

ELECTRON MICROSCOPE STUDY OF SURFACE
IMMUNOGLOBULIN-BEARING HUMAN
TONSIL CELLS*

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A currently popular concept of events leading to activation of the immune response includes the interaction of antigen with specific receptors on the lymphoid cell surface (1). Much experimental evidence has accumulated indicating that these cell surface recognition units are composed of immunoglobulins (Ig). For example, lymphocytes transform into immunoblasts when treated with anti-immunoglobulins (2-7). Furthermore, lymphocytes adhere to red cells to which they have been sensitized (8), to red cells coated with a sensitizing antigen (9), or to red cells coated with gamma globulin and treated with anti- γ -globulins to form "rosettes" (10, 11). In addition, immunoglobulins have been detected on the surface of mouse, rabbit, and human lymphocytes by immunofluorescence (12-14) and radioautography (12, 15-17), and have been removed semiquantitatively from the surface of mouse lymphocytes (14, 18). It seems likely that these immunoglobulins are synthesized by the cells on which they are found (14, 19) even though these cells need not be responsible for the production of circulating antibody. On the other hand, it has been claimed that normal (13) and neoplastic (20) plasma cells do not contain surface immunoglobulins because their cell membranes do not fluoresce when supravivally stained with fluorescein-conjugated anti-immunoglobulins. Thus, the presence or absence of surface gamma globulin does not necessarily indicate whether a particular cell is cytologically equipped to elaborate humoral antibody or not.

For these reasons, human tonsil lymphocytes with surface immunoglobulins were studied by high resolution electron microscopy. This would permit more precise characterization of the immunoglobulin-bearing cells and would also detect modifications of the plasma membranes and the submembranous cytoplasm at the site of antigen attachment. To quantitate the cells with Ig surface receptors, and to facilitate their localization by electron microscopy, the reverse immune cytoadherence technique was employed (21). This technique utilizes a hybrid antibody possessing activity against both gamma globulin and ferritin. The antibody is thus able to react with immunoglobulin-bearing lymphoid cells, on the one hand, and ferritin-coated sheep red cells on the other, to form rosettes. Our study demonstrates that the vast majority

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of "rosette-forming lymphocytes" contain profiles of rough endoplasmic reticulum (ER)¹ and polyribosomes. For the first time, connecting "bridges" were resolved between the ferritin particles and the lymphocyte membrane so that the cytoplasm immediately underlying the site of attachment could be examined and analyzed. The incidence of rosette-forming cells was correlated with the incidence of cells possessing surface or cytoplasmic gamma globulin by the supravital or fixed-smear fluorescent antibody technique. Approximately 6% of human tonsillar lymphocytes carry surface immunoglobulins when studied by these methods.

Materials and Methods

Lymphoid Cells.—Freshly excised tonsils were obtained from nine children and five adults. The tissue was placed into culture medium 199 and gently teased apart with needles to release the cells. Debris was removed by centrifugation at 100 g for 5 min. The supernatant cells were washed three times in medium 199 and resuspended in an amount of medium to yield 1×10^6 cells/ml. The cells were kept at room temperature throughout all procedures.

Erythrocytes.—Sheep red blood cells (SRBC) stored in Alsever's solution were "tanned" (22) the day before each tonsil specimen was obtained. After three washes, 0.6 ml of packed SRBC was suspended in 10 ml phosphate-buffered saline (PBS) and incubated with 10 ml of 0.01% tannic acid in PBS for 15 min at 37°C. After another wash, the cells were stored overnight at 4°C. The morning of the experiment, 0.2 mg ferritin ($5 \times \text{CsSO}_4$ precipitated horse spleen ferritin, Pentex Biochemical, Kankakee, Ill.) (23) was added to each 10^3 red cells and the cells were incubated for 30 min at 37°C. They were washed once before admixture with hybrid-coated tonsil cells.

Preparation and Purification of Antisera to Ferritin.—Antibody to ferritin was prepared by injecting 0.5 mg of horse spleen ferritin in complete Freund's adjuvant into the footpads of rabbits. They were boosted weekly with 0.5 mg of antigen injected subcutaneously. The antibody was separated from whole serum by adding sufficient ferritin, as determined by a precipitin curve, to produce maximum precipitation at equivalence. Purified antibody was isolated from the washed precipitate by dissolving it in glycine HCl buffer, 0.1 M, pH 2.8, incubating it for 30 min at room temperature, and spinning it for 2 hr at 40,000 rpm in a Spinco Model L centrifuge (Spinco Div., Beckman Instruments, Inc., Palo Alto, Calif.), to remove the dissociated ferritin. The supernatant containing purified antibody was dialyzed against distilled water, lyophilized, and stored.

Preparation and Purification of Antisera to Human Gamma Globulin.—Antibody to human gamma-G-globulin was prepared by injecting 0.5 mg of diethylaminoethyl- (DEAE-)purified fraction II (Lederle Laboratories Div., American Cyanamid Co., Pearl River, N. Y.) in complete Freund's adjuvant into the footpads of rabbits which were boosted weekly with 0.5 mg antigen subcutaneously.

The antibody was separated by absorbing it with glutaraldehyde-insolubilized human gamma-G-globulin (24). To produce the immunoabsorbant, 500 mg DEAE-cleaned gamma-G-globulin was dissolved in 10 ml of 0.1 M phosphate buffer, pH 7.0. 2 ml of 2.5% aqueous glutaraldehyde was added dropwise to this solution which resulted in the formation of a gel. After standing at room temperature for 3 hr to permit total insolubilization, the protein was dispersed in 200 ml of 0.2 M phosphate buffer, pH 7.3, homogenized in a loose-fitting hand homogenizer, and centrifuged at 2500 rpm for 10 min at 4°C. Homogenization was repeated twice with phosphate buffer and twice with 0.1 M glycine-HCl, pH 2.8. The immunoabsorbant

¹ *Abbreviations used in this paper:* aFe-aFe, anti-ferritin recombinants; aG-aFe, hybrid recombinants; aG-aG, anti-gamma-G-globulin recombinants; EM, electron microscopy; ER, endoplasmic reticulum; PBS, phosphate-buffered saline; RER, ribosome-associated endoplasmic reticulum; SRBC, sheep red blood cells.

was then washed with phosphate buffer until the supernatant was free of protein. Rabbit antiserum to human gamma globulin was added to the immunoadsorbant and gently stirred at room temperature for 30 min. After centrifugation, the immunoadsorbant-antibody complex was washed with pH 7 buffered saline until the supernatant was protein free. Subsequently, the adsorbed antibody complex was incubated at room temperature for 15 min with 10 ml glycine-HCl buffer, 0.1 M, pH 2.8 until all antibody had been eluted. After passage through a 0.45 μ Millipore filter (Millipore Corp., Bedford, Mass.), the protein was dialyzed against distilled water, lyophilized, and stored. The immunoadsorbant was regenerated by washing once more with glycine-HCl and pH 7 buffered saline.

Hybridization of Antibody.—Antibody was hybridized according to the method of Nisonoff (25, 26) as modified by Hämmerling et al. (27). 100 mg of purified antibody to ferritin and 100 ml of purified antibody to human gamma-G-globulin were dissolved separately in 10 ml of 0.1 M acetate buffer, pH 4.5, after which they were incubated with 2 mg of pepsin for 16 hr at 37°C. Each mixture was neutralized with NaOH and the F(ab')₂ fragments were purified on a Sephadex G-100 column (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.). After concentration, equal amounts of F(ab')₂ anti-ferritin and anti-gamma-G-globulin were dissolved in 0.1 M acetate buffer, pH 5.0, and incubated with 0.015 M 2-mercaptoethanolamine-HCl under N₂ at 37°C for 1 hr. The reducing agent was removed on an AG 50 W \times 4 100–200 mesh column (Bio-Rad Laboratories, Richmond, Calif.) equilibrated with acetate buffer at pH 5.0. The protein eluate was neutralized and reoxidized by gentle stirring in an oxygen atmosphere for 2 hr at room temperature. Subsequently, nondimerized Fab' fragments were removed on a Sephadex G-100 column, and the 5S recombinant fraction was dialyzed against distilled water and lyophilized.

Purification of Hybrid Antibody.—Hybrid antibody was purified according to the method of Paraskevas et al. (21). Glutaraldehyde-insolubilized gamma-G-globulin was added to the mixture containing recombinants with activity against ferritin (aFe-aFe), against human gamma-G-globulin (aG-aG), and hybrid (aG-aFe), and allowed to react for 30 min with gentle stirring at room temperature. The sediment was washed with PBS until no protein was found in the supernate. The adsorbed recombinants (aG-aG and aG-aFe) were eluted with 0.1 M glycine-HCl at pH 2.8 and neutralized. This eluate was then added to glutaraldehyde-insolubilized ferritin which retained the hybrid aG-aFe and left aG-aG in the supernate. The desired hybrid was then harvested from the ferritin by elution with glycine pH 2.8 buffer. Upon immunodiffusion, the hybrid formed a precipitin line against a mixture of ferritin and gamma-G-globulin, but failed to react with either antigen alone. The pure hybrid antibody was dialyzed against distilled water, lyophilized, and redissolved in culture medium 199.

Formation of Rosettes.—1 million tonsil cells/ml were incubated with 0.1 mg of purified hybrid for 30 min at 37°C. At the end of this period they were washed once and combined with 10⁸ ferritin-coated SRBC giving a final ratio of 200 SRBC to each lymphoid cell. The cell suspension was incubated at 37°C for 2–4 hr with intermittent gentle agitation. Rosette formation was monitored by phase microscopy. Specimens were kept for not more than 6 hr before fixation for electron microscopy because incubation at 4°C overnight, which was recommended for studies by light microscopy (21), destroyed the ultrastructure of the cells within the rosettes almost beyond recognition. In order to test the specificity of rosette formation, control specimens were incubated without antiserum as well as with the 3.5S anti-ferritin and anti-immunoglobulin fragments instead of the hybridized antibody.

Electron Microscopy.—After rosette formation was confirmed by phase-contrast microscopy, the cells were sedimented and resuspended in 3% phosphate-buffered glutaraldehyde overnight. On some occasions, the cell suspensions were fixed before sedimentation with three times the volume of glutaraldehyde. Postfixation was carried out with 2% osmium tetroxide. The specimens were dehydrated in increasing concentrations of ethanol and propylene oxide, and embedded in Epon 812 (28). Each specimen was divided about evenly into three to six "blocks." For the purpose of counting leukocytes and for assessing the incidence of rosette

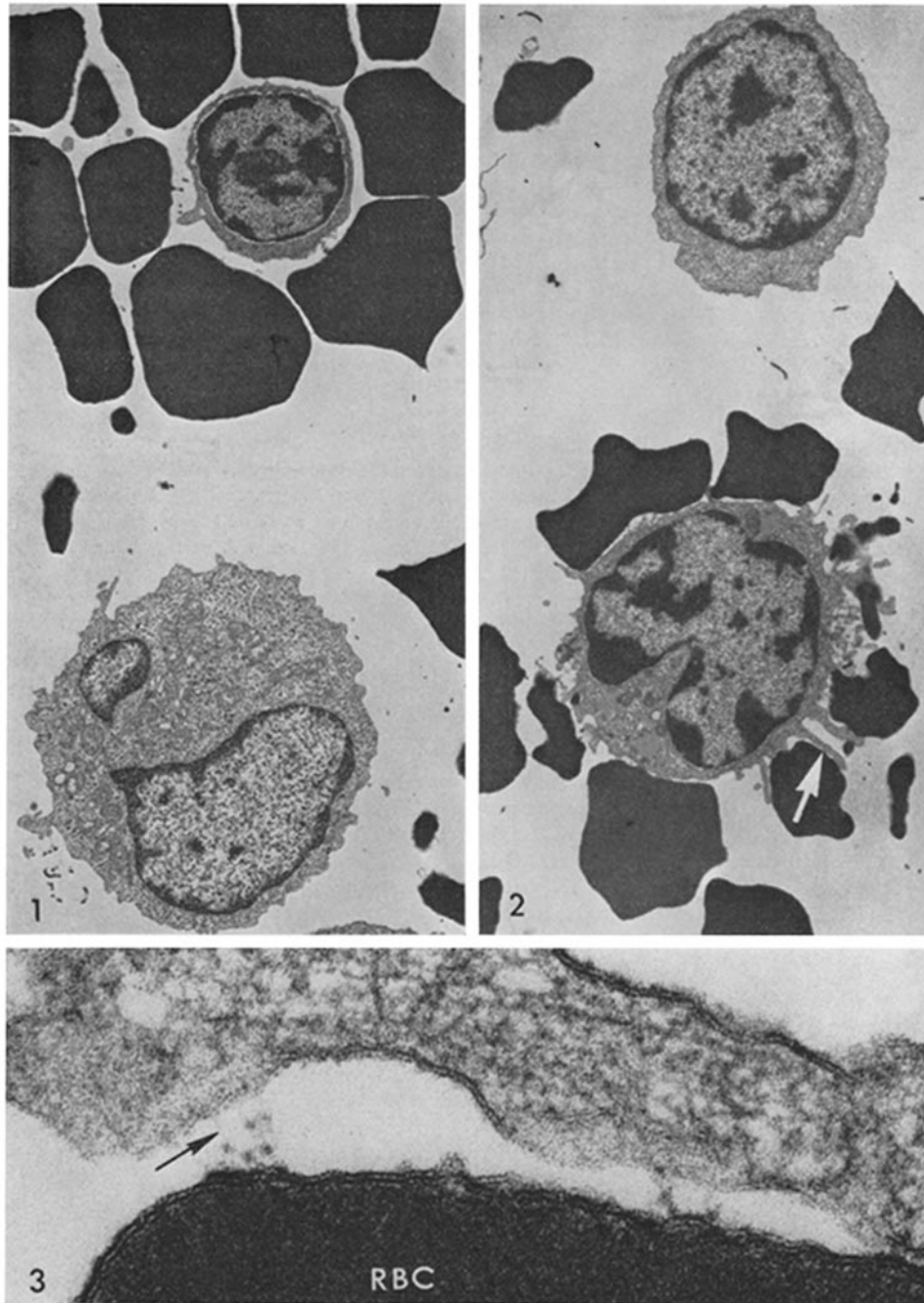


FIG. 1. Tonsil cells incubated with ferritin-coated SRBC and hybrid antibody. The small lymphocyte is seen to have formed a rosette whereas the large lymphocyte is devoid of adhering red cells. $\times 6000$.

formation, large-face thick sections (about $1\ \mu$) were studied by phase-contrast microscopy. Rosettes were called "incomplete" when the surface of the lymphocyte was not entirely surrounded by SRBC but when at least three adjacent erythrocytes formed a "cap" or when half the circumference of the white cell was covered with SRBC. Since the distribution of white cells varied in different block faces of the same specimen, all blocks were sectioned and a minimum of 500 leukocytes were counted in each sample. Subsequently, the block face was trimmed around the rosettes to permit thin sectioning and ultrastructural analysis of the lymphoid cells.

A total of 6662 leukocytes was counted, 336 rosettes were examined by electron microscopy, and about half of these were photographed. Rosettes which on electron microscopy contained damaged or unidentifiable leukocytes (*vide infra*) were not included in the final count. The thin sections were contrasted with uranyl acetate (29) and lead citrate (30). Micrographs were taken with a Siemens Elmiskop I electron microscope (Siemens Corp., Iselin, N. J.) equipped with a cooling device. Original magnifications ranged from 800 to 40,000.

Fluorescence Microscopy.—In order to correlate the percentage of cells containing immunoglobulin by fluorescence techniques, aliquots of washed tonsil cells were smeared, fixed with 100% ethanol, and stained with polyvalent fluorescein-conjugated rabbit anti-human Ig essentially as described elsewhere (31). The antiserum was prepared in rabbits and conjugated with fluorescein isothiocyanate on Celite by the method of Goldstein (32). The same antiserum was used to stain the surface of living lymphoid cells by a modification of the method originally described by Cerottini (33). For this purpose, aliquots of 0.1 ml cell suspension (containing 5×10^6 tonsil cells/ml) were combined with 0.1 ml fluorescein-conjugated antiserum for 30 min at room temperature. The cells were then washed three times in PBS and a drop of this preparation was examined with a Leitz fluorescence microscope (E. Leitz, Inc., Rockleigh, N. J.) equipped with an OSRAM HBO-200 lamp, a UG5 or BG 12 excitor, and a blue absorbing barrier filter.

RESULTS

Electron microscopy of control human tonsil cells showed between 2 and 10% unmistakable plasma cells, about 5% monocytes, and a negligible number of granulocytes. Thus, the bulk of cells consisted of lymphoid cells of varying size and cytoplasmic development. The per cent of white cells which formed rosettes ranged from a low of 2.1% to 11.2% in a single specimen. There were no obvious reasons for the difference in these particular samples. The incidence of rosettes in the remainder of the specimens averaged 6%. Upon examination by electron microscopy, it became immediately apparent that the cells within rosettes were morphologically heterogeneous (Fig. 1-6). Some were so damaged that they could not be included in the final count (Fig. 5). In general it can be stated that neither size, nor nucleo-cytoplasmic ratio, nor the amount of rough ER, nor the

FIG. 2. A medium-sized lymphocyte in the center of a rosette has thin processes extending between adhering erythrocytes. The relationship of one of these (*arrow*) to the red cell membrane is shown at higher magnification in Fig. 3. The other lymphocyte in the same field has not formed a rosette. $\times 5000$.

FIG. 3. Higher magnification of area indicated by arrow in Fig. 2. Both red cell and lymphocyte membranes appear intact. Where the lymphocyte membrane has been sectioned tangentially, ferritin particles on the red cell are seen connected to the lymphocyte by thin strands of electron-opaque material 80-100 A in width (*arrow*). RBC, red blood cell. $\times 150,000$.

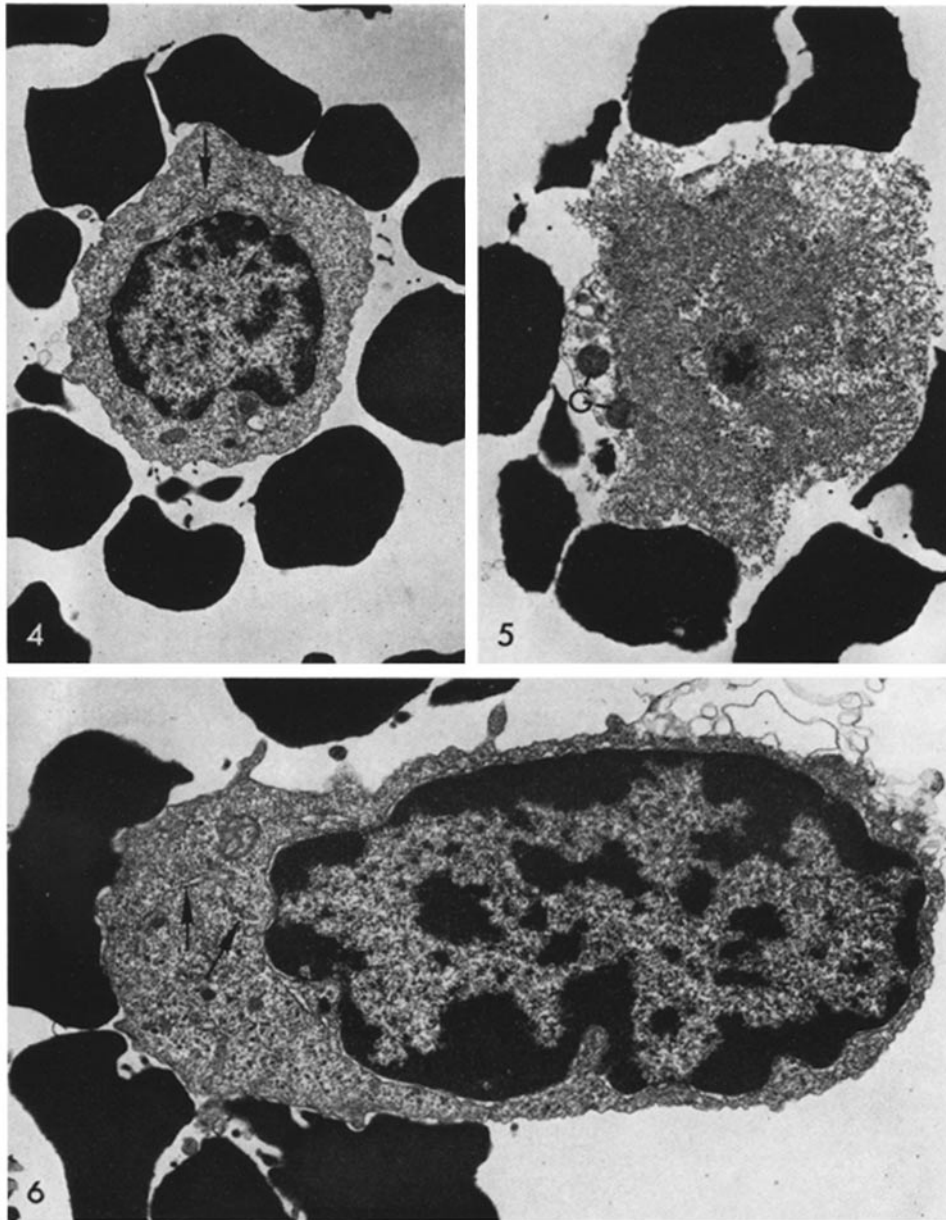


FIG. 4. Example of a small rosette-forming lymphocyte showing polyribosomes and a flat profile of RER (*arrow*). $\times 6000$.

FIG. 5. Ultrastructure of a severely damaged cell in the center of a rosette which on light microscopy appeared reasonably intact. The two granules (*G*) suggest that the cell may have been a monocyte. $\times 8000$.

FIG. 6. Incomplete rosette showing lymphocyte with "polarized" cytoplasm. Five erythrocytes are seen to adhere to the pole nearest the RER (*arrows*). Only a thin rim of cytoplasm is seen at the opposite pole. $\times 12,500$.

development of the Golgi apparatus could be used to predict whether any particular cell was likely to possess surface immunoglobulins. Nevertheless, with the exception of a rare small lymphocyte as shown in Fig. 1, all rosette-forming cells possessed profiles of rough ER (e.g., Fig. 4), and many of the cells were ostensibly plasmocytoid or intermediate type lymphocytes (34). About 20% of the rosettes were incomplete. Most of these cells were polarized, with the erythrocytes adhering to the pole of the cell with the largest amount of cytoplasm containing the Golgi zone, centrioles, spindle tubules, mitochondria, polyribosomes, and some profiles of rough ER (Fig. 6 and 7). Connections between lymphoid cells and adhering erythrocytes, which will be referred to as bridges, were noted whether the rosettes were complete or only partial. Examples of such bridges are illustrated in Fig. 3, 8, and 9 *a-c*. Here, one or more ferritin particles attached to the red cell membrane can be seen connected to the lymphoid cells by a strand of material measuring 300–600 Å in length and 80–100 Å in width. Rarely two strands resolved at the lymphocyte membrane converged on the same ferritin particle (Fig. 9 *b*). In some illustrations no bridges are seen (Fig. 1), but it may be assumed that they existed at a different level as could be deduced from serial sections. The distance between lymphocyte and red cells varied unpredictably in different planes of section except at sites where bridges had formed between them. At such sites, the distance between the cells appeared relatively constant. It has not been possible to determine precisely where on, or in, the lymphoid cell the connecting bridge is attached, but some observations warrant reporting. In Fig. 9 *a* the unit membrane of the lymphocyte is seen in cross-section. The strands (*arrows*) seem to be in continuity with similar structures in the cytoplasm. In close proximity to the bridge, a single ribosome is seen under a shallow invagination of the plasma membrane where it has been cut tangentially. In Fig. 9 *b* another ribosome is seen close to the intact surface membrane while the bridge is barely within the plane of this section. Grazing sections, at times, helped to define cytoplasmic structures which have relevance to the bridges. Thus in Fig. 9 *c* the ferritin particles within the indicated area appear to be in continuity with an electron-opaque region in the lymphoid cytoplasm which may represent tangentially sectioned rough ER.

In addition to lymphocytes, about half of the plasma cells in each specimen formed rosettes (Figs. 10 *a-c*). Plasma cells were usually surrounded by more than a single layer of erythrocytes. Such cells revealed many processes or pseudovilli which extended at a considerable distance from the body of the cell. Apposition of membranes was seen at multiple sites, but typical bridge formation could not be resolved. The surface membrane of the plasma cells which did not form rosettes seemed to be smoother with fewer cellular processes (compare Figs. 10 *a* and *c* with 10 *b*). Less than 1% of rosette-forming cells appeared to be monocytes according to morphologic criteria defined elsewhere (35). A few of these cells showed inclusions and vesicles but the small diameter and nucleocytoplasmic ratio did not permit them to be classified as monocytes (see Fig. 2).

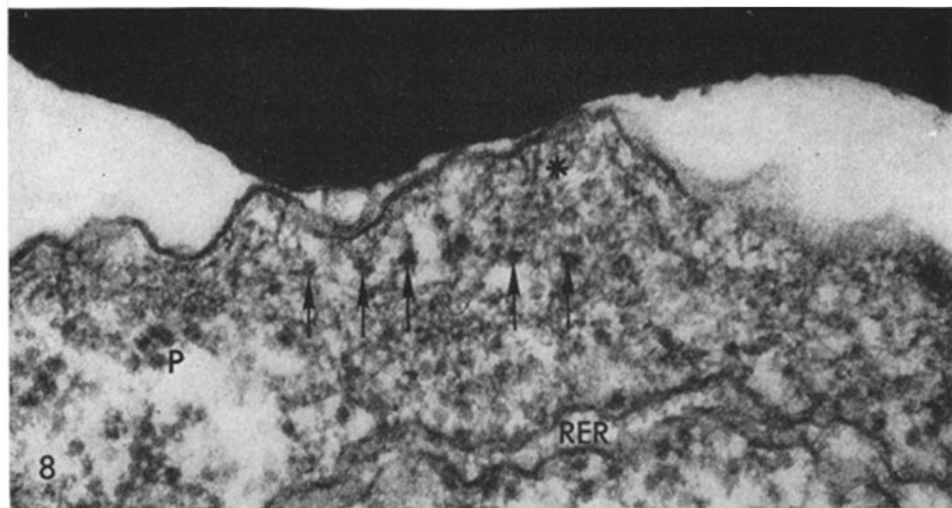
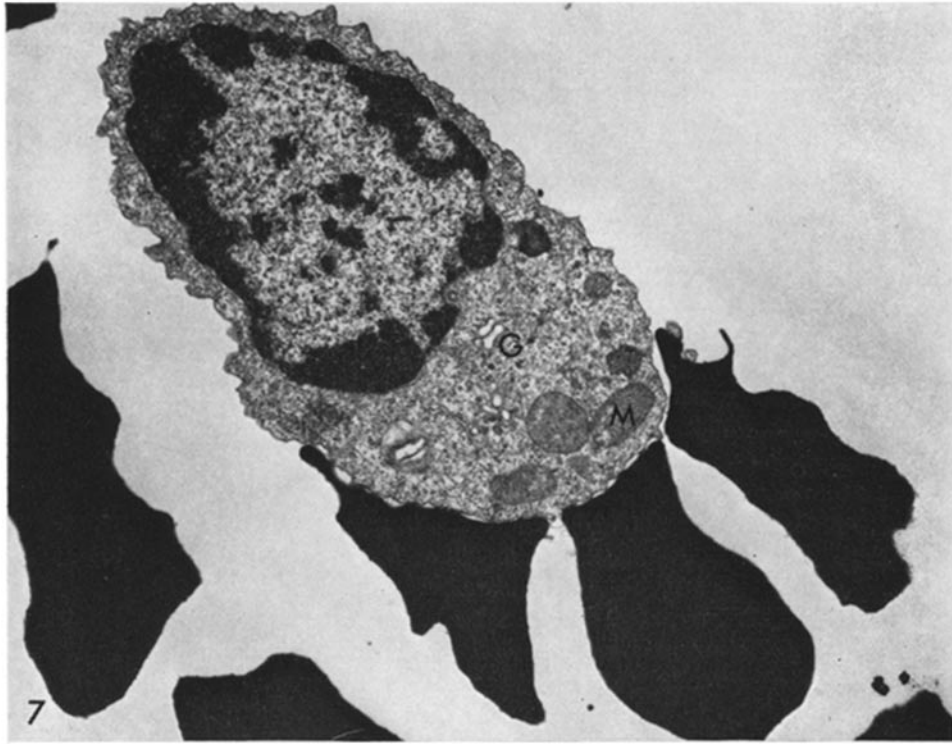


FIG. 7. Cap of erythrocytes adhering to part of the cell nearest Golgi (*G*), mitochondria (*M*), RER, and many polyribosomes. The centriole and spindle tubules can barely be seen at this magnification. $\times 11,000$.

FIG. 8. Detail of the submembranous cytoplasm at attachment site. *Arrows* indicate single ribosomes. *P*, polyribosome; RER, ribosome-associated endoplasmic reticulum. Also note membranes near surface (*asterisk*) and orientation of cytoplasmic strands between ribosomes and surface. $\times 99,000$.

Not a single rosette was found in control specimens in which lymphoid cells were mixed with ferritin-coated SRBC with or without the addition of anti-serum to ferritin or gamma globulin only.

Since the incidence of lymphoid cells bearing surface Ig in the human tonsil seemed unexpectedly low, experiments were conducted to confirm this observation with fluorescence microscopy. When living human tonsil cells were stained with a polyvalent fluorescein-conjugated anti-human γ -globulin, the incidence of cells showing surface fluorescence was also between 5 and 7% (Figs. 11 *a* and *b*). About 20% of these fluorescing cells showed caps or "crescents" which correlated with the number of polarized incomplete rosettes seen during electron microscopy (EM). On the other hand, when the cells were smeared and fixed permitting entry of the fluorescent antiserum into the cells, the number of fluorescent cells was two to three times as high. In this case, the majority of the staining cells were typical plasma cells (Fig. 11 *c*) but a few cells which on the basis of size and nucleo-cytoplasmic ratio looked like lymphocytes (Fig. 11 *d*) also had fluorescent cytoplasm. It is possible that these cells represent intermediate type lymphocytes which have a considerable amount of rough ER and are believed to function like plasma cells (34).

DISCUSSION

Electron microscopy of human tonsil cells revealed that the incidence of plasma cells as well as the number of lymphocytes possessing surface Ig was relatively low. This observation was confirmed by fluorescence microscopy. Supravital staining with fluorescein-conjugated polyvalent antiserum showed that less than 10% of the cells had surface Ig, and with the conventional fixed-smear fluorescent antibody technique, the majority of the cells with fluorescing cytoplasm had the appearance of typical plasma cells. If the relationship between surface Ig and cell origin observed in the mouse (36) and chicken (37, 38) holds true for man, it may be assumed that the larger proportion of tonsil cells are thymus- rather than bone marrow-derived. A similar low incidence of cells with surface Ig has been found in normal human peripheral blood by ourselves and others (21).

The ultrastructure of cells which appeared to possess surface Ig was as variable as the morphology of cells not able to form rosettes. Although it seemed that all rosette-forming cells had some rough ER, often located near the surface, it could not be definitely concluded on the basis of these studies that ribosome-associated ER (RER) is an absolute prerequisite for the elaboration of surface Ig. Conversely, many lymphoid cells with conspicuously developed granular ER as well as half the plasma cells did not react with ferritin-coated SRBC at all. However, it may be significant that where bridge formation between lymphocyte and erythrocyte had occurred, profiles of RER were often seen to be very close to this membrane site. In addition, incomplete rosettes or caps of SRBC always occurred at the pole of the lymphocyte nearest the Golgi ap-

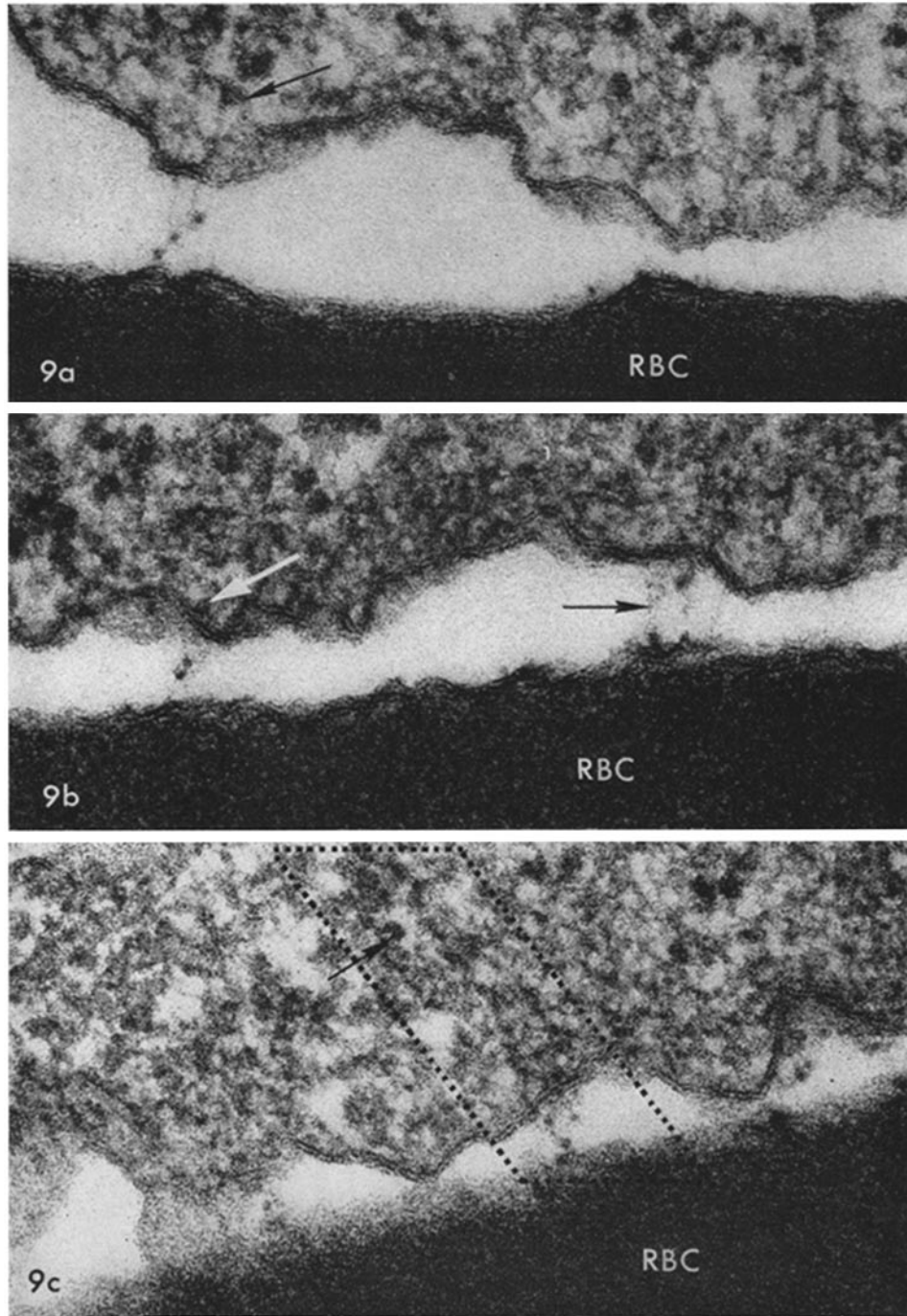
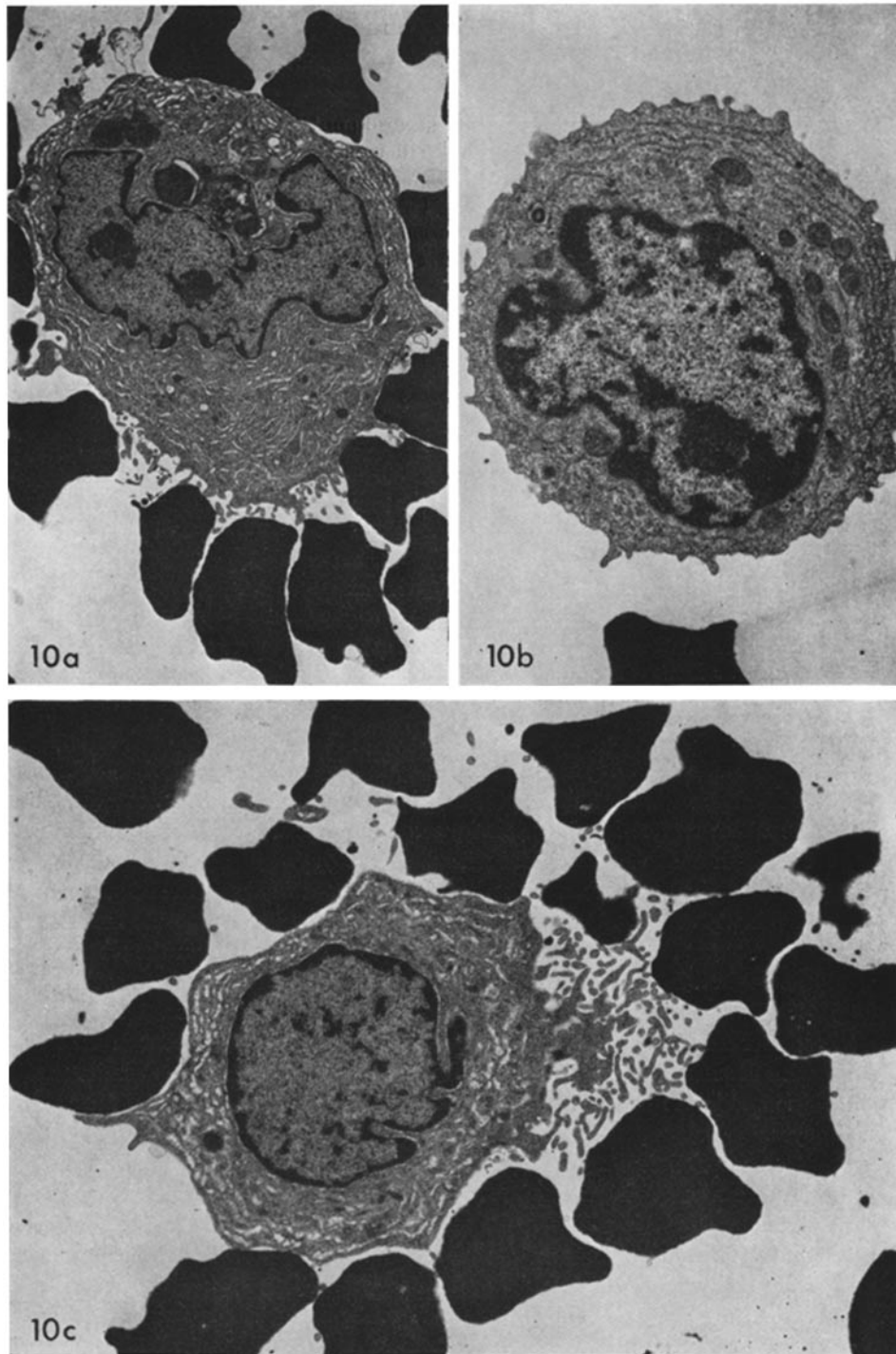
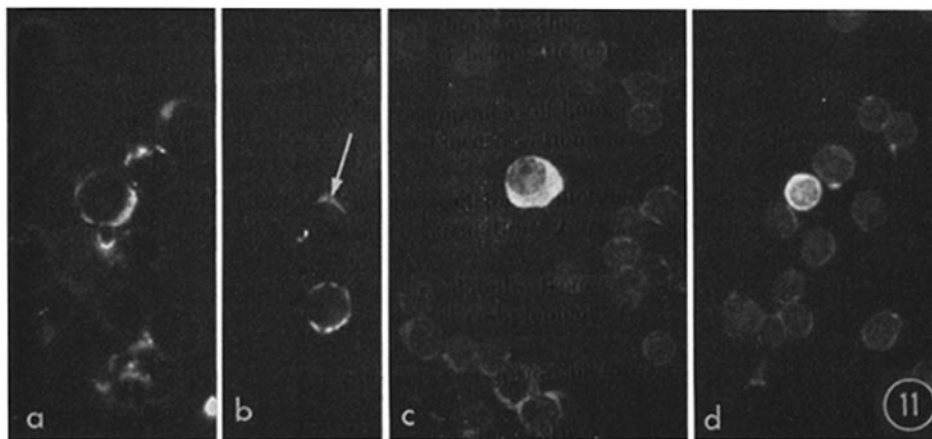


FIG. 9. Examples of bridge formation showing the submembranous cytoplasm to better advantage. (a) Only two of the three ferritin particles are connected by bridges with the lymphocyte membrane. Where this membrane appears to invaginate it has been sectioned tangentially. A single ribosome (*arrow*) is seen in close proximity to this invagination. (b) A density (*white arrow*) which could represent a ribosome is also seen contiguous to the membrane site where a bridge has formed. On the right, three connections between *RBC* and lymphocyte are seen. (c) The demarcated area shows a density in the lymphocyte cytoplasm which is likely to represent tangentially sectioned RER. *Arrow* indicates ribosome. This cistern approximates the membrane site where a bridge has formed. $\times 150,000$.



FIGS. 10 *a-c*. Three plasma cells seen in one section of the same specimen. Two of the cells have formed rosettes, the third (10 *b*) bears no erythrocytes. The cells seen in the center of the rosettes show many extensions, some of which are located at some distance from the main body of the cell. The surface of the plasma cell devoid of adhering SRBC (10 *b*) appears to be more smooth. (*a*), $\times 6000$; (*b*), $\times 7500$; (*c*), $\times 7000$.



FIGS. 11 *a-d*. Human tonsil cells stained with fluorescein-conjugated antiserum to γ -globulin. The supravitaly stained cells are depicted in *a* and *b*. Most of these showed fluorescing spots over the entire membrane but occasional crescents (*arrow* in 11 *b*) are also seen. *c* and *d* represent smears which were dried and fixed before staining with fluorescent antibody. *c* shows a typical plasma cell surrounded by unstained lymphocytes. *d* shows a lymphocyte with cytoplasmic fluorescence of the same intensity as the plasma cell in *c*. $\pm 2000\times$.

paratus and RER, organelles presumably involved in the synthesis and secretion of immunoglobulin. A similar observation was reported by Storb et al. who used the direct rosette-forming technique to study surface-associated antibody on cells from animals immunized with SRBC (39).

Although most of the rosette-forming cells contained some RER, the paucity of this organelle is unusual for a protein-secreting cell and suggests the existence of an alternative mechanism by which Ig is transported from the ribosomes to the surface. In some instances, the submembranous cytoplasm near the bridge site displayed only single ribosomes with very questionable connections to the membrane (Fig. 9 *a*). Though these cytoplasmic strands appear in continuity with the bridge, it is possible that they represent irrelevant cytoplasmic material oriented in the direction of stress resulting from the attachment of the erythrocyte.

The biochemical nature of the bridge itself also remains to be defined. Since the distance between the lymphocyte membrane and the ferritin particle usually did not exceed 400 A, it may be postulated that the bridge consisted of the hybrid antibody plus the fraction of the immunoglobulin molecule which protrudes from the lymphocyte. It has also been suggested that the γM molecule is more extended when it is bridging two particulate antigens than when it is free as resolved by the negative staining technique (40). This may account for the larger distance sometimes observed in the illustrations shown here. In addition, it is known that current methods of fixation, dehydration, and embedding alter

the electrolyte and phospholipid composition of membranes as well as the spatial relationships between various membrane constituents (41, 42). Therefore, it is possible that after preparatory procedures for EM, the bridge is composed not only of denatured γ -globulin but also of some membrane component which may be "buried" in the living cell. It is noteworthy that the ferritin particles are not found in contact with the erythrocyte membrane either, but that, even here, a distance of up to 200 Å may occur. The precise physical relationship of ferritin particles with tanned red cell membranes remains to be elucidated.

The fact that bridges were not readily resolved when plasma cells occupied the center of rosettes is compatible with the observation by others that plasma cells may not have membrane-associated γ -globulin recognition units (21). Most plasma cells found in the center of rosettes extended multiple processes to some distance from the main body of the cell resulting in several layers of adhering erythrocytes (Figs. 10 *a* and *c*). It is possible that such cells may have been damaged permitting intracellular Ig to react with the hybrid antibody. Alternatively, the rosette-forming plasma cells may be in the process of secretion which, even under normal conditions, has been postulated to take place by a mechanism of peripheral cytolysis (43). If the latter were the case, rosette formation by plasma cells would be temporary or reversible, a hypothesis which could be tested.

It should be reiterated that severely damaged cells, even those partially devoid of surface membrane, formed rosettes (Fig. 2). This was also observed in control specimens when no specific reaction could be implicated. Therefore, any unexpected rosette formation between SRBC and unsensitized lymphoid cell must be regarded with caution unless the integrity of the ultrastructure of such cells has been verified.

No comments can be made here on the number of reactive sites present on lymphoid cell surfaces since bridge formation was entirely dependent on the number of ferritin particles which had become attached to the erythrocyte membrane by means of the tanning procedure.

SUMMARY

Surface immunoglobulin-bearing cells were selected from suspensions of human tonsil cells by the reverse immune cytoadherence technique. The method employed a hybrid antibody directed against Ig on lymphoid cells and against ferritin bound to sheep red blood cells (SRBC). Only 6% of the cells formed rosettes. When subjected to electron microscopy they were shown to consist of a morphologically heterogeneous population of cells. However, most cells in the center of rosettes showed ribosome-associated endoplasmic reticulum (RER) and polyribosomes. Usually these organelles were located in close proximity to membrane sites where a 400–600 Å bridge was resolved between the lymphocyte and the ferritin particle on the SRBC. The bridge is postulated to consist at least in part of Ig. Only 50% of the plasma cells formed rosettes and bridges

could not be resolved. The surface of the plasma cells within rosettes differed from that of plasma cells which had not reacted with ferritin-coated sheep erythrocytes. The incidence of plasma cells and γ -globulin-bearing lymphoid cells was corroborated with the help of fluorescent antibody techniques.

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