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Immunoglobulin systems of human tonsils

I. CONTROL SUBJECTS OF VARIOUS AGES: QUANTIFICATION OF Ig-PRODUCING CELLS, TONSILLAR MORPHOMETRY AND SERUM Ig CONCENTRATIONS

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SUMMARY

Specimens of clinically normal palatine tonsils were studied by morphometry and immunohistochemistry, with regard to the relative tissue contribution and the content of Ig-producing immunocytes of four morphological compartments: the germinal centres of lymphoid follicles, their mantle zones, the extrafollicular area and the reticular parts of the crypt epithelium. Ig-producing cells occurred in all compartments; most of them were located in the extrafollicular area, although their density was highest in the reticular epithelium. There was a general predominance of IgG cells—including the blasts present in germinal centres. In subjects 4–25 years old, the tonsillar immunocyte population showed overall IgG:IgA:IgM:IgD class ratios of 65·2:30·1:3·5:1·2. IgE-producing cells were virtually absent.

A reticular distribution of non-diffusible immunoglobulins, especially IgM, was observed in the germinal centres—apparently bound to dendritic reticular cells. The mantle zones commonly contained numerous lymphocytes with membrane-related immunofluorescence, particularly prominent for IgD and less distinct for IgM. These B-cells were probably derived from local clonal expansion processes. There was no indication of active immunoglobulin transport through the tonsillar epithelium, which was devoid of 'secretory component'.

In subjects 30–81 years old, lymphoid elements of the tonsils were reduced, especially the follicular mantles and the reticular crypt epithelium, as well as their content of Ig-producing immunocytes. Such cells were also fewer in the germinal centres and in the extrafollicular area. Moreover, some shifts in the immunocyte class ratios had occurred in the various tissue compartments. In this age group, the number of tonsillar IgA cells showed a significant negative correlation with the rate of synthesis of serum IgA.

INTRODUCTION

Davis (1912) reported that typical plasma cells occur in human tonsils 2–3 weeks after birth, and are present there even to advanced age. He observed the first cells just beneath the crypt epithelium and believed that they appeared 'due to a chronic infectious or absorptive process'. By means of various membrane markers, recent studies have demonstrated both B-and T-cells in suspensions made from human tonsils. The balance of evidence suggests that there are proportionately more B-cells than in the peripheral blood (Delespesse *et al.*, 1976; Willson *et al.*, 1976). The tissue distribution of such membrane markers has indicated that T-cells are chiefly located in the extrafollicular areas (Takeuchi,

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Tabata & Kitashoji, 1976; Olah & Törö, 1975), whereas B-cells seem to be concentrated in the lymphoid follicles (Takeuchi *et al.*, 1976). The function of the tonsillar B-cell system has been studied by the quantification of immunoglobulins in extracts of tonsils (Siegel & Wilke, 1976), by estimating the formation of immunoglobulins and antibodies in cultures (Smith, Sherman & Newcomb, 1974; Platts-Mills & Ishizaka, 1975) and by localizing Ig-producing cells in tissue sections. Chiappino & Corbetta (1962) initially reported immunohistochemical studies on the presence of intracellular and extracellular immunoglobulins in human palatine tonsils. The first attempt to differentiate between various classes of Ig-producing immunocytes was made by Crabbe & Heremans (1967) in inflamed human pharyngeal tonsils (adenoids). They found a predominance of IgG cells over IgA cells and relatively few IgM and IgD cells.

At about the same time, some less specified immunohistochemical reports appeared, concerning the human palatine tonsils (Rossen *et al.*, 1968; Zaccheo & Pallestrini, 1968; Ricci & Russolo, 1970). These investigations were carried out on heterogeneous pathological material obtained at tonsillectomy. Moreover, no attempt was made to enumerate the immunocytes present in the different morphological compartments. Here we report such quantitative studies performed on specimens of clinically healthy palatine tonsils. In addition to basic information on the immunological function of tonsils, this investigation provided control material for a subsequent study of tonsils with clinical signs of disease (Surjan, Brandtzaeg & Berdal, 1978).

MATERIALS AND METHODS

Tonsil specimens. Among patients who needed surgery in the Department of Oto-rhino-laryngology, Rikshospitalet, those who had clinically normal tonsils and no history of recurrent throat infections, and who (or whose parents) consented to participate in the study were selected. While the patient was under general anesthesia for the necessary operation, a small specimen was excised from one of the palatine tonsils. One group of patients (two females and six males) was under 26 years of age, while another (three females and six males) was over 29 years of age (see Table 1). In the younger group three were operated on because of facial traumas, others had a cervical cyst or abscess, and one had otitis. In the older group most of the patients had benign or malignant tumours.

Immunohistochemistry. The biopsy specimen was collected in ice-chilled saline and subsequently divided into small pieces, which were fixed directly in cold alcohol or extracted for 48 hr in phosphate-buffered saline before fixation (Brandtzaeg, 1974). Only the latter tissue blocks were used for the enumeration of Ig-containing immunocytes. After paraffin embedding, serial sections were cut at 6 μm and incubated with 'green' (fluorescein) and 'red' (rhodamine) rabbit IgG conjugates specific for the five human Ig classes, $F(ab')_2$ or 'secretory component' (SC). The characteristics of these reagents and the fluorescence microscope conditions with incident light illuminator have been reported before (Brandtzaeg, 1976a).

The first section in a series was routinely stained with haematoxylin and eosin (HE) for morphological evaluation, the second was incubated with 'green' anti-IgG combined with 'red' anti-IgA and the third with 'green' anti-IgD combined with 'red' anti-IgM. This paired immunofluorescence staining was done to substantiate the immunological specificity of the reagents (cells should show pure colours) and to obtain reliable differential cell counts by relating the number of one immunocyte class to that of another in the same microscopic field. The anti-IgE reagent was applied as a 'green' or 'red' conjugate in combination with anti-$F(ab')_2$, anti-IgD or anti-IgM of contrasting colours. A 'red' anti-SC reagent was used in combination with a 'green' anti-IgA conjugate on the sections of directly alcohol-fixed specimens for the evaluation of epithelial staining.

Enumeration of Ig-containing cells. The density of each immunocyte class was based on counts made in a tissue unit (0·1 mm^2), defined by the outer frame of an ocular grid (Leitz code No. 519902) when the section was viewed through a Leitz × 22 immersion objective and a × 10 ocular. Green cells were first counted under narrow-band excitation for fluorescein staining; after switching to selective conditions for rhodamine fluorescence, red cells were enumerated in the same unit area. Cells with a discernible nucleus and a pure cytoplasmic colour, as well as green or red cell-like bodies with a diameter of about 8 μm or more, were included. Truly double-stained cells were extremely rare (see Fig. 6), in agreement with previous observations made on cell suspensions (Ferrarini *et al.*, 1976).

The enumerations were performed in a systematic pattern; starting below the subepithelial conncetive tissue in the upper left part of the section, every second unit was counted throughout the entire section including, at random, parts of four defined tissue compartments (Fig. 1). A quantitative record was kept of the fluorescent cells in each tissue unit evaluated, and of their distribution among the four compartments. On this basis, cell density could be determined in an average 'tonsillar tissue unit'. Moreover, by taking into account the morphometric data (see below), the cell density in an imaginary 'compartment unit' could be calculated; this parameter was useful in comparisons with pathological tonsils (Surjan *et al.*, 1978). All enumerations were made by one observer. Good reproducibility was confirmed by counting the IgG cells in the same section five times, giving a coefficient of variation of 2·1%.

Tonsillar morphometry. The proportions of the four tissue compartments (Fig. 1) were determined in HE sections by a point-counting method, essentially as described by Weibel & Elias (1967). Briefly, using the ocular grid, the number of

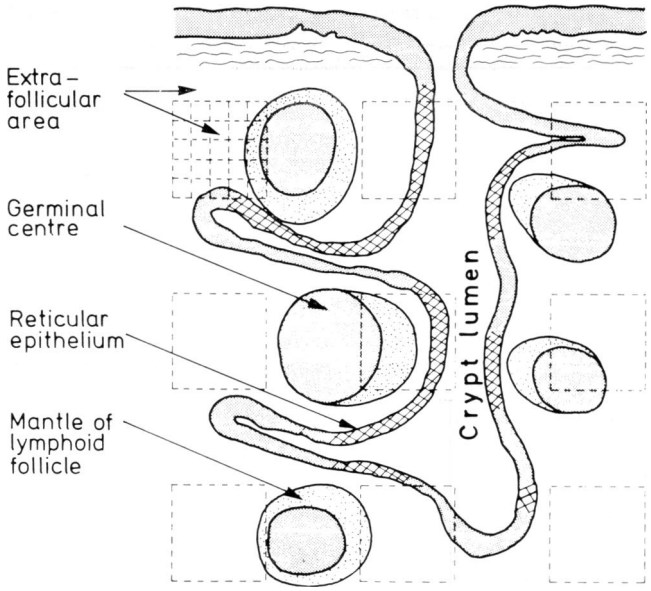


FIG. 1. Schematic representation of a section through a human palatine tonsil. Immunocyte enumerations were based on counts of Ig-containing cells in a defined unit tissue area which, as indicated, included at random parts of four morphological compartments: the extrafollicular area (excluding the faucial epithelium at the top and the adjacent connective tissue), the germinal centres of the lymphoid follicles, their mantle zones and the reticular parts of the crypt epithelium.

cross-points falling on each tissue type was counted and compared with the point number corresponding to the entire section area, avoiding the surface epithelium and the connective tissue beneath it. In the final calculations, the non-reticular crypt epithelium was excluded, so the four compartments studied do not comprise 100% (see Table 2).

Serum immunoglobulins. The blood samples were allowed to clot at room temperature and the separated serum was stored at −70°C. IgG, IgA and IgM were quantified by single radial immunodiffusion with reference to defined standard proteins (Brandtzaeg, Fjellanger & Gjeruldsen, 1970). Monospecific rabbit antisera were used in the agarose plates. IgD was measured in the same way with reference to the British Research Standard 67/37. On the basis of comparison with a purified IgD myeloma protein, 1 unit (u) was estimated to be 1·76 μg. All quantifications were done at least in duplicate.

In order to obtain a relative expression for the rate of synthesis of serum immunoglobulins, two variables had to be taken into account: the fractional catabolic rate and plasma volume. For IgG, the fractional catabolic rate increases in direct proportion to the serum concentration (Waldmann & Strober, 1969). The IgG values were therefore individually adjusted, as recently detailed by Turesson (1976). Further multiplication by approximate body weight (according to the Norwegian average values) afforded adjustment relative to the plasma volume. IgA and IgM values were only adjusted by the latter multiplication, since their rates of synthesis are directly proportional to the serum concentrations (Waldmann & Strober, 1969). There is some indication of an inverse relationship between the IgD fractional catabolic rate and IgD concentration (Waldmann & Strøber, 1969), but adjustments were only based on body weight.

Statistical analyses. Immunocyte enumerations were expressed as arithmetic means of absolute counts and of class ratios within a unit tissue area. Plotting on a normal probability paper indicated a fairly normal distribution of the data, and group comparisons were made by the Student's *t*-test. An analysis of variance was applied to demonstrate the homogeneity of the data for various specimens from a single tonsil. Immunoglobulin concentrations were transformed logarithmically to produce a more normal distribution before comparisons were made by the Student's *t*-test. Differences showing $P \leq 0{\cdot}05$ were accepted as statistically significant. By linear regression analyses, correlations were sought between immunocyte numbers, age and rates of synthesis of the immunoglobulins.

RESULTS

Serum immunoglobulins (Table 1)

Excepting IgD, the immunoglobulin concentrations were slightly higher in the older age group, but due to wide individual variations these differences were not statistically significant. The same held true when the immunoglobulin levels were expressed as relative rates of synthesis.

TABLE 1. Serum immunoglobulin levels as geometric means of concentrations and as relative rates of synthesis (mean ± s.d.) in two subject groups of various ages

	Group 1 (mean age: 14 years; range: 4–25; $n = 8$)			Group 2 (mean age: 60 years; range: 30–81; $n = 9$)		
Ig class	g/l		(g/l) × kg*	g/l		(g/l) × kg*
IgG	10·79	(6·29–18·52)†	41·8 ± 29·2	11·96	(4·75–30·13)	71·6 ± 34·7
IgA	2·46	(< 0·01–8·53)	147·2 ± 148·5	2·86	(1·38–5·93)	202·9 ± 85·2
IgM	0·96	(0·41–2·24)	52·7 ± 40·7	1·07	(0·41–2·79)	76·7 ± 33·0
IgD	0·035	(0·004–0·280)	4·4 ± 9·0	0·030	(0·002–0·397)	3·3 ± 2·8

* Relative rate of synthesis indicated by the product of serum concentration and body weight, and for IgG this is multiplied by the fractional catabolic rate as well.

† 95% probability limits.

TABLE 2. Relative areas of tonsillar morphological compartments studied in the two subject groups

	Mean area percentage ± s.d.	
Tissue compartment	Age: 4–25 years	Age: 30–81 years
Germinal centre	18·1 ± 7·5	15·2 ± 7·6
Mantle of the follicle	12·8 ± 4·3	7·4 ± 4·8*
Extrafollicular area	51·1 ± 7·5	61·0 ± 14·1*
Reticular epithelium	6·5 ± 2·2	4·0 ± 3·7*

*Difference statistically significant.

Tonsillar histology and morphometry (Table 2)

The faucial epithelium and the connective tissue beneath it, as well as the connective tissue septa, were virtually devoid of lymphoid cells. Activated lymphoid follicles were numerous, but of fairly small size. In the older age group, the extrafollicular area was significantly increased and some fibrosis was seen. Moreover, only a small part of the crypt epithelium was invaded by lymphoid cells. The contribution of the reticular epithelium was consequently significantly reduced compared with the younger age group; the same held true for the mantle zone of the lymphoid follicles.

Extracellular and epithelial immunoglobulin components

In directly alcohol-fixed specimens, extracellular immunoglobulins are retained in the connective tissue ground substance (Brandtzaeg, 1974). The staining was always, as expected, of high intensity for IgG, less for IgA and relatively faint for IgM. The greatest accumulation of extracellular immunoglobulins was seen in the connective tissue beneath the faucial epithelium and adjacent to the crypts (Fig. 2). In these areas, most IgG-containing cells were poorly visualized because of the bright background fluorescence, whereas IgA and IgM cells were usually fairly well discerned (Fig. 3). 'Leakage' of immunoglobulins, especially IgG, between epithelial cells was frequently indicated in the crypt epithelium (Figs 2b and 3a); staining for the three major Ig classes was, moreover, seen in the contents of the crypt (Fig. 3). The squamous faucial epithelium was generally negative, except for epithelial cells —single or in clusters or bands—showing cytoplasmic staining, especially for IgG (Fig. 2). SC was neither revealed in the faucial nor in the crypt epithelium.

In the deeper parts of the tonsil, extracellular fluorescence was less prominent, except in the vessel

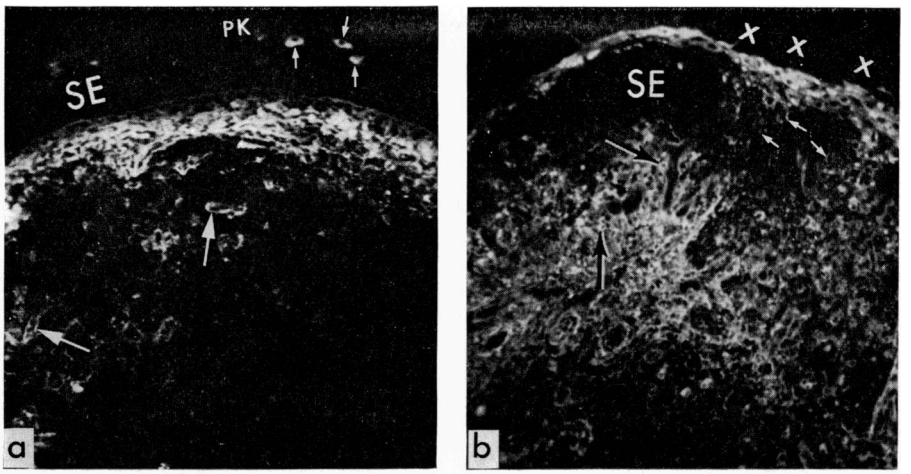


FIG. 2. (a) Immunohistochemical red staining for IgG in a section of a directly alcohol-fixed specimen of a clinically normal palatine tonsil from a 5-year-old boy. There is an accumulation of extracellular IgG in the connective tissue adjacent to the faucial surface epithelium (SE). In the underlying lymphoid tissue, IgG is mainly present in the vessel walls (large arrows) and in a few plasma cells. In the surface epithelium IgG is seen in three epithelial cells (small arrows) beneath the parakeratotic layer (PK). (b) Immunohistochemical red staining for IgG and green for IgA (double exposure) in a section of a directly alcohol-fixed tonsillar specimen from a 19-year-old man with hyperplastic tonsillitis. There is a more extensive extracellular immunoglobulin staining in the connective tissue beneath the faucial surface epithelium (SE), and numerous Ig-producing cells (large arrows) are more or less concealed by the bright background staining. A band of Ig-containing epithelial cells is seen, being especially prominent at the entrance of a crypt (× × ×). Traces of intercellular immunoglobulins (small arrows) are present in the crypt epithelium. (Magnification of (a): × 90; of (b): × 120.)

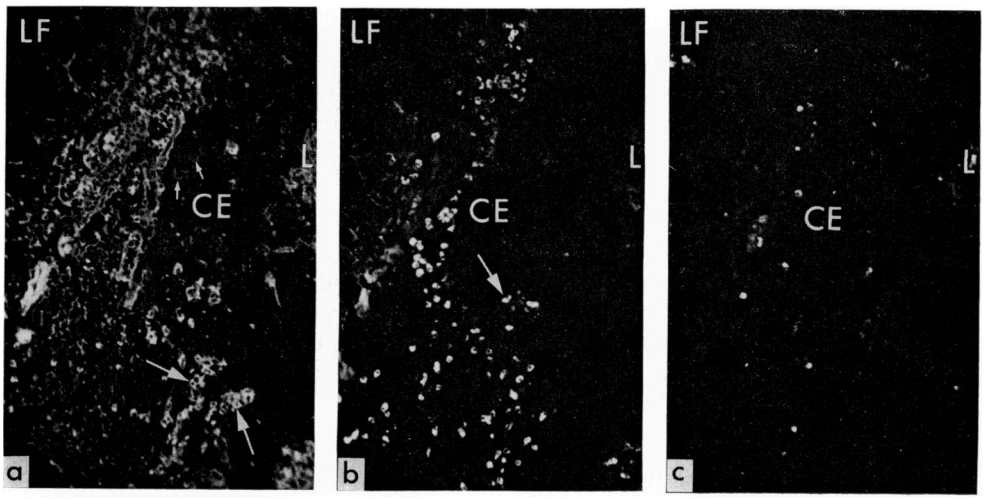


FIG. 3. Immunohistochemical red staining for (a) IgG, (b) IgA and (c) IgM in three adjacent sections of a directly alcohol-fixed specimen of a clinically normal tonsil (same as in Fig. 2a) showing comparable fields from a crypt region. Numerous IgG-producing cells lying between a lymphoid follicle (LF) and the crypt epithelium (CE) are more or less concealed by extracellular staining, whereas IgA- and IgM-producing cells are easily seen. Note epithelial traces of intercellular IgG (small arrows) and Ig-producing immunocytes (large arrows) in the reticular crypt epithelium. Some Ig-positive material is seen in the crypt lumen (L). (Magnification for (a), (b) and (c): × 75.)

walls (Fig. 2) and germinal centres. In the latter site, there was commonly an intense reticular staining, especially for IgM and IgG. It was not possible to decide whether this represented extracellular, membrane-associated or intracellular immunoglobulins. In saline-extracted specimens, where most diffusely distributed proteins were removed (Brandtzaeg, 1974), the germinal centre-staining persisted for IgG and IgM, especially for the latter (Fig. 4b), and sometimes also for IgA (Fig. 5a).

Extracellular IgE and IgD were not detected. A possible exception was a frequently observed IgD-staining in the mantle zone of the lymphoid follicles, indicating that the lymphocytes were surrounded by IgD. This feature persisted in saline-extracted specimens, and at high magnification the fluorescence

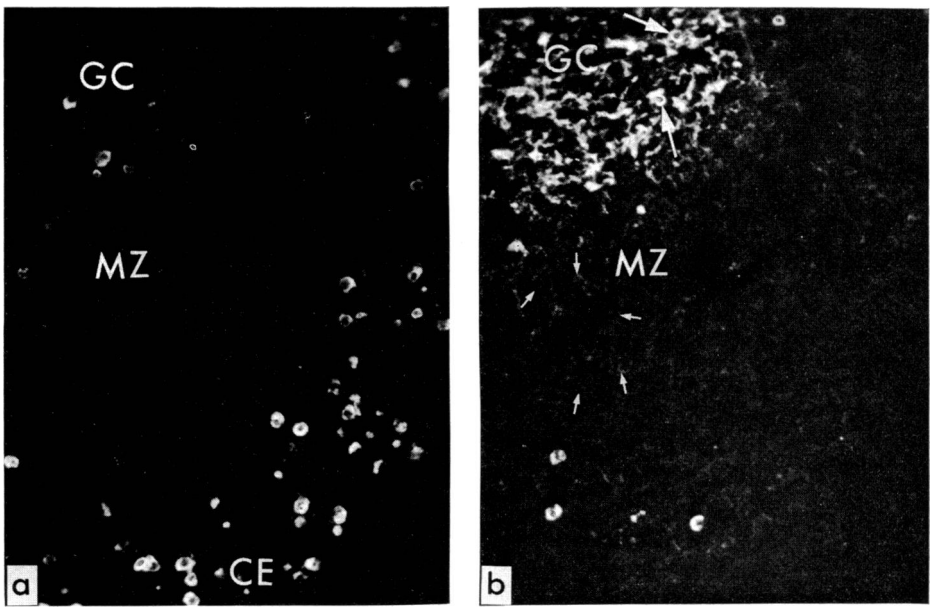


FIG. 4. Paired immunohistochemical green staining for (a) IgA and red for (b) IgM in a section (same field) of a saline-extracted specimen of a clinically normal palatine tonsil from a 5-year-old boy. There is a group of IgA-producing cells between a lymphoid follicle and the crypt epithelium (CE); some IgA cells are also present in the germinal centre (GC) and in the reticular crypt epithelium. Few IgM-producing cells are present, and some of them (large arrows) are partly concealed by a bright reticular staining in the germinal centre. Some faint IgM staining (small arrows) is seen in the mantle zone (MZ) of the follicle, perhaps related to lymphocyte membranes. (Magnification for (a) and (b): × 185.)

pattern was compatible with membrane-associated IgD (Fig. 5b and c). Very little extracellular IgG and IgA occurred in the mantle zone, and only small amounts persisted in the saline-extracted specimens (Figs 4a and 6d to i). Some IgM was commonly retained (Fig. 4b), but usually in a more diffuse pattern than IgD, precluding a distinction between extracellular and membrane localization.

Quantification of Ig-producing immunocytes

The enumeration of Ig-containing cells was performed in sections of saline-extracted tissue from which most extracellular immunoglobulins had been removed. Some retention was seen along epithelia and vessel walls, but this did not usually disturb the cell counting. The same held true for the reticular staining of germinal centres, although visualization of the IgM-producing immunocytes was occasionally jeopardized (Fig. 4b).

Most Ig-producing cells were found in the extrafollicular area, and they were concentrated especially between the follicles and the crypt epithelium (Fig. 6). A considerable number of cells with cytoplasmic staining were likewise seen in the interstices of the reticular epithelium (Fig. 6c). The numbers of such

cells in the germinal centres varied greatly, whereas they were always low in the mantle zone of the lymphoid follicles (Fig. 6d to i).

To obtain representative data, the quantification of Ig-containing cells had to be based on enumerations throughout the tissue section (Fig. 1) because the immunocytes were so irregularly distributed. This problem is illustrated for IgG and IgA cells in Fig. 7. Stable, and hence true, estimates of the number of these immunocytes in an average tonsillar tissue unit were not obtained until the calculations had been based on more than twenty-five examined units. It was therefore decided to count at least thirty units from each tonsil for each immunocyte class.

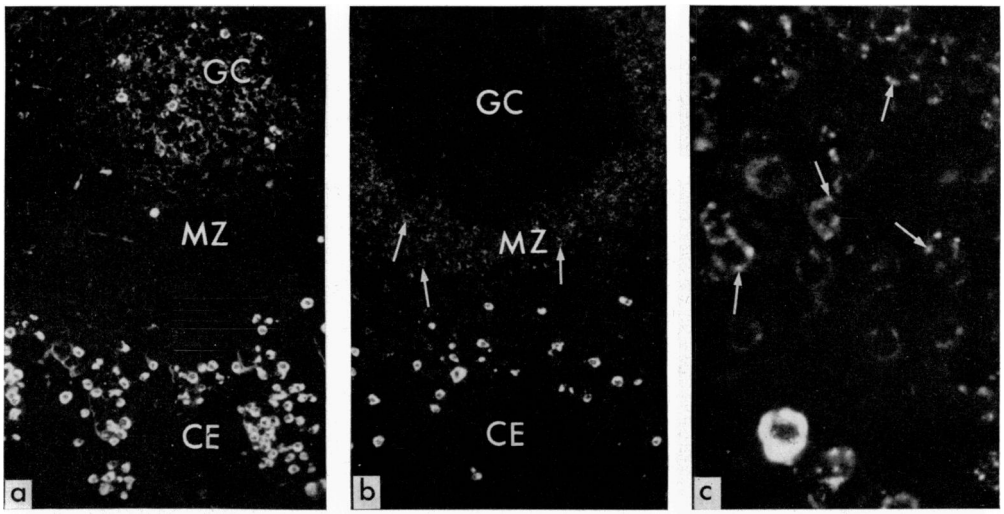
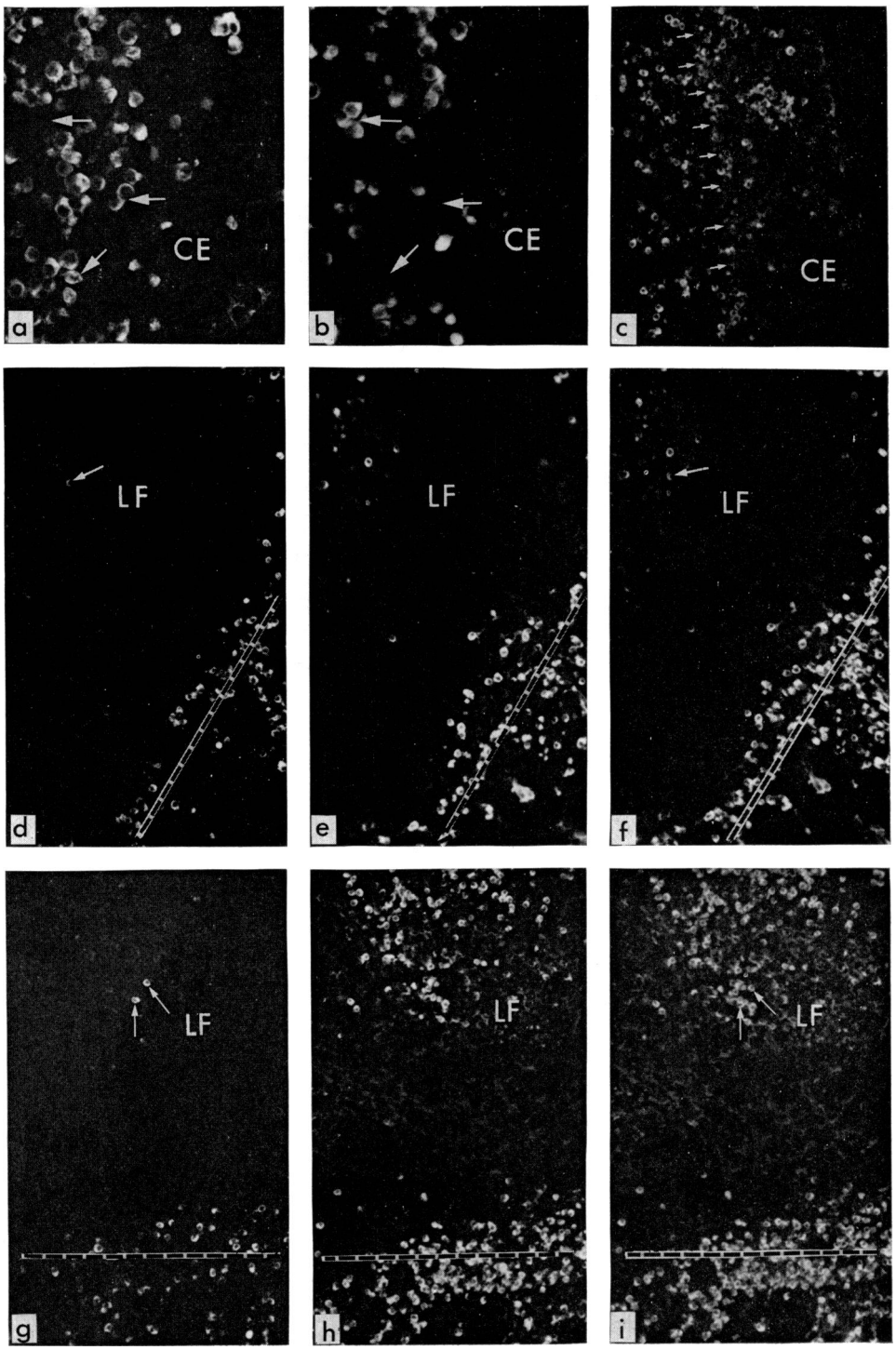


FIG. 5. Immunohistochemical red staining for (a) IgA and (b) IgD in two adjacent sections (comparable fields) of a saline-extracted specimen of a clinically normal palatine tonsil from a 5-year-old boy. There are numerous IgA- and some IgD-producing cells adjacent to, and in, the reticular crypt epithelium (CE). A few IgA-producing cells are present in the germinal centre (GC) of a lymphoid follicle, partly concealed by a reticular staining. Note that virtually no IgA is present in the mantle zone (MZ) where, on the contrary, there is a distinct IgD staining (arrows), apparently related to the periphery of the lymphocytes. In (c) the granular appearance of this membrane-staining (arrows) is shown along with the cytoplasmic fluorescence of an IgD-producing plasma cell. (Magnification of (a) and (b): × 105; of (c) × 580.)

An efficient extraction of the extracellular immunoglobulins depends on a small-size tissue specimen (Brandtzaeg, 1974), and only 10–15 units could usually be counted in a tonsillar section. Enumerations for individual tonsils therefore had to be based on several tissue blocks. The effect of such data pooling was tested with five tonsils. The homogeneity of the mean cell density numbers from two to four tissue blocks was examined by an analysis of variance, and the hypothesis of equal means held true in each case.

The three major immunocyte classes were found in every specimen studied. In the younger age group, IgG-producing cells predominated clearly in all tissue compartments (Table 3). Most of these cells were located in the extrafollicular area, as were the other immunocyte classes (Table 4). The average number of IgA cells was slightly less than half of the IgG cell number; their proportion was increased above this level only in the reticular epithelium (Table 3), where the highest immunocyte density was found. Remarkably few IgA-producing immunocytes were present in the germinal centres. The average number of IgM cells was only 5–6% of the IgG cell number, but their relative share was increased in the lymphoid follicles, where they occurred about as frequently as the IgA cells (Tables 3 and 4). The average number of IgD cells was approximately 33% of the IgM cell number; their relative share was usually greatest in the reticular epithelium (Tables 3 and 4), although most of them were encountered between the epithelium and the adjacent lymphoid follicles (Fig. 5b). IgE-containing immunocytes were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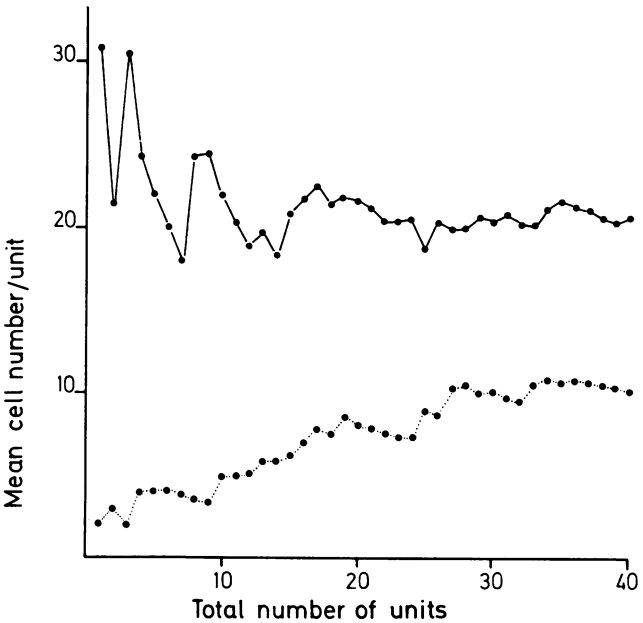

FIG. 7. Influence of the total number of tonsillar tissue units evaluated on the estimated specimen mean number of immunocytes per tissue unit. (●—●) IgG-containing cells; (● · · · ●) IgA-containing cells. The counts were made in sections from a clinically normal tonsil.

not found in the tonsils, except for a few cells in occasional sections. This class was therefore excluded from the calculations referred to above.

In the older age group, the overall immunocyte class ratios were similar to those in the younger group (Table 3), but the average cell number per tissue unit was reduced almost to half (Table 4). This decrease was most uniform for the IgG class. A scatter diagram (Fig. 8) indicated that after 18 years, the reduction in average cell number, including all classes, shows a linear negative correlation with age ($r = -0{\cdot}824$, $P<0{\cdot}01$). This correlation was not so strong when the older age group was analysed separately ($r = -0{\cdot}639$, $0{\cdot}05<P<0{\cdot}1$). In the younger age group there was only a very weak positive correlation ($r = 0{\cdot}199$).

Apparent shifts in the immunocyte class ratios had occurred in some of the tonsillar tissue compartments in the older age group (Tables 3 and 4). Thus the share of IgM cells had increased in the germinal

FIG. 6. (a) and (b) Paired immunohistochemical green staining for IgA and red for IgG in a section of saline-extracted specimen of a clinically normal palatine tonsil from an 11-year-old boy. In this field adjacent to the crypt epithelium (CE) there is a slight predominance of IgA-producing cells, and this was one of the three control specimens where the overall proportion of IgA cells exceeded 40%. Identical positions in the two pictures are marked by arrows for orientation. Note absence of double-stained cells. (c) Red staining for Ig light chains in a section of a saline-extracted specimen of a clinically normal tonsil from a 5-year-old boy. Note infiltration of Ig-producing cells in the reticular part (arrows) of the crypt epithelium (CE). (d) to (f) Paired immunohistochemical green staining for IgA (d), red for IgG (e) and double exposure of same field (f) in a section of a saline-extracted specimen of a hyperplastic palatine tonsil from a 6-year-old boy. A lymphoid follicle (LF) contains a single IgA cell (arrow) and a few IgG cells in its germinal centre, whereas there are numerous immunocytes of both classes adjacent to, and in, the reticular crypt epithelium (to the right of stippled line). By close inspection it will be seen that no double-stained cell is present. (g) to (i) Same conditions as in (d) to (f), but a specimen from a 9-year-old girl with hyperplastic tonsillitis and repeated episodes of pharyngitis during the last 6 months. A highly activated lymphoid follicle (LF) contains numerous IgG-producing cells in its prominant germinal centre, but there are only two cells (arrows) of the IgA class. Numerous immunocytes of both classes are present adjacent to and in the reticular crypt epithelium (below stippled line). Again there are no double-stained cells. Although (d) to (i) illustrate clinically diseased tonsils, similar staining patterns were seen in clinically normal specimens. (Magnification of (a), and (b): × 230; of (c) to (i): × 105.)

TABLE 3. Class distribution (percentage) of Ig-containing cells within various tonsillar tissue compartments in two age groups

Localization and class of immunocytes	Mean percentage ± s.d.	
	Age: 4–25 years	Age: 30–81 years
Total specimen		
IgG	65·2 ± 11·8	64·8 ± 9·6
IgA	30·1 ± 11·7	30·1 ± 7·8
IgM	3·5 ± 2·0	4·0 ± 1·8
IgD	1·2 ± 1·1	1·1 ± 2·1
Germinal centre		
IgG	72·3 ± 12·5	65·2 ± 14·2
IgA	12·6 ± 9·7	9·3 ± 6·6
IgM	14·3 ± 11·9	25·0 ± 16·9
IgD	0·8 ± 1·0	0·5 ± 0·9
Mantle of the follicle		
IgG	68·4 ± 22·0	49·9 ± 23·6
IgA	21·1 ± 14·0	48·4 ± 24·0
IgM	9·8 ± 9·3	4·4 ± 9·0
IgD	0·7 ± 1·9	1·3 ± 3·5
Extrafollicular area		
IgG	67·0 ± 11·4	66·8 ± 10·2
IgA	29·7 ± 11·3	29·4 ± 9·2
IgM	2·3 ± 1·4	2·7 ± 1·5
IgD	1·0 ± 1·3	1·1 ± 2·3
Reticular epithelium		
IgG	56·3 ± 13·6	43·2 ± 11·9
IgA	38·1 ± 13·4	50·6 ± 11·5
IgM	3·6 ± 3·8	2·9 ± 3·4
IgD	2·0 ± 2·6	3·3 ± 4·7

centres, obviously because their number had remained fairly stable in this site. A relative increase in IgA cells was noted in the mantle zone and reticular epithelium, but there were wide individual variations. In this age group, there was, moreover, a fairly strong positive correlation ($r = 0{\cdot}782$, $0{\cdot}05 < P < 0{\cdot}1$) between the tonsillar IgM cell density and the rate of synthesis of this Ig class as measured in the serum, whereas the tonsillar IgA cell density, and to a lesser degree that of IgM cells, were negatively correlated with the rate of synthesis of serum IgA ($r = -0{\cdot}811$, $P = 0{\cdot}05$, and $r = -0{\cdot}521$, respectively). In both age groups the tonsillar IgD cell density was positively correlated with the rate of synthesis of IgD ($r = 0{\cdot}460$ and $r = 0{\cdot}499$, respectively), but there were too large individual variations to expect a possible association to show up as statistically significant. In the older age group two subjects lacked tonsillar IgD cells, and one of these had undetectable serum IgD. Other correlations between the tonsillar immunocyte density and the rate of synthesis of immunoglobulins turned out to be negligible in both groups.

DISCUSSION

This is the first extensive investigation of Ig-producing cells in clinically normal human tonsils. The predominant immunocyte was found to be of the IgG class in all of the seventeen palatine tonsils examined from subjects between 4 and 81 years of age. Our result is in contrast to previous limited studies on similar material. Ricci & Russolo (1970) reported IgA cells to be most frequent in normal palatine tonsils, but did not give methodological details or quantitative data. Chen & Izui (1971) examined four autopsy specimens and found a slight preponderance of IgA over IgG cells. The other

TABLE 4. Quantification of the Ig-containing cells in an average tonsillar tissue unit and their distribution between various tissue compartments in two age groups

Immunocyte class and tissue compartment	Mean cell number per unit ± s.d.	
	Age: 4–25 years	Age: 30–81 years
IgG		
Germinal centre	4·88 ± 3·9	1·69 ± 1·1*
Mantle of the follicle	0·90 ± 0·5	0·56 ± 0·8*
Extrafollicular area	26·32 ± 5·3	15·80 ± 3·8*
Reticular epithelium	7·95 ± 2·4	3·77 ± 5·4*
Total unit	40·05 ± 5·7	21·82 ± 6·8*
IgA		
Germinal centre	0·78 ± 0·6	0·30 ± 0·2
Mantle of the follicle	0·25 ± 0·2	0·40 ± 0·5
Extrafollicular area	12·64 ± 7·5	6·14 ± 1·4*
Reticular epithelium	6·10 ± 3·4	3·10 ± 3·5
Total unit	19·77 ± 10·6	9·94 ± 3·3*
IgM		
Germinal centre	0·70 ± 0·4	0·50 ± 0·5
Mantle of the follicle	0·11 ± 0·1	0·04 ± 0·1
Extrafollicular area	0·91 ± 0·6	0·55 ± 0·6
Reticular epithelium	0·53 ± 0·7	0·21 ± 0·2
Total unit	2·25 ± 1·4	1·30 ± 0·6
IgD		
Germinal centre	0·05 ± 0·1	0·01 ± 0·0
Mantle of the follicle	0·00 ± 0·0	0·01 ± 0·0
Extrafollicular area	0·39 ± 0·4	0·26 ± 0·3
Reticular epithelium	0·25 ± 0·3	0·09 ± 0·2*
Total unit	0·69 ± 0·5	0·37 ± 0·7
All immunocyte classes	62·76 ± 11·3	33·43 ± 8·6*

* Statistically significant decrease.

TABLE 5. Previously reported overall class distribution (percentage) of Ig-containing cells from human tonsils

Authors	Material	Class of cells				
		IgG	IgA	IgM	IgD	IgE
Turesson (1976)	Suspensions of seven (palatine ?) tonsils from patients (age ?) with recurrent tonsillitis	61	31	9	n.d.*	n.d.
Ferrarini *et al.* (1976)	Suspensions of five (palatine ?) tonsils obtained at tonsillectomy of patients 2–30 years old	81·6	13·8	10·2	5·6	n.d.
Chen & Izui (1971)	Immunohistochemistry of four clinically normal palatine tonsils from young adults	43·4	50·1	6·5	n.d.	n.d.
Tada & Ishizaka (1970)	Immunohistochemistry of thirty-three palatine tonsils from patients (age ?) with hyperplastic tonsillitis	++++	++++	+	+ (++)	5

* n.d. = not determined.

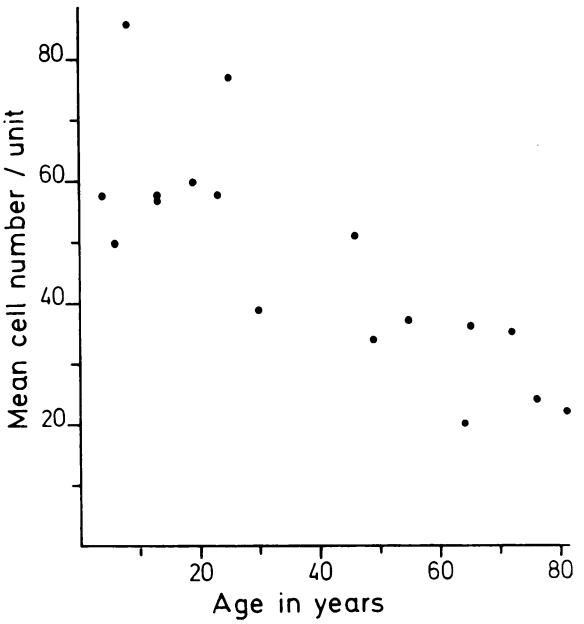

FIG. 8. Scatter diagram of relation in seventeen subjects between age and the number of Ig-containing cells (all classes) per tonsillar tissue unit ($r = -0{\cdot}809$, $P < 0{\cdot}01$).

quantitative data in the literature (Table 5) have been derived from unspecified samples obtained at tonsillectomy, and are based on cell suspensions, except for the study of Tada & Ishizaka (1972), that focussed on the quantification of IgE cells. It will be seen that our immunocyte class ratios (Table 3) conform best with those recently reported by Turesson (1976). His specimens were obtained from patients with recurrent tonsillitis, but as shown in our subsequent report, there are only small overall shifts in class proportions associated with disease (Surjan *et al.*, 1978).

Several technical problems may explain the discrepant findings. IgG-producing cells are easily concealed by the abundance of retained extracellular IgG, even in frozen sections (Crabbe & Heremans, 1967), and especially in directly fixed material such as that used by Chen & Iziu (1971). Moreover, Gitlin & Sasaki (1969) reported that the immunoglobulin content per tonsillar immunocyte is decreased in the order IgA:IgM:IgG by the proportions 5·6:3·7:1·0. This probably explains why IgG cells may show a relatively faint staining, and special precautions must be taken to detect all of them in tissue sections (Brandtzaeg, 1974).

High quality of the fluorochrome conjugates is an additional requirement for reliable immunohistochemical results. This is particularly exemplified by discrepant observations on IgE-containing cells (Brandtzaeg & Baklien, 1976a). We found this immunocyte class to be virtually lacking in palatine tonsils, in agreement with the results of Pesak (1971). Tonsillar tissue cultures have likewise failed to reveal IgE synthesis (Platts-Mills & Ishizaka, 1975). Conversely, Tada & Ishizaka (1970) reported an astonishingly high percentage of IgE cells in human and monkey tonsils. Subsequent studies have indicated that mast cells showing bright membrane staining for IgE may erroneously be taken as IgE-producing immunocytes (Feltkamp-Vroom *et al.*, 1975).

Quantification of Ig-producing cells by immunohistochemistry is of little value unless the enumerations are done in a reproducible manner that facilitates comparisons between individual tissue specimens and between studies in different laboratories. Our method for quantification of Ig-producing cells in the intestinal mucosa (Brandtzaeg & Baklien, 1976b) could not be adopted for the tonsils because of the very irregular distribution of immunocytes. We therefore recorded their number per tissue unit throughout the section within four tissue compartments, which include the lymphoid elements where important disease-associated changes may occur. The latter aspect will be dealt with in a subsequent publication

(Surjan *et al.*, 1978). The present work was carried out partly to establish a normal reference material. However, the selection of controls was largely determined by availability. Thus in the younger group there were only two girls, and most subjects in the older group suffered from neoplasia. There was no indication of any sex-dependent influence on the data, and in a histological study of tonsillar lymphoid elements Awaya (1969) found no sex-related differences. Evaluation of a possible influence of malignancy on the data from the older age group will have to await future studies.

The tonsils may exert immunological functions in different ways. Firstly, they may be central lymphoid organs involved in the development of the B-cell system, although such a bursa homologous function has not been demonstrated. Secondly, they may be important organs of contact between foreign material and lymphocytes, and hence sites of clonal expansion and cellular differentiation, for which much supporting evidence exists. Thus the reticular crypt epithelium is specialized for the absorption of foreign material (Olah & Everett, 1975), and the tonsils contain numerous activated lymphoid follicles. Moreover, they hold the complete sets of cells necessary for a primary antibody response (Watanabe *et al.*, 1974), and in sensitized children they contain T-and B-cells able to mount secondary immune responses (Platts-Mills & Ishizaka, 1975). A third important possibility—that stimulated tonsillar lymphoid cells are disseminated to other sites, for example mucosal glands, and there become effector cells—has, as yet, not been studied. Nevertheless, indirect suggestive evidence was provided by Ogra (1971), when he showed that the ability of children to produce nasopharyngeal secretory IgA antibodies against polio virus was markedly decreased after the combined removal of palatine and pharyngeal tonsils.

Our results demonstrate that, contrary to some suggestions in the literature, the B-cell responses in palatine tonsils do not give rise to the production of secretory immunoglobulins *in situ*. Tissue culture experiments have, moreover, provided a ratio of 3·7:1 for tonsillar IgG:IgA synthesis, and antigen stimulation *in vitro* has regularly given rise to a prominent IgG antibody production (Platts-Mills & Ishizaka, 1975). Indeed, IgG-committed B-cells that show little susceptibility to T-cell suppressor effects have been identified in tonsillar suspensions (Janossy *et al.*, 1977). Moreover, most IgA-containing cells in the palatine tonsils produce monomers without J chains (Brandtzaeg, 1976b). This result has also been supported by tissue culture experiments (Smith *et al.*, 1974; Platts-Mills & Ishizaka, 1975), and agrees with the fact that no SC could be detected in the tonsillar epithelium. Previous reports on this point have been contradictory (Rossen *et al.*, 1968; Schmedtje & Batts, 1973). Our results indicate that epithelial immunoglobulin transmission in the palatine tonsils mainly takes place as intercellular passive diffusion. This may well give a local protective effect, but it is known that IgG-mediated immune reactions can enhance epithelial penetrability to unrelated macromolecules (Brandtzaeg & Tolo, 1977). The Ig-positive groups of cells found in the surface epithelium correspond to observations made with other squamous epithelia (Brandtzaeg, 1975). Conversely, in the pharyngeal tonsils the surface epithelium may produce SC and show intracellular transport of secretory IgA and IgM (Brandtzaeg, unpublished observations).

Reports on the distribution of membrane immunoglobulins borne by tonsillar B-cells have been inconsistent, especially with regard to the IgG and IgA class (Delespesse *et al.*, 1976; Willson *et al.*, 1976), but IgM or IgD is most frequently encountered, and some cells bear both these classes (Ferrarini *et al.*, 1976). This agrees with the immunohistochemical indication of membrane-associated IgD, and possibly IgM, in the mantle zone of tonsillar lymphoid follicles. Ishikawa, Wicher & Arbesman (1972) likewise observed a prominent IgD-staining of the lymphocytes in that zone. Autoradiographic studies of rabbit tonsils have suggested that these lymphocytes are mainly derived from clonal expansion processes taking place in the germinal centre (Koburg, 1967). Antigens and immune complexes are trapped by antibody-binding in this site, resulting in an efficient stimulation of B-cells (Hirokawa, Easki & Nariuchi, 1973). The reticular staining noted, particularly for IgM, in germinal centres most likely represents antibodies bound to dendritic reticular cells via complement or Fc receptors, and has likewise been observed in activated lymphoid follicles in the gut (Baklien & Brandtzaeg, 1976). Contrary to the report of Tada & Ishizaka (1970), we did not see IgE in the tonsillar follicles.

B-cell differentiation in tonsillar germinal centres was found to give rise mainly to IgG-producing

blasts. However, heavy chain expression can switch in the direction $\mu \rightarrow \gamma \rightarrow \alpha$ (Cooper *et al.*, 1976), and tonsillar cells may well reach the blood and end up as IgA producers in glandular sites. B blasts generated in the tonsillar germinal centres are prone to be J chain-positive, which is a characteristic of gland-associated immunocytes (Brandtzaeg, 1976b). The studies of Koburg (1967) indicated that some of the stimulated cells migrate to extrafollicular areas and the reticular epithelium, and he postulated that they there receive a second signal for maturation. Our findings showed increasing proportions of IgA-producing cells in the latter sites, especially at an older age (Table 4), but, in contrast to the situation in gland-associated immunocytes, their J chain-synthesizing capacity was much decreased (Brandtzaeg, 1976b).

Except for IgD, serum levels and especially rates of synthesis of immunoglobulins were increased in the older age group. Nevertheless, there was a reduction of tonsillar lymphoid elements, including Ig-producing cells, lymphocyte mantle zones and reticular epithelium. The histological studies of Awaya (1969) likewise indicated an involution of tonsillar lymphocytopoiesis with advancing age. This change is more significant than indicated by our cell density measurements and morphometric analyses, since the total volume of the tonsils is also known to decrease.

The tonsillar density of IgD- and IgM-containing cells was positively correlated with the rate of synthesis of these immunoglobulins. This was especially so for IgM in the older age group. Conversely, the number of tonsillar IgA cells, and to a lesser degree IgM cells, showed in this age group a negative correlation with the rate of synthesis of IgA measured in serum. Since IgA probably is important in immunological homeostasis, due to its lack of phlogistic properties, it is conceivable that compensatory mechanisms may be reflected in inversely related levels of local and systemic IgA synthesis.

In conclusion, our study showed that the B-cell system of clinically normal tonsils is highly activated between the ages of 4 to 81 years. After 18 years a steady decrease in this immunological activity takes place. The tonsillar capacity to produce IgA may be retained better when there is a relatively low level of systemic IgA.

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