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Mucosal and Glandular Distribution of Immunoglobulin Components

IMMUNOHISTOCHEMISTRY WITH A COLD ETHANOL-FIXATION TECHNIQUE

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Summary. Precise immunohistochemical information about the mucosal distribution of diffusible immunoglobulin (Ig) components and the local occurrence of Ig-containing cells can be obtained by studying in parallel directly fixed and saline-extracted biopsy specimens. This combined approach is useful for evaluating systemic and local contributions to the mucosal Ig supply in patients with immunodeficiency. Their mucosal populations of Ig-bearing cells can also be demonstrated immunohistochemically.

A new model for the secretory Ig system is based on experience with this technique applied to normal mucosal specimens from various levels of the human respiratory and gastrointestinal tract. The serous-type secretory epithelial cell produces secretory component (SC) and is also responsible for the selective external transfer and molecular completion of secretory IgA and secretory IgM. Cell surface-associated SC most likely mediates the epithelial affinity for dimeric IgA and 19S IgM, and Ig–SC complexes are probably formed and mobilized in the cell membrane; they may then reach the cytoplasm outside the Golgi apparatus by pinocytosis or facilitated diffusion. The composite molecules finally appear to be extruded into the gland lumen along a general secretory pathway.

INTRODUCTION

During a 10-year period following the pioneering immunohistochemical studies of Coons, Leduc and Connolly (1953), only sections of frozen tissue were used for the localization of immunoglobulins (Ig) and antibody activities. In 1962 Sainte-Marie successfully employed a cold ethanol-fixation and paraffin-embedding technique to process rabbit lymphoid tissue for immunohistochemistry. He reported that antibody-producing cells had retained their activity well, as revealed by indirect immunofluorescence, and that they exhibited a much better morphology than comparable cells in frozen sections. Brandtzaeg and Kraus (1965) first used this technique to locate human Ig. They found that the relative immunofluorescence intensities emitted from sections of oral mucosa reflected the extravascular distribution of IgG, IgA and IgM. Because of the proportionately large amounts of extravascular IgG (Waldmann and Strober, 1969), the

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corresponding tissue fluorescence was especially intense and tended to mask the staining of cells containing this immunoglobulin. In order to reveal clearly the mucosal distribution of Ig-containing immunocytes it was, therefore, necessary to remove extracellular, diffusible Ig by saline extraction prior to tissue fixation.

We have used this modified method for almost 10 years to study normal and diseased mucosae from individuals with or without Ig deficiencies. In combination with the original Sainte-Marie technique, it provides useful information about the mucosal supply of serum-derived and locally formed Ig. The biopsy specimens are routinely divided into two pieces; one is fixed directly by alcohol precipitation, the other is first extracted by washing in isotonic saline for 48 hours at 4°. This approach permits parallel examination of the extravascular Ig distribution and the local occurrence of Ig-producing cells even in highly vascularized and inflamed tissue. The combined method thus has obvious advantages over other procedures currently used for immunohistochemical studies of Ig. In sections of unfixed, frozen specimens there are poor morphological details and an uncontrolled loss of diffusible proteins; with the cold formaldehyde method of Eidelman and Berschauer (1969) there is satisfactory morphology but the loss of diffusible Ig is still uncontrolled, although much smaller than from unfixed material.

The aims of this article are: (1) to report some of our general experiences with the combined, modified ethanol-fixation method applied to studies on the mucosal and glandular distribution of Ig components; and (2) to point out the potential of this procedure for characterizing local aspects of immunodeficiency states. Parts of this work have been briefly presented elsewhere (Brandtzaeg and Baklien, 1972; Brandtzaeg and Berdal, 1973); and particular features relating to different tissue sites in health and disease will be detailed in subsequent publications.

MATERIALS AND METHODS

Tissue source

Human mucosal specimens were obtained by excision or suction biopsy from various levels of the respiratory and gastrointestinal tracts. Each tissue site was represented with samples from at least five individuals with normal levels of serum Ig; nasal mucosa was also obtained from patients with immunodeficiency (Brandtzaeg and Berdal, 1973). Experimental granulomas induced in rabbit oral mucosa served as a source of tissue containing antibody-producing cells of known specificity. Egg-white lysozyme (Worthington Biochemical Corporation, Freehold, New Jersey) was co-polymerized with normal rabbit serum (NRS) in the ratio 100:1 (mg/ml) by means of ethyl chloroformate (Avrameas and Ternycnk, 1967). An amount of insolubilized lysozyme, corresponding to 0·4–0·8 mg measured by antigenicity, was injected above the upper incisors of systemically immunized rabbits. After 2–4 weeks the mucosal tissue surrounding the antigen depots was removed.

Tissue processing

The specimens were collected in ice-cold, isotonic saline. Within 30 minutes they were divided into small pieces (approximately 2×3×5 mm) which were placed in bags of cheese cloth and treated in one of the two following ways: (1) direct fixation was immediately initiated in cold ethanol to retain the 'total' content of Ig components in the tissue; (2) prior to fixation the specimens were extracted by washing in PBS (0·15 M NaCl, 0·01 M phosphate buffer, pH 7·5) with 9 r.p.m. (stirrer Type 30, gear Type SS9; Heidolph-

Elektro KG, Kelheim, Germany) in a 1-litre beaker at 4° for 48 hours. After fixation for 18–24 hours in 96 per cent ethanol at 4°, the pieces were kept in cold absolute alcohol for about 4 hours; next in cold xylene for 20–44 hours; and finally in paraffin for 3–4 hours at 56°. The blocks of embedded tissue were stored at 4°. Serial sections (6 μm) were stretched on acetone-cleaned microscope glass slides at 40° by means of a small amount of water which was quickly soaked away. Thereafter the sections were dried at 37° for 30 minutes and stored at 4°. Deparaffinization was carried out the same or the next day at 8–10° according to Sainte-Marie (1962); the slides were finally rinsed briefly in deionized water and air-dried just before conjugate incubation.

Immunohistochemical procedures

Monospecific rabbit antisera were produced and labelled with fluorescein isothiocyanate (FITC), tetramethylrhodamine isothiocyanate (MRITC), or lissamine rhodamine B sulphonyl chloride (RB200SC) as detailed elsewhere (Brandtzaeg, 1973a). A MRITC-labelled goat reagent to rabbit Ig was purchased from Hyland Laboratories (Los Angeles, California) and fractionated by anionic exchange chromatography (Brandtzaeg, 1973a). The characteristics of representative conjugate fractions appear in Table 1. In the direct immunofluorescence test, human tissue sections were incubated with various combinations

TABLE 1

CHARACTERISTICS OF ANTIBODY CONJUGATES IN WORKING DILUTIONS *

Specificity	Fluorochrome	OD ratio	Concentration (mg/ml)	Precipitating units
Rabbit anti-γ-chain (human)	FITC	1·8	0·3	2/3
	MRITC	3·6	0·4	2/3
	RB200SC	3·9	0·9	3/2
Rabbit anti-α chain (human)	FITC	2·0	0·3	1/2
	RB200SC	5·1	1·0	2
Rabbit anti-μ chain (human)	FITC	2·2	1·0	3/4
	MRITC	2·9	1·1	3/4
Rabbit anti-SC† (human)	FITC	1·4	1·4	1/4
	MRITC	4·5	1·5	1/4
Rabbit anti-lysozyme (egg-white)	FITC	2·4	1·7	2/5
Goat anti-Ig (rabbit)	MRITC	1·6	0·7	1/2

* For details, see Brandtzaeg (1973a, b).
† SC = secretory component.

of 'green' and 'red' conjugates (Brandtzaeg, Baklien, Fausa and Hoel, 1974) for 30 minutes at room temperature, and were thereafter washed and mounted as previously described (Brandtzaeg, 1973b). A paired direct–indirect technique was used for the simultaneous demonstration of intracellular Ig and antibody activity in rabbit oral mucosa. The sections were first reacted with a mixture of 'red' anti-Ig conjugate and unlabelled egg-white lysozyme (5 μg/ml); after thorough washing they were next incubated with NRS, and finally with the 'green' anti-lysozyme conjugate diluted in NRS. The introduction of NRS was necessary to inhibit binding of the latter conjugate to antibody sites of the goat reagent in the first layer.

Fluorescence microscopy and photography

The microscope was a Leitz Ortholux equipped with an Osram HBO 200 W lamp for rhodamine excitation, a XBO 150 W lamp for fluorescein excitation, and Leitz immersion

objectives. A Ploem-type vertical illuminator combined with interference filters was used for narrow-band excitation and selective filtration of green and red fluorescence (Brandtzaeg, 1973b). The findings were recorded on 'Anscochrome' 500 daylight film.

RESULTS

EFFECT OF THE WASHING PROCEDURE

IgG-containing immunocytes could not be discerned with certainty in directly fixed specimens because of the intense fluorescence of the connective tissue ground substance.

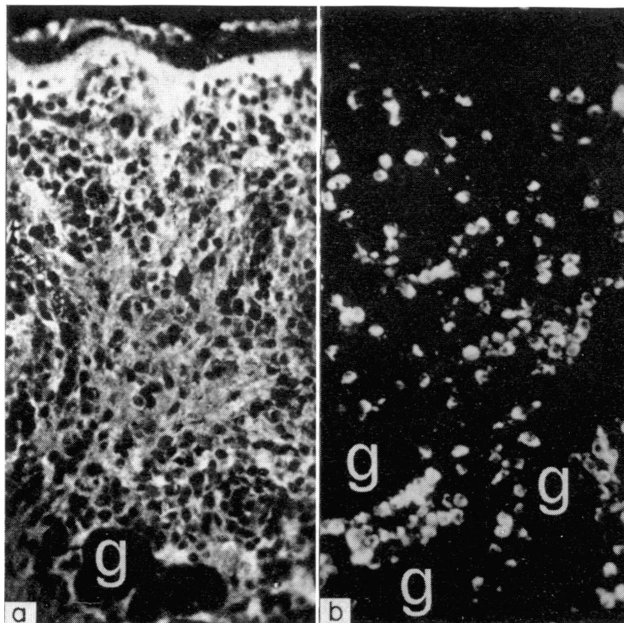

Fig. 1. Effect of the saline extraction procedure on the immunohistochemical demonstration of IgG immunocytes in the nasal mucosa of a patient with rhinitis and IgA deficiency. (a) Section of directly alcohol-fixed specimen from concha inferior. Diffuse specific staining of high intensity throughout the connective tissue completely masks fluorescing immunocytes. Glands (g) are unstained whereas surface epithelium (at the top) contains superficial cells with IgG. (b) Comparable field in a section of washed specimen from the same mucosa. Numerous IgG immunocytes are distinctly revealed in the connective tissue surrounding the glands (g) and beneath the surface epithelium. (Magnification × 180).

This was especially so with inflamed tissue (Fig. 1a). Extraction in PBS for 48 hours at 4° removed most of the diffusible IgG from the ground substance; the immunocytes were consequently clearly revealed against a fairly dark background (Fig. 1b). Extracellular IgA was similarly extracted whereas small granular IgM deposits were often retained, particularly in basement membrane zones of vessel walls and epithelia. A general retention of Ig deposits was noted in traumatized tissue along the periphery of the specimens. This was partly due to diffusion of IgG into damaged cells, for example smooth muscle fibres.

The morphological quality of the washed specimens was highly dependent upon an appropriate biopsy technique and a prompt and careful processing of the tissue. Partial loss or dislocation of columnar surface epithelium commonly occurred; and areas with disintegrated plasma cells were always present if the specimen had been unduly traumatized

before the washing procedure. However, following adequate sample handling the morphology of the Ig-containing cells was surprisingly well preserved (Figs. 1b and 6a–c). The possibility of inward leakage of diffusible Ig from the tissue fluid was excluded by paired immunofluorescence which demonstrated that each immunocyte contained only one Ig class (Fig. 6a–c). The technique was thus of great advantage in differential enumeration of immunocytes since all cell types were equally well discerned (Brandtzaeg *et al.*, 1974).

Immunocytes were readily detected in directly fixed rabbit mucosa when located by their anti-lysozyme activity; there was virtually no fluorescence of the connective tissue ground substance, indicating that the extracellular concentration of relevant antibody was

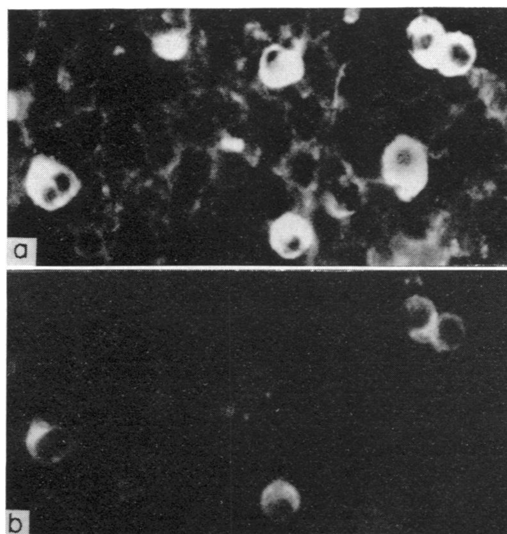

FIG. 2. Paired immunohistochemical demonstration of Ig and antibody to egg-white lysozyme in a washed specimen of rabbit oral mucosa. (a) Red filtration reveals that although some Ig has been retained around inflammatory cells, Ig-containing immunocytes are readily discernible. (b) Green filtration of the same field reveals that four of the immunocytes exhibit antibody activity to lysozyme. (Magnification ×430.)

very low. But the simultaneous study of cytoplasmic Ig was impossible because of the intense, diffuse interstitial fluorescence which masked the cellular localization. In sections of washed specimens, on the other hand, the Ig-containing cells were clearly revealed even in this severely inflamed tissue (Fig. 2a); and paired tracing demonstrated that they had retained their antibody activity (Fig. 2b).

When tissue specimens from hypo-γ-globulinaemic patients were subjected to the washing procedure, extraction of the reduced quantities of IgG was very efficient as evidenced by complete lack of diffuse background staining. This favoured high sensitivity for the detection of specific immunofluorescence features, especially when rhodamine conjugates were used (Brandtzaeg, 1973b). Since selective excitation of this fluorochrome with green light also induces negligible tissue autofluorescence, such preparations could be photographed with exposure times that were more than 10 times increased. In consequence, it was

possible to detect and record the presence of small amounts of IgG associated with the surface membranes of mononuclear cells in the nasal mucosa of immunodeficient patients (Fig. 3).

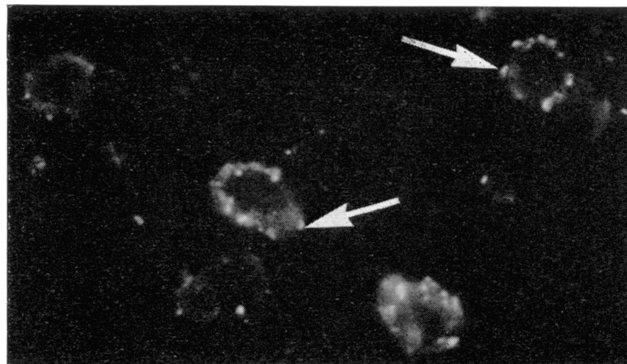

Fig. 3. Immunohistochemical demonstration of IgG in a washed specimen of the nasal mucosa from a patient with 28 mg IgG/100 ml serum. Mononuclear cells with specifically fluorescing (red), apparently membrane-associated granules (arrows) are clearly disclosed against a dark background. (Magnification ×960.)

NORMAL MUCOSAL AND GLANDULAR DISTRIBUTION OF Ig COMPONENTS

The diffuse connective tissue immunofluorescence of directly fixed specimens was normally very intense for IgG, intermediate for IgA and quite faint for IgM (Figs 4a–c and 5a,b). For all classes the brightest staining was related to the basement membrane zones of vessel walls and epithelia. These observations supported the conclusion that there is complete retention of Ig in directly alcohol-fixed tissue, and that the immunofluorescence truly reflects the relative extravascular distribution of the different Ig classes.

Despite the distinct dominance of diffusible IgG in the interstitial fluid, glandular epithelia were virtually devoid of this immunoglobulin (Figs 1a and 4a) except for occasional intercellular traces (Fig. 5a). Surface epithelia, on the other hand, commonly exhibited intercellular IgG staining especially in inflamed specimens (Fig. 4a). This apparently represented passive external diffusion from the tissue fluid. Some 'leakage' can probably also occur through goblet cells (Brandtzaeg *et al.*, 1974). Degenerated superficial epithelial cells often contained all three Ig classes (Fig. 4a–c). This accumulation should most likely be ascribed to protein uptake through damaged cell membranes (Kent, 1967); the source of Ig could either be the interstitial fluid or the secretion *in vivo*, or contaminating blood during biopsy.

Distinct fluorescence for IgA was present intercellularly in secretory epithelia throughout the respiratory and gastrointestinal tract. The serous-type cells also showed specific cytoplasmic fluorescence, especially adjacent to the lumen; this was relatively faint for glands of the respiratory tract (Fig. 4b), gastric mucosa, and small intestine (Fig. 5b), but quite bright for glands of the large intestine (Fig. 6d). Mucous acinar cells and goblet cells were virtually devoid of IgA (Figs 5b and 6d). Respiratory and gastric surface epithelia generally contained less IgA than IgG intercellularly (Fig. 4); this also applied to the epithelium lining the crypts of the palatine tonsils and the tips of the villi in the small

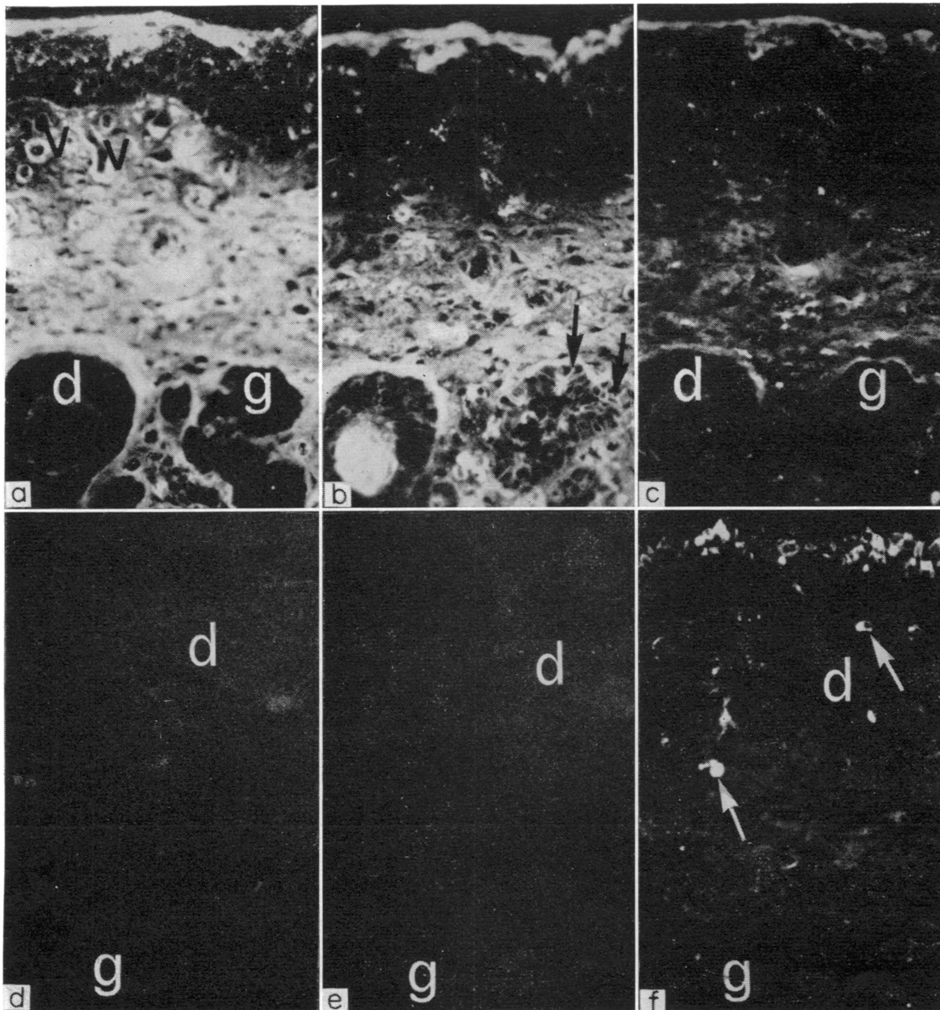

FIG. 4. Immunohistochemical demonstration of IgG (a), (d), IgA (b) (c), and IgM (c), (f) in directly alcohol-fixed specimens from slightly inflamed nasal mucosa of a normal subject [comparable fields in serial sections (a), (b) and (c)], and of a patient with hypo-γ-globulinaemia [comparable fields in serial sections (d), (e) and (f)]. IgG is normally present throughout the connective tissue ground substance, especially concentrated in basement membrane zones of vessel walls (V) and epithelia. Glands (g) are virtually devoid of IgG, whereas surface epithelia (at the top) commonly contain this immunoglobulin intercellularly and in superficial cells. The extracellular distribution of IgA is normally similar to to that of IgG, although signified by less intense staining. Glands contain IgA particularly concentrated intercellularly (arrows). Very little IgM is normally present in the connective tissue, and IgM can hardly be detected in glands of the respiratory mucosa. In hypo-γ-globulinaemia reduced serum levels of Ig are generally reflected by lowered intensity or complete absence of corresponding mucosal immunofluorescence. However, this patient had some local synthesis and secretion of IgM as indicated by a small number of positive immunocytes (arrows) and distinct immunofluorescence of superficial epithelial cells adjacent to the opening of a glandular duct labelled (d). (Magnification $\times$ 150.)

intestine. The surface epithelium of the large intestine contained small amounts of both inter- and intra-cellular IgA (Brandtzaeg *et al.*, 1974). When the luminal content of acini, ducts or intestinal crypts had been retained, it stained brightly for IgA (Figs 4b and 6d).

IgM could hardly be detected in the glands of most normal respiratory tract specimens (Fig. 4c); and secretory epithelia of the gastric mucosa and small intestine also contained very little of this immunoglobulin. The small amounts detected, however, appeared to be distributed like IgA. This was substantiated by observations on the glands of the large intestine where the distribution of IgM clearly mimicked that of IgA, although with less intense fluorescence (Brandtzaeg *et al.*, 1974). IgM was rarely detectable intercellularly in surface epithelia (Fig. 4c).

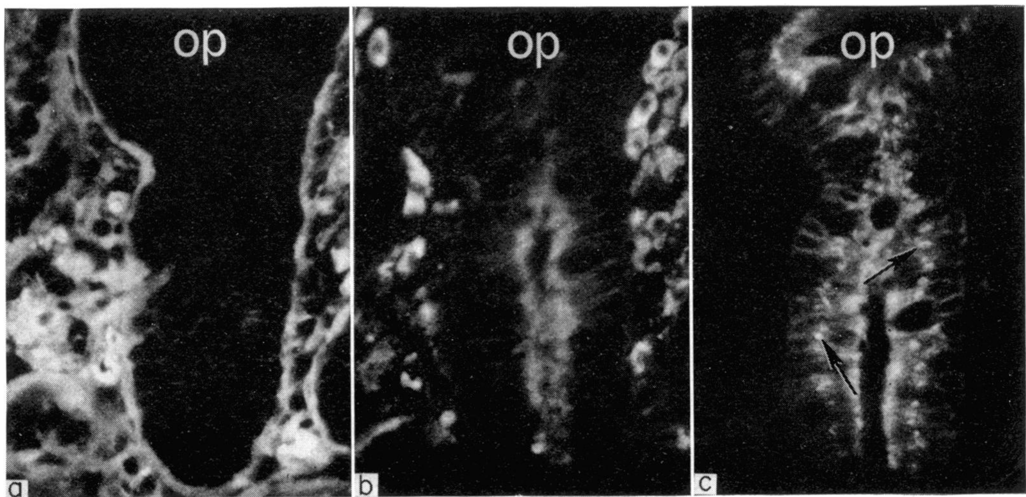

FIG. 5. Immunohistochemical demonstration of IgG (a), IgA (b), and SC (c), comparable fields in neighbouring sections of directly alcohol-fixed normal jejunal mucosa. IgG dominates in the connective tissue ground substance and basement membranes, but is barely detectable intercellularly in the gland. IgA is present in numerous immunocytes, and inter- and intra-cellularly in the gland. The glandular distribution of SC is similar to that of IgA except for additional distinct localization in the Golgi region (arrows). The epithelial concentrations of IgA and SC gradually decrease above the gland openings (op). Goblet cells are virtually devoid of both components. (Magnification ×350.)

The 'secretory component' (SC) was ubiquitously present in serous acinar and duct cells of the respiratory tract, whereas mucous acinar cells were virtually or completely devoid of this component. The presence of SC in the surface epithelium of the nasal mucosa was generally confined to degenerated superficial cells and columnar cells in the vicinity of duct openings. Epithelial cells lining the crypts of the palatine tonsils did not contain this component. In the gastric mucosa SC was chiefly located in the mucous neck cells and the transitional cells lining the bottom of the foveolae (compare Fig. 7b in Brandtzaeg (1973b)). In the small intestine it was present in the columnar epithelium of the crypts (Fig. 5c) and in a decreasing concentration gradient extending into the epithelium covering the villi, but rarely reaching their tips. In the large intestine SC was located in glandular cells (Fig. 6f) and generally also in the surface lining cells (Brandtzaeg *et al.*, 1974). Goblet cells did not contain detectable SC (Figs 5c and 6f).

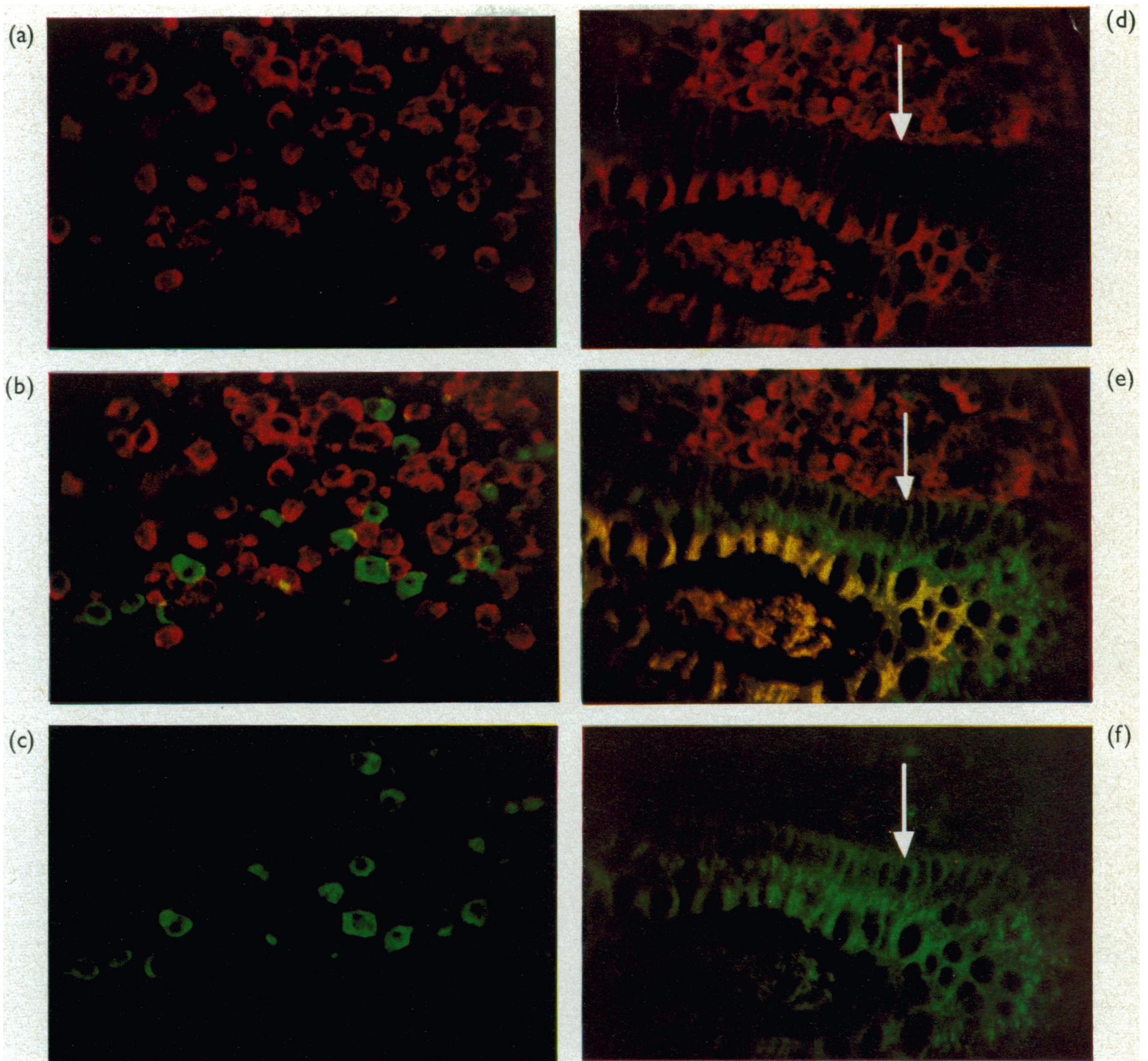

Fig. 6. Paired immunohistochemical tracings of IgG and IgA in tonsillar tissue, and of IgA and SC in colonic mucosa: (a) (red filtration); (b) (double exposure); and (c) (green filtration), a field including crypt epithelium (at the bottom) in a section of washed specimen from the palatine tonsil. Immunocytes are readily discernible against a dark background after incubation with 'red' anti-IgG+'green' anti-IgA. Differential immunocyte enumeration can easily be obtained from the double-exposed slide since the cells exhibit satisfactory morphology and pure colours. Comparison of the field after selective red and green filtration substantiates that each immunocyte contains only one Ig class: (d) (red filtration); (e) (double exposure); and (f) (green filtration), a field including glandular epithelium (at the bottom) in a section of directly alcohol-fixed specimen from inflamed colon. Incubation with 'red' anti-IgA+'green' anti-SC has produced diffuse red staining throughout the connective tissue, partly masking IgA immunocytes; in the gland pure green colour indicates the presence of SC in the Golgi region of columnar epithelial cells, whilst mixed colour signifies the presence of both SC and IgA in the rest of the cytoplasm and in the cell membranes (arrows). Both components are also present in luminal content, whereas goblet cells are unstained. (Magnification ×370.)

Although the epithelial distributions of SC and IgA in general were similar, the detailed fluorescent patterns of the two components were not completely congruent. A common feature was the fluorescence apparently related to the cell membranes of secretory epithelia (Figs 5b, c and 6d, f). Intracellularly both SC and IgA were present in the apical portion of the cytoplasm, but SC alone was distinctly located in a granular pattern corresponding to the region of the Golgi apparatus (Fig. 5b, c). This differential distribution was best revealed by paired tracing (Fig. 6e). The same pattern was observed for both the respiratory and the intestinal tract, although more distinctly for the latter.

IgA immunocytes were generally quite well discerned in normal specimens which had been directly fixed in alcohol (Fig. 5b); in inflamed specimens, however, the cellular fluorescence was more or less masked by a relatively intense background staining which probably in part represented exudation of serum IgA (Figs 4b and 6d). As described above, the presence of IgG immunocytes was even more obscured (Fig. 1a). The true mucosal distribution of all three classes of Ig-containing cells could therefore be revealed only in sections of washed tissue. As expected on the basis of previous observations in several laboratories, the dominating gland-associated immunocyte was of the IgA type throughout the respiratory and gastrointestinal tract. None the less, the share of IgG and IgM cells in the local immunocyte populations varied greatly among different mucosal sites; this will be detailed in subsequent publications. A marked numerical increase of local IgG immunocytes was observed in association with inflammatory disease of the respiratory (Fig. 1b) and colonic mucosa (Brandtzaeg *et al.*, 1973).

MUCOSAL ASPECTS OF IMMUNODEFICIENCY

Immunofluorescence examination of directly fixed mucosal specimens was a useful supplement to the quantitation of serum Ig in patients with immunodeficiency. The fluorescence intensities of the ground substance generally reflected the serum concentrations of the respective Ig classes when these were above certain levels. Intensified fluorescence for IgG could, for example, be clearly observed following administration of γ-globulins. In addition, the immunofluorescence patterns provided information about the local Ig supply which could not be obtained from serum analyses. This is illustrated in Figs 4d–f which show serial sections of nasal mucosa from a patient with 58 mg IgG, 3 mg IgA, and 10 mg IgM per 100 ml serum. There was no fluorescence for IgA (Fig. 4e) and very little diffuse staining for IgG (Fig. 4d). IgM, on the other hand, was distinctly present in the ground substance and in a small number of local plasma cells (Fig. 4f). Superficial epithelial cells contained IgM also, indicating that the patient had some transfer of this immunoglobulin into his nasal secretions (Fig. 4f). Two other clear discrepancies between the serum Ig levels and the nasal Ig supply were found in a group of eight hypo-γ-globulinaemic and four IgA-deficient individuals. The best idea of the local *versus* the systemic contribution to the mucosal Ig supply was obtained by comparing directly fixed and washed specimens.

IgG-containing immunocytes were never encountered in the nasal mucosa from patients whose serum IgG levels were below 60 mg/100 ml. However, six out of seven patients with a concentration between 60 and 2 mg/100 ml had numerous 'IgG-bearing' mononuclear cells in their mucosae. In most cases these cells were distinctly demonstrated only in the washed specimens as described above. The specific fluorescence was, apparently, related to the cell membranes and chiefly distributed in a granular or lumpy pattern (Fig. 3).

The morphology was for most of the positive cells compatible with that of lymphocytes; they were, moreover, occasionally present in collections which by conventional microscopy of neighbouring sections were characterized as aggregates of lymphocytes. Nevertheless, some fluorescent cells appeared morphologically more like macrophages or mast cells.

DISCUSSION

When immunocytes were first studied in alcohol-fixed tissue (Sainte-Marie, 1962), they were located by their antibody activity. With this technical approach there is no cellular masking due to background fluorescence, because the interstitial fluid contains very low concentrations of relevant antibody. When localization of cells by their Ig content is attempted, however, great difficulties are encountered because of diffuse, interstitial fluorescence of high intensity. Since the proteins of the connective tissue fluid are precipitated *in situ* by alcohol, the Sainte-Marie technique should result in a fairly complete retention of diffusible Ig components. This was substantiated by the relative fluorescence intensities of the three major Ig classes, which were in accordance with their known extravascular distribution (Waldmann and Strober, 1969).

In order to exploit the technical advantages offered by the Sainte-Marie technique, and at the same time eliminate cellular masking, Brandtzaeg and Kraus (1965) introduced a simple modification whereby the diffusible Ig components are extracted from the tissue prior to fixation. The present study confirms and extends the applicability of this immunohistochemical approach. Immunocytes of all Ig classes can be readily demonstrated even in inflamed areas with exudation; and their integrity is well preserved as demonstrated by paired staining. Differential enumeration of Ig-containing cells can thus be performed with great precision in tissue processed by the modified technique (Brandtzaeg *et al.*, 1974). The cells, moreover, retain their antibody activities after the extraction procedure; paired immunohistochemical tracing of antibody specificity and Ig class is therefore made possible. The modification is also useful for investigation of more subtle intracellular protein affinities (Brandtzaeg, 1973c). The blocks of tissue exhibit Ig antigenicity for at least 8 years when stored at 4°. Our observation of preservation of antibody activity is so far limited to 17 months.

In previous studies of respiratory and intestinal tissues, techniques have been employed that neither permit complete retention nor satisfactory removal of extracellular Ig components. Firstly, therefore, a wrong impression of the composition of the extravascular Ig compartment has been created; and, secondly, difficulties have been encountered in the enumeration of immunocytes, especially those containing IgG. We routinely divide our biopsy specimens into two pieces, one for direct alcohol-fixation and another for saline extraction. This allows a parallel study of the distribution of diffusible Ig components in the tissue site, and adequate enumeration of local Ig-containing cells.

The combined technical approach may provide insight into the mucosal aspects of immunodeficiencies. Evaluation of systemic and local contributions to the mucosal Ig supply is a valuable supplement to the data obtained from analyses of serum Ig and blood lymphocytes. Moreover, the high immunofluorescence sensitivity resulting from the combination of modified tissue processing and use of rhodamine conjugates, permits detection of mucosal IgG-bearing mononuclear cells in immunodeficiency. Most of these probably belong to the ill-defined population of lymphocytes with Fc-receptors, which can be neither classified as B cells nor as T cells (Fröland, Natvig and Wislöff, 1973). The

demonstration of a large number of such lymphocytes in the nasal mucosa of hypo-γ-globulinaemic patients may add to the understanding of their origin and function. Investigations in a patient with defective B-cell maturation have indicated that the mucosal IgG-bearing cells can be differentiated immunohistochemically from locally occurring IgM-bearing cells which probably represent B lymphocytes (Brandtzaeg and Fröland, unpublished).

Our immunohistochemical studies of the normal secretory Ig system have produced results that are partly in accordance and partly in direct contrast with reports from other laboratories. Several investigations have concerned the cellular origin of the Ig moiety and the 'secretory component' (SC) which make up the composite secretory IgA molecule. In agreement with most of the relevant information, but contrary to the concept of Rossen, Morgan, Hsu, Butler and Rose (1968), we conclude that SC is an epithelial product. However, there are many discrepancies when it comes to defining the type of the SC-forming cell. Unlike Rossen *et al.* (1968), and Tourville & Tomasi (1969), we did not detect SC ubiquitously in mucosal surface-lining epithelia such as those of the palatine tonsillar crypts and nasal mucosa. When SC was present in the latter it was as a rule confined to the vicinity of gland openings, probably being carried by duct cells which were moving outwards. Söltoft and Söeberg (1972) reported the presence of SC in the complete epithelium covering the villi of the small intestine, whereas we, like Munster (1972), rarely found it located in the tips of the villi. The only surface epithelia regularly containing SC, although in relatively small concentrations, were those of the bronchial mucosa and the large intestine (Brandtzaeg *et al.*, 1974), indicating that they have a secretory function. Tourville, Adler, Bienenstock and Tomasi (1969) reported the goblet cells to be the major intestinal source of SC; they also detected it in mucous-type acinar cells of the respiratory tract and salivary glands. This was not confirmed with our technique despite the fact that mucin was well retained in alcohol-fixed material as demonstrated by Alcian Green staining (Culling, 1963). Moreover, Söltoft and Söeberg (1972), using frozen tissue like Tourville *et al.* (1969), reported negative goblet cells after application of anti-SC conjugate.

Since the conjugates used in our studies were better defined (Brandtzaeg, 1972, 1973b) than those previously employed for the localization of SC, we feel confident that the serous-type secretory epithelial cell should be regarded as the general source of this component. The same cell is most likely also responsible for the selective external Ig transmission. Several workers have noted that columnar crypt cells of the large intestine contain cytoplasmic IgA (Gelzayd, Kraft and Kirsner, 1968; Schofield and Atkins, 1970; Savilahti, 1972). In addition, Tourville *et al.* (1969) and Tourville and Tomasi (1969) pointed out that IgA is particularly concentrated intercellularly in secretory epithelia, and proposed a model for its external transfer. According to this, IgA diffuses along intercellular channels until it reaches the tight junctions; then it passes into the apical part of the epithelial cell and further into the gland lumen.

Two observations indicate that the above transport model is wrong. Firstly, if IgA reaches the intercellular space by simple diffusion, this should also apply to IgG, which is present in the basement membrane zones in higher concentrations than IgA. However, as also noted by Tourville and Tomasi (1969), the intercellular spaces of secretory epithelia normally contain very little or no IgG. Secondly, IgM was detected inter- as well as intra-cellularly (especially in colonic and rectal glands) despite its large size and relatively low concentration adjacent to the epithelium. Quantitative studies of pure glandular secretions have likewise suggested preferential external transmission and comparable

secretory dynamics for IgM and IgA (Brandtzaeg, Fjellanger and Gjeruldsen, 1970; Brandtzaeg, 1971a, b). It is hard to believe that the underlying molecular selection takes place in the basement membranes.

Hence, 'intercellular' staining for IgA and IgM in secretory epithelia should rather be interpreted as cell membrane fluorescence, and I propose a model for the external transport of these immunoglobulins as outlined in Fig. 7. In accordance with presented observations SC may be associated with the surface of the epithelial cells where it may act as a specific receptor for dimeric IgA and 19S IgM, thus mediating the first step of their selective external transfer. The SC–Ig complexes may then become mobilized and float along the cell membrane; this would be compatible with the fluid mosaic model proposed for

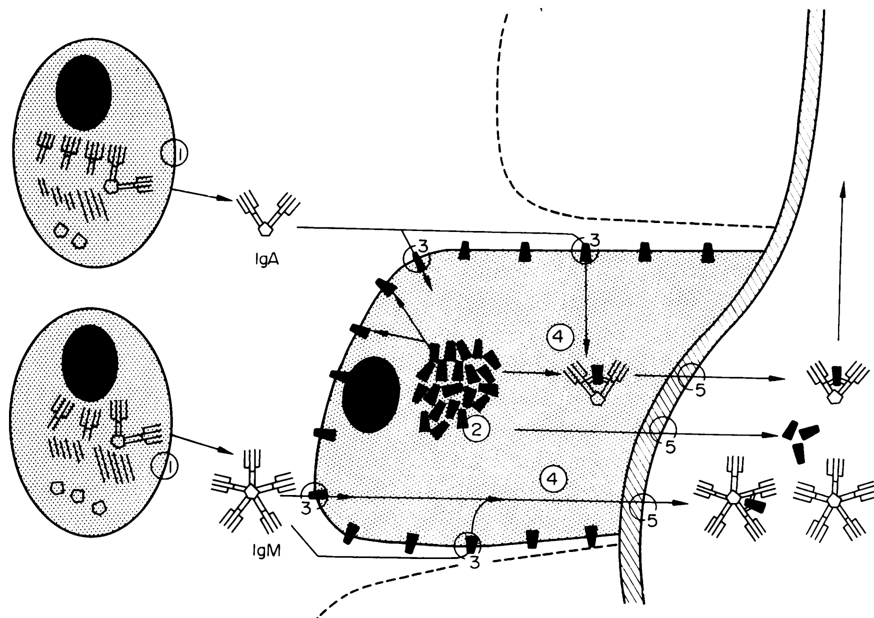

FIG. 7. Schematic representation of gland-associated synthesis and selective external transfer of dimeric IgA and 19S IgM. It is proposed that SC acts as a specific receptor for these two immunoglobulins, and that SC–Ig complexes are formed and become mobilized in the membrane of secretory epithelial cells. The completed secretory immunoglobulins finally reach the gland lumen (to the right) via the cytoplasm outside the Golgi region. While the combination of IgA with SC is efficient and gives rise to very stable complexes, this is so for only 60–70 per cent of the IgM; the rest of the secreted IgM contains SC in a loose association which depends on an excess of free SC. (1) Synthesis of immunoglobulin light, α, μ and J chains. (2) Synthesis and accumulation of SC. (3) Selective reception of IgA and IgM. (4) Conjugation of SC with IgA and IgM (5) External transport of secretory IgA, free SC and IgM.

such structures by Singer and Nicolson (1972). The complexes may next enter the cell cytoplasm outside the Golgi region either by pinocytosis or by facilitated diffusion. As independently shown by Munster (1972), SC is especially concentrated in areas corresponding to the Golgi apparatus. In a subsequent study (Brandtzaeg, 1974a) it has been substantiated that, while these accumulations represent free SC, the apical part of the cytoplasm does indeed contain SC combined with IgA. The final extrusion of the complexes into the gland lumen probably follows common secretory pathways (Kagnoff, Serfilippi and Donaldson, 1973). The columnar epithelial cell thus appears responsible for both the

transport and the molecular completion of secretory IgA. Recent experiments (Brandtzaeg, unpublished) have demonstrated that 60–70 per cent of purified secretory IgM contains bound SC; this immunoglobulin is therefore most likely handled by the same cellular mechanism as dimeric IgA, but the complexing seems to be less stable.

Results from *in vitro* experiments support the above transport model. When dimeric IgA is mixed with free SC spontaneous complexing occurs (Mach, 1970). This holds true also for 19S IgM (Brandtzaeg, 1974b). Dimeric IgA and 19S IgM contain a common polypeptide called J chain (Mestecky, Zikan and Butler, 1971); this may be responsible for SC affinity, thus determining epithelial Ig reception (Eskeland and Brandtzaeg, 1974). Finally, most gland-associated IgA immunocytes produce dimeric molecules which hence are readily available for external transmission by the proposed mechanism (Brandtzaeg, 1973c). In subsequent experiments with cold formaldehyde-fixation and unfixed or acetone-fixed cryostat sections of human colon, we have been unable to demonstrate significant immunofluorescence for SC in the mucigen of goblet cells.

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