agglutinins to this bacterium is normally present in salivary IgA and in salivary IgM of individuals with selective IgA deficiency (Arnold, Cole, Prince & McGhee, 1977). In this connection it is of interest that those immunodeficient individuals who do not have a compensatory secretion of salivary IgM show an increased caries susceptibility (Arnold *et al.*, 1977). Altogether these results suggest that local production of secretory immunoglobulins can influence bacterial activity in the oral cavity, and that basic knowledge about the immunocyte populations of the salivary glands therefore may be of value in future health measures.

In several studies attempts have been made to relate salivary IgA to diseases other than dental caries. No reproducible associations have appeared, except an increased level of salivary IgA in patients with oropharyngeal cancer (Mandel, Dvorak & Decosse, 1973; Brown, Lally, Frankel, Harwick, Davis & Rominger, 1975). Although it cannot be excluded that this finding reflects an influence of tobacco and alcohol habits on the secretory immune system (e.g. on the production of precursor cells in Peyer's patches and tonsils or on the secretory dynamics of oral glands), IgA immune responses to tumour-associated antigens is a challenging possibility. Thus, salivary IgA antibodies to Epstein Barr virus have been found in 50% of patients with nasopharyngeal carcinoma (Desgranges, Li & De-Thé, 1977). Since IgA may act as a tumour-enhancing antibody (O'Neill & Romsdahl, 1974), further work is needed to study the response pattern of salivary gland immunocytes in relation to neoplastic lesions.

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