ANTIGENS IN IMMUNITY

XV. Ultrastructural Features of Antigen Capture in Primary and Secondary Lymphoid Follicles*

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In the preceding paper (1), we described the events in the rat lymph node medulla which follow the injection of *Salmonella adelaide* flagella. Another important area of antigen localization is the lymph node cortex. Here, antigen can be retained for long periods in lymphoid follicles, both primary and secondary (2). Whereas all antigens are captured by medullary macrophages, though to varying degrees, follicular localization of antigens is by no means universal (3). The most important single factor which determines whether a particular antigen will be trapped by follicles is the level of specific antibody exhibited by the animal concerned. This antibody may have been acquired by active or passive immunization (4) or may represent a natural opsonin (5).

Previous studies using immunofluorescent staining of antigen depot sites (6, 7) suggested that the fundamental processes at work in follicular localization differ from those involved in medullary antigen pinocytosis. This notion was strengthened when preliminary observations using electron-microscopic radioautography (8) showed the follicular antigen to be mainly extracellular and plasma membrane associated. The present paper describes the detailed distribution of ^{125}I-labeled *Salmonella* flagella in lymphoid follicles as revealed by high resolution radioautography. The distinctive nature of the phenomenon of follicular localization has been confirmed.

Materials and Methods

These have been fully described in the accompanying paper (1). The same 24 rats killed at intervals ranging from 3 min to 21 days after the injection of ^{125}I-labeled flagella formed the basis of most of the present study. Follicular areas, as distinct from medullary areas, were recognized on light microscopic radioautographs of 1 μ sections which matched the ultrathin

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sections. The relevant area was then readily located and studied in the electron microscope. Frequently, a full low power photomontage of a complete follicle and surrounds was made before high-power examination of specific antigen depots.

Three small experiments involving a total of five rats were performed to study the follicular localization of three other ^{125}I-labeled proteins. These were rat IgG; human serum albumin complexed to rat anti-human albumin; and monomeric flagellin. Rat IgG was prepared by DEAE–Sephadex A-50 chromatography of an ammonium sulphate precipitate of normal adult rat serum. 60 μg of ^{125}I-labeled material, 0.9 g atoms ^{125}I per mole of protein, was injected into one rat which was killed 24 hr later. Human serum albumin (HSA) was labeled with ^{125}I (substitution rate 1.2 g atoms ^{125}I per mole of protein) and a soluble complex was made using a 15-fold excess of antibody. Three rats were injected, each receiving 15 μg of HSA as determined by scintillation counting. They were killed 2 hr, 1 day, and 8 days later.

Monomeric flagellin, a protein of 38,000 mol wt made by acid treatment of intact flagella and purified by differential centrifugation, was prepared as previously described (2). One rat was injected with 7.5 μg, substitution rate 1.1 g atom ^{125}I per mole of flagellin, and killed 3 days later.

RESULTS

Description of Rat Popliteal Lymph Node Follicles.—In previous publications of this series (2, 4, 7) we have described the differences between primary and secondary lymphoid follicles as revealed by study of methyl green–pyronin–stained sections. Primary follicles simply represent rounded aggregations of small lymphocytes and reticular cells, with few if any blast cells, and are not demarcated sharply from the surrounding cortex. Frequently, the only way of delineating the full extent of a follicle is to cause radioactive antigen to become deposited there, and to perform a radioautograph. Secondary follicles represent the end result of the deposition of an appropriate antigen in the primary follicle. They consist of a pyroninophilic germinal center composed of rapidly-dividing lymphoid cells and characteristic "tingible body" macrophages; and of a cuff of surrounding small lymphocytes, of varying degrees of prominence. Careful examination reveals the presence of a crescentic cap of reticular cells on the outer aspect of the center; i.e., on the side subjacent to the circular lymph sinus. This cap stains a salmon pink rather than bright red with pyronin. The edges of a germinal center are clearly demarcated from the surrounding, nonpyroninophilic cortex.

Electron microscopic study has confirmed this general picture, but has also revealed a number of intermediates between the extreme situations just described. Perhaps the most characteristic cell type in germinal centers is the tingible body macrophage (TBM). These are large cells, up to 25 μ in diameter, possessing an ovoid, clear nucleus with diffuse but widely dispersed chromatin occasionally showing narrow peripheral condensation and containing a great number and variety of large, electron-opaque inclusions in the cytoplasm. Some of these have a moth-eaten appearance (Fig. 7); others are myelinic figures; still others are smaller and homogeneous dense bodies. Many of the inclusions represent ingested nuclear material in various stages of breakdown.

Our study has shown that some follicles which, by their low content of dividing or blast cells appeared to be primary follicles, still contained an occasional TBM, presumably indicating at least some degree of past or present germinal center activity. Moreover, other follicles with well-developed germinal centers, as judged by high TBM content, still contained small lymphocytes rather than blasts as the dominant cell type. Thus this electron microscope study accentuated the fact that it is not always possible to categorize a follicle into either of the two major divisions determined by chromatic staining. Finally, even the most active germinal centers encountered in the popliteal nodes of our secondarily stimulated rats were considerably smaller than the very large germinal centers found in the same rats' mesenteric nodes or Peyer's patches. This is a factor important to the understanding of some of the differences between the descriptions which follow and previous descriptions in the literature on germinal centers.

Antigen Entry Pattern into Follicles.—Already 3 min after antigen injection, light labeling was apparent in the circular sinus. This intensified over the next 4 hr. Sectioned afferent lymphatics containing free, scattered label were encountered. In the sinus itself, some of the label appeared to be free, even as long as 24 hr after antigen injection; some was contained in vacuoles of phagocytic cells. These were usually monocytes but occasionally lightly labeled granulocytes were seen.

The sinus-lining cells showed a typical appearance after antigen injection. The outer wall of the sinus consisted of flattened cells with scanty cytoplasm and these displayed little interest in the antigen. Occasional grains could be seen adherent to their surface or inside a pinocytic vacuole, but labeling was never extensive. The inner wall of the sinus was formed by much bulkier cells. Some of these had deeply indented nuclei typical of blood monocytes (Fig. 1). While they usually had less cytoplasm than medullary macrophages (1), they contained numerous vacuoles and granules of a basically similar type, exhibiting a moderate degree of labeling. Many of these sinus-lining cells had a somewhat contorted shape suggesting active movement. This concept was supported by the occasional finding of similar cells in the sinus itself and in the subjacent cortex. The likelihood that the sinus-lining cells were far from static also gained support from the fact that they rarely showed labeling at intervals longer than 1 day after antigen injection. In view of the much longer persistence of antigen in the phagolysosomes of medullary macrophages, one likely explanation is that the cells lining the sinus and showing label, say 4 hr after antigen injection, had migrated elsewhere by 2 days and had been replaced by unlabeled monocytic cells. A dynamic concept of the inner sinus lining has also been proposed by Clark (9). Not every cell lining the inner aspect of the sinus was phagocytic. Some were medium lymphocytes and others were reticular cells. No endothelial cells or basement membrane were noted.

Labeling of phagocytic cells lining the sinus was never as intense as that observed in the medulla. Much antigen eluded these cells and apparently percolated between them. As cells of phagocytic character formed a layer of only one or at most two cells' thickness, such antigen soon reached the underlying lymphocytes. Antigen permeation into the cortex was intense only over lymphoid follicles. For a transient period of about 4 hr in previously uninjected rats and about 30 min in preimmunized rats, intercellular antigen could be seen in the thin layer of lymphocytes which separated the follicle from the sinus. After this period, progressively more and more of the total antigen retained in the lymph node cortex was confined to the follicles themselves.

Antigen Retention in Primary Lymphoid Follicles.—A typical portion of a primary lymphoid follicle is shown in Fig. 2. The great difference in antigen localization pattern from that seen in the medulla is evident even under low power. The bulk of the antigen is *between* cells rather than *in* them.

Higher power observation shows primary follicles to contain basically two types of cells, lymphocytes and reticular cells. The lymphocytes are usually small lymphocytes, though the median amount of cytoplasm may well be somewhat greater than that of small lymphocytes in the diffuse cortex. The reticular cells are recognized by their lighter nuclear chromatin pattern, their bigger size, and their profusion of long, thin cytoplasmic processes. An outstanding feature of follicle architecture is the intricate profusion of tenuous cytoplasmic extensions emanating from both reticular cells and lymphocytes (Figs. 4 and 5). Even in areas where cells appeared to be tightly packed together, close inspection frequently revealed one or more thin filamentous process between them. Often, areas of follicle were encountered, as in the center of Fig. 2, where the nuclei of lymphocytes were grouped around a heavily-labeled zone free of cell nuclei. Closer examination always revealed such areas to be very complex. A multitude of cell processes of varying sizes were seen to interdigitate extensively with each other and the bulk of the label appeared to be associated with the surfaces of processes or the related intercellular spaces (Figs. 2–5).

Details of Follicle Reticular Cells.—Sections which included favorably cut reticular cell processes (Figs. 2 and 4) showed that they suffered rapid attenuation at their origin in the basal regions of the cell. Even the broadest portion of the processes measured only 1–2 μ in width; this is, in general, considerably less than the broad blunt type of cytoplasmic extension of medullary macrophages. A further difference between follicle reticular cells and medullary macrophages was the relative paucity of dense granules and phagocytic vacuoles, and a somewhat lower content of mitochondria in the former. The cytoplasm of reticular cells contained numerous Golgi-type vesicles, occasional isolated strands of rough-surfaced endoplasmic reticulum, and a moderate number of free polyribosomes. There was little or no evidence of pinocytosis of antigen; the characteristic location of silver grains was on or very near the plasma mem-

brane (Fig. 4). Processes varied in thickness throughout their length; the terminal regions displayed marked exility with featureless cytoplasmic content. The profusion of fine processes and the relative infrequency of reticular cell bodies in any plane of section suggested that each cell emitted a large number of processes which interdigitated between lymphoid cells and with processes of lymphocytes to create an extensive dendritic web or "labyrinth" (10). The finest processes encountered were less than 100 mμ across.

Occasionally, where two reticular cell processes met, an increased focal thickening of apposing membranes (macula adhaerens) was noted, but well-developed desmosomes were encountered rarely in primary follicles.

It could not be claimed that every thin process seen in the follicle came from the reticular cell, nor was every antigen depot necessarily associated with a reticular cell process. Fig. 5 shows that lymphocytes, too, can engender delicate cytoplasmic extensions and these when examined in transverse or tangential section, are not distinguishable from the more tenuous of reticular cell processes cut in similar plane. In fact in many cases, where a complexity of processes was encountered, it was not possible to determine from which type of cell a particular process was derived. In most cases label was at or near the surface of a process. Occasionally, however, label could be seen between two lymphocytes that were in very close apposition. The resolution of the system does not enable us to determine whether such labeled antigen was attached to or embedded in a lymphocyte plasma membrane, or whether it lay in the extracellular space.

While most reticular cells in follicles were as described above, an apparently different variety was occasionally encountered. An example is shown in Fig. 6. The cytoplasm of these cells was filled with multivesicular bodies of various types and sizes. In contradistinction to most reticular cells, these cells actually engulfed antigen. Grains were scattered throughout their cytoplasm, both within and outside the multivesicular bodies. Unfortunately, such cells were encountered too infrequently for us to gain an appreciation of the detailed way by which antigen had entered them.

The Question of Antigen Entry into Lymphocytes.—The great majority of lymphocytes encountered in follicles had no grains over either their cytoplasm or their nucleus. No lymphocytes exhibited heavy labeling except at their very surface as described above. However, occasional cells had from one to five grains overlying them, usually over the nucleus. An example is shown in Fig. 6. Lightly labeled lymphocytes were often close to heavy intercellular antigen depots. This rendered interpretation of the significance of single, scattered grains difficult. For example, the grains over lymphocyte nuclei might have represented labeled molecules dragged across the surface of the section by the knife edge; or they may have been caused by vigorous Auger electrons taking an oblique path from the isotope source. Accordingly, we studied serial sections of several

lightly labeled cells, and found that in over half the cases, one or more grains also appeared over the nuclear profile of sections adjacent to the original one. Though no formal, statistical study was made, this lead us to conclude tentatively that small amounts of antigen or antigen fragments did occasionally enter follicle lymphocytes. This point will be discussed further below.

Antigen Localization in Secondary Follicles.—As our rats were not germfree, a small proportion of follicles present in popliteal lymph nodes before the first injection of flagella were secondary follicles with germinal centers. A much greater proportion were evident when rats had been intentionally preimmunized with nonradioactive flagella 6 wk before the injection of ^{125}I-flagella. Interestingly, apart from a greater initial rate of antigen entry in the preimmunized rats, no differences in antigen localization were noted between the above two situations.

There were four chief differences noted between primary (Figs. 2, 4, and 6) and secondary (Figs. 3, 5, 7, and 8) follicles: (*a*) the secondary follicles contained a greater proportion of large lymphoid cells with an extensive development of polyribosomes, and numerous cells in mitosis; (*b*) the reticular cells of secondary follicles tended to cluster in a crescentic manner over the outer aspect of the follicle; (*c*) in some of the intercellular spaces between cell processes in secondary follicles, a homogeneous, electron-opaque material (Fig. 8) was seen; and (*d*) a greater number of TBM were present in secondary follicles.

Antigen localization in secondary follicles followed the same general pattern as described for primary follicles. While the antigen concentration over the crescentic "cap" region rich in reticular cells was higher than over the deep aspect of the germinal center, there was still a diffuse labeling pattern over the whole of most germinal centers. Grains lay mainly between cells and cell processes. In general, the pattern of distribution of label was similar to that of the homogeneous, electron-opaque material (Fig. 8), suggesting that the latter could have been a complex of antigen and antibody. Tingible body macrophages (TBM) showed great variation in their tendency to capture antigen. Many TBM were unlabeled. Others showed antigen on or near their surface, but only a few scattered grains inside them. Still others showed moderate to heavy labeling over the characteristic inclusions, and especially over myelinic figures (Fig. 7). However, the intensity of labeling over TBM did not ever reach the level of that over medullary macrophages. Even in germinal centers containing many TBM, the total amount of label inside TBM would have represented only a very small percentage of the total antigen stably trapped in that follicle.

As discussed above, some follicles exhibited only occasional TBM, presumably indicating a low level of past or present germinal center activity. These were intermediates between primary follicles and typical germinal centers. The rare TBM in such follicles showed similar inconsistencies of labeling intensity as did the more numerous TBM of germinal centers.

Comparison of our pictures with previously published reports of the electron microscopic features of germinal centers (10–13) revealed many similarities but also some differences. The accumulation of electron-opaque intercellular material was less in our studies. Also, desmosomes between reticular cell processes were less prominent. We gained the general impression that our germinal centers were "younger" and not so well developed relative to the germinal centers of organs such as the mesenteric nodes which are subjected to continued, intense antigenic stimulation.

Persistence of Antigen in Follicles.—In general, the kinetic aspects of our study of antigen retention in follicles were not revealing following the first few hours after injection. In secondary follicles, TBM labeling may have reached maximal intensity somewhat later than membrane-associated labeling. There was an impression that follicular labeling may have declined from a peak at 24 hr somewhat more rapidly than did medullary labeling but no quantitative grain counts were carried out. In general, however, the overall pattern of labeling in the follicle remained constant between 4 hr and 21 days after antigen injection, with intercellular, membrane-associated grains dominating the picture. The usual well known changes of the immune response occurred in the lymph node cortex after injection of labeled antigen. They included increased germinal center activity and appearance of blast cells in the diffuse cortex. Apart from a tendency of the follicular antigen to assume a more peripheral, crescentic distribution as germinal centers developed in primary follicles (2), the cellular changes had no influence on cortical antigen distribution.

Labeling in Other Areas of the Lymph Node Cortex.—Trabecular sinuses joined the circular sinus to the medulla. The cells lining these sinuses and lying within them exhibited all the features of antigen capture already described for their medullary counterparts (1).

Occasionally, a lone labeled macrophage of medullary type was encountered in the diffuse cortex. Typical postcapillary venules were frequently observed but these were not detectably labeled in the present study. As light microscopic radioautography has shown these venules to become heavily labeled in their deeper aspects following intravenous, rather than foot-pad, injection of ^{125}I-flagella (14), this point deserves further study.

In general, however, the absence of any label over extensive areas of diffuse cortex was in striking contrast to the heavy labeling of follicles and medulla.

Follicular Labeling with Materials other than Flagella.—Study of the localization of HSA–anti-HSA and of autologous gamma globulin fully confirmed previous experience (15) with light microscopic radioautography. In both cases, strong follicular localization was noticed. The pattern of labeling resembled that seen following injection of ^{125}I-flagella. If anything, both the HSA and the autologous globulin showed proportionately somewhat more tendency to enter surrounding lymphocytes than did flagella. However, it must be stressed that

the vast bulk of follicular antigen was intercellular and associated with membranes of processes.

Monomeric flagellin showed considerably less intense follicular localization than did an equivalent dose of flagella. However, the ultrastructural aspects of localization pattern were identical.

DISCUSSION

In both the medullary sinuses and the cortical follicles, antigen is trapped and retained for long periods. In the medulla, the events are fairly conventional and well known. Phagocytic cells capture the antigen and sequester it into digestive inclusions. In the follicles, the mechanism of antigen retention is more obscure and more unusual. Here antigen reaches a largely extracellular location at or near the surface membranes of a dendritic web of interlocking cell processes, and in some way, it is held there for at least several weeks.

There now appears to be little argument about the chief anatomical features of the specialized follicular reticular cells which constitute the nonlymphoid framework of the follicle. Many of the observations recorded by us have simply confirmed and extended descriptions of these cells by previous authors (10–13), though we appear to have been the first to draw attention to the reticular cells as a crucial part of primary follicles as well as germinal centers. However, the main novel feature of the present study has been the demonstration of the role of follicle reticular cells in antigen handling. Two chief viewpoints of this role can be stated. On the one hand, the surface of the dendritic processes may have no special biochemical properties, but may serve a purely structural role. As Milanesi (10) has claimed, they may, by their extensive intertwinings, simply construct an elaborate labyrinth through which antigen percolates very slowly, creating an illusion of stable trapping. This formulation could explain the greater tendency of particulate antigens to go to follicles compared to low molecular weight, soluble antigens. It does not readily account for the comparatively poor ability of nonantigenic particles such as colloidal carbon to lodge there (7) nor does it fit with our observation that autologous globulin, labeled and reinjected subcutaneously into rats, shows strong follicular localization.

On the other hand, it is possible that the dendritic cells play a more active role. Perhaps they possess on their surface certain receptors which recognize a site on the globulin molecule. Antigen might then become attached to reticular cell processes because of its prior union with natural or immune antibody, which in its turn adheres to follicle cells. Finally, the two mechanisms are not mutually exclusive. The labyrinthine nature of the extracellular space could slow down the flow of fluid between cells, which might increase the chance of attachment of antigen to cell surfaces.

It might be possible to decide between the two hypotheses if one knew whether antigen was firmly bound to reticular cell plasma membranes, or was distributed evenly in the extracellular space between cells and processes. Unfortunately, the resolution of our radioautographic method did not allow us to determine this point. A critical study with ferritin as antigen might be helpful, and this is an avenue which we hope to explore.

Although study of the follicular localization using materials other than flagella was confined to three small experiments, these were sufficient to show quite clearly that the essential features of trapping are identical in all cases. As four substances of such differing physical and chemical properties all followed the same pattern, it seems reasonable to conclude that most substances which have been shown by light microscopic radioautography to lodge in follicles will be found, on electron-microscopic study, to be largely associated with reticular cell processes and other cell membranes.

Regardless of the mechanism involved, follicular localization presents an extraordinary opportunity for contact of lymphocyte surfaces with antigen. The total surface area of the antigen-retaining web must be enormous, and recent studies in our laboratory (C. M. Austin, manuscript in preparation) have shown a significant traffic of recirculating lymphocytes through primary follicles. Antigen deposited in follicles may cause some lymphocytes to undergo blast cell transformation and division, the end result being the genesis of a germinal center. It is possible that surface contact between antigenic determinants and natural antibody receptors on certain lymphoid cells triggers the blast cell change. There is a good model for such a mechanism in the in vitro blast transformation induced by contact between globulin allotype determinants on the lymphocyte surface acting as antigen, and antiallotype sera (16). We do not know whether entry of some antigen molecules into the lymphocyte cytoplasm or nucleus is either a necessary or a sufficient condition for blast cell transformation. Our radioautographs were consistent with the view that small amounts of antigen may have passed from the follicular depot into lymphocyte nuclei, a conclusion previously reached (17) on the basis of light microscopic radioautography. However, it should be stressed that the amount of antigen used in the electron-microscopic study, i.e. 20 μg, was thousands of times greater than the minimal immunogenic dose. Accordingly, it is quite possible that the finding of antigen in lymphoid cells is an epiphenomenon of no importance to immune induction. Furthermore, there is no way of knowing whether a particular lymphocyte noted to contain antigen would have gone on to blast transformation. A more critical analysis of the way in which antigen modulates the immune response awaits a technology whereby antigen-reactive cells could be either definitively identified or obtained in pure suspension, and then induced in defined in vitro conditions. Recent progress in this area has been so rapid (18, 19) that this may not be an entirely vain hope.

Our studies suggest that the TBM so characteristic of germinal centers play only a secondary role in follicular antigen capture. Labeling of these cells was very variable and, more importantly, antigen localization was equally efficient in follicles containing few or no TBM. One gained the impression that TBM labeling may have represented little more than the inevitable consequence of the presence of a highly phagocytic cell in a region of a heavy concentration of extracellular antigen. Clearly the main duty of TBM is to phagocytize lymphocytes that have died locally. Why so many lymphocytes, most of which have undergone recent division, should die in germinal centers remains a mystery. TBM are a different type of cell from the nonphagocytic dendritic reticular

cells. Very speculatively, we suggest that they may have developed from monocytes that arrived in the node via the circular sinus, transiently formed the inner sinus lining, and subsequently moved down into a stimulated lymphoid follicle, there to assume the characteristic function and morphology.

We attach some importance to the observation that the homogeneous, electron-opaque intercellular material so prominent in germinal centers of other workers (10, 13) was absent in our primary follicles and not very extensive in the young, lightly stimulated germinal centers of rat popliteal nodes. Previous reports (20–22) have shown the presence of immunoglobulins in germinal centers in a distribution broadly similar to that of the antigen depots noted by us. This suggests that the electron-opaque material may well represent antigen-antibody complexes. The question is then raised as to which came first—the antigen, or the antibody? If germinal centers are the site of active antibody production, may the localization of antigen in germinal centers not be merely a trivial consequence of high local antibody concentration? Several lines of reasoning speak against this view, and suggest that antigen, albeit linked to antibody, initiates the changes: (*a*) the ontogeny of follicular localization shows the development of a trapping web well before the first germinal centers appear in popliteal nodes (23); (*b*) in mature animals, appropriate antigens reach peak concentrations in primary follicles within hours, well before the first appearance of detectable antibody or antibody-forming cells (2); (*c*) animals not forming antibody to an antigen, but given antibody passively, show follicular localization equal in extent to that of actively immunized animals (4); and (*d*) every follicle of every animal can be labeled with a sufficient dose of ^{125}I-flagella (4), and it appears somewhat unlikely that every single follicle would be actively synthesizing antiflagellar antibody within 4 hr of antigen injection.

We thus speculate that the sequence of events may be the following. An antigen enters the tissues; it may encounter natural or immune antibody. A soluble complex is formed and it moves in the lymph to the circular sinus of the draining lymph node; some of the complexed antigen passes by the sinus-lining phagocytes and reaches the intercellular spaces of the follicle. By an as yet unclear mechanism, a proportion of this material is specifically retained within the follicle. If the complex is in antigen excess, free antigenic determinants remain and are capable of causing blast cell transformation in appropriate antigen-reactive cells which are in slow but constant motion throughout the follicle web. The blast change may or may not involve the passage of small amounts of antigen into the lymphocyte. Some of the transformed cells leave the follicle and move to the medullary cords where they differentiate further into antibody-forming plasma cells. Others remain within the follicle and continue to divide, eventually forming a germinal center which may be a factory for memory cells. Antibody to the antigen concerned is made, chiefly in the medullary cords of lymph nodes and the red pulp of the spleen, and enters the

lymph and the serum. It circulates and, on reaching any unsaturated antigenic determinants in follicles, unites with them. Initially this deposit is relatively inconspicuous either by immunofluorescence or by electron microscopy. With the passage of time, more antigen of the same or different type reaches the follicle and is trapped in its turn. The cycle is repeated, and continued stimulation results in the progressive accumulation of considerable quantities of immunoglobulins. Examination of lymph nodes chronically stimulated either by the investigator or by naturally present antigens then reveals these substantial deposits. The mechanical stresses involved in the progressive expansion of the germinal center fall largely on the dendritic reticular cells. As a result, their processes hypertrophy and numerous obvious desmosomes may develop between them. At this stage, lymphocyte traffic from germinal centers is mainly export; recirculating lymphocytes have great trouble entering. If antigen entry into the node diminishes or ceases, antibody deposition on antigen and catabolism of antigen eventually terminate the inductive stimulus. Thus the rapid lymphocyte division ceases and the germinal center regresses. Renewed antigenic barrage may initiate another similar cycle.

SUMMARY

This paper describes the trapping of antigen in lymphoid follicles of rat popliteal lymph nodes as revealed by electron microscopic radioautographs following injection of ^{125}I-labeled *Salmonella adelaide* flagella and other materials.

The antigen was taken up vigorously, and to an approximately equal extent, by both primary and secondary follicles. The rate of uptake was faster in preimmunized than in virgin adult rats. The bulk of the antigen in follicles was extracellular, and persisted in this location for at least 3 wk. Label was most frequently found at or near the surface of fine cell processes. Many of these were branches of dendritic follicular reticular cells. Such processes interdigitated with equally fine processes of lymphocytes, creating an elaborate meshwork. In some cases, antigen was found between lymphocytes which appeared to be in close apposition. Occasionally, a few grains appeared over lymphocyte nuclei and study of serial sections suggested that this probably represented true entry of small amounts of antigen into lymphocytes.

The characteristic "tingible body" macrophages (TBM) of germinal centers appeared to play only a secondary role in follicular antigen retention. They showed degrees of labeling over their phagocytic inclusions varying from negligible to moderately heavy. Moreover, follicles lacking or poor in TBM retained antigen just as effectively as those containing numerous TBM. The hypothesis is advanced that TBM may be derived from monocytes that migrate down from the circular sinus.

Follicular localization of three other materials was also studied, though not

in such detail. These were ^{125}I-HSA complexed to anti-HSA: ^{125}I-labeled autologous IgG; and ^{125}I-monomeric flagellin. All of these showed the basic features of intercellular, membrane-associated deposition noted with ^{125}I-flagella.

The role of follicular antigen depots in immune induction is discussed. The tentative conclusion is reached that follicular antigen in a primary follicle encounters natural antibody on the surface of certain antigen-reactive lymphocytes. The resultant reaction causes blast cell transformation and eventually the genesis of a germinal center.

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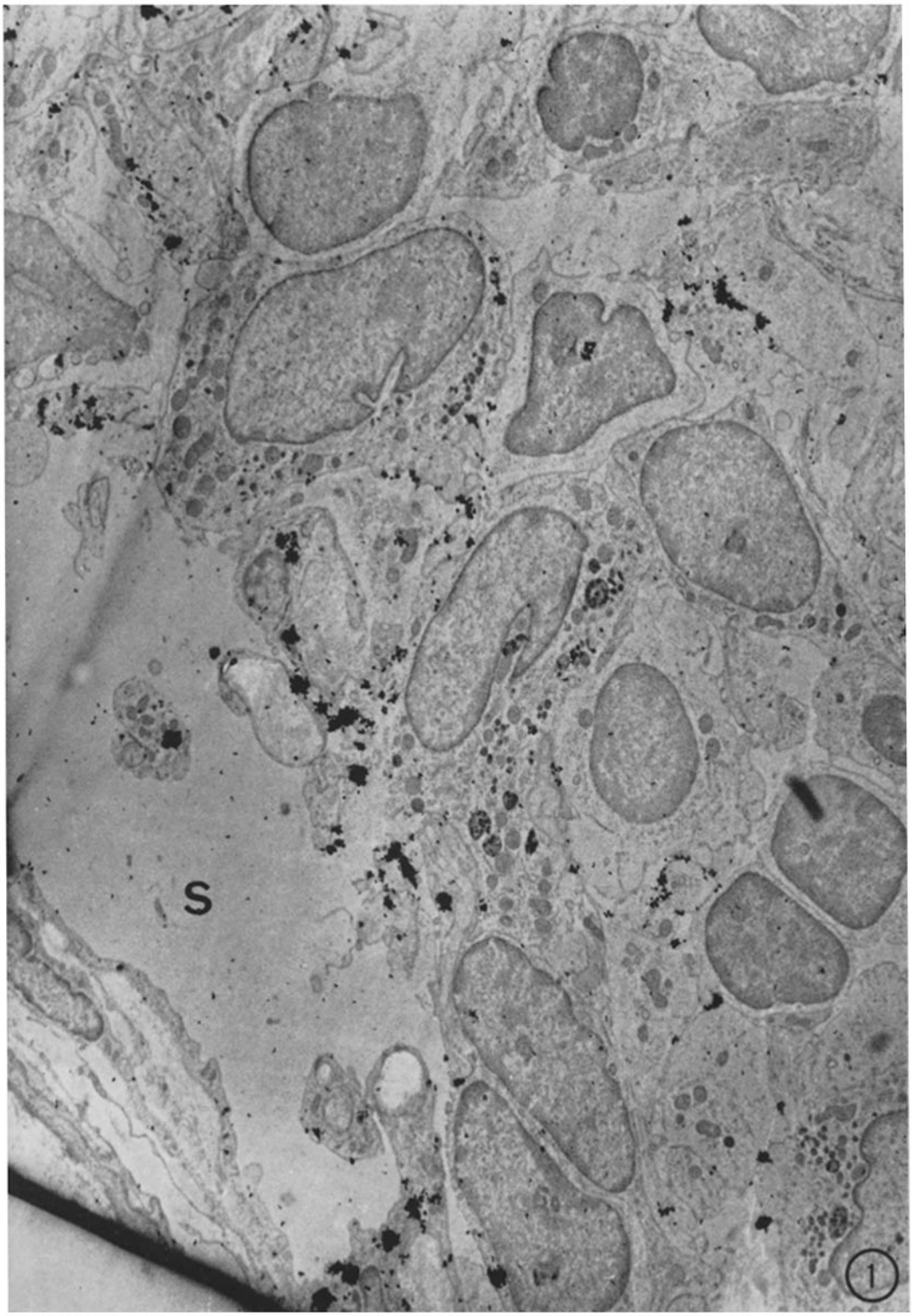
EXPLANATION OF PLATES

In all figures fixation was by osmium tetroxide; uranyl acetate stain. The labeled antigen was ^{125}I-flagella unless stated.

PLATE 43

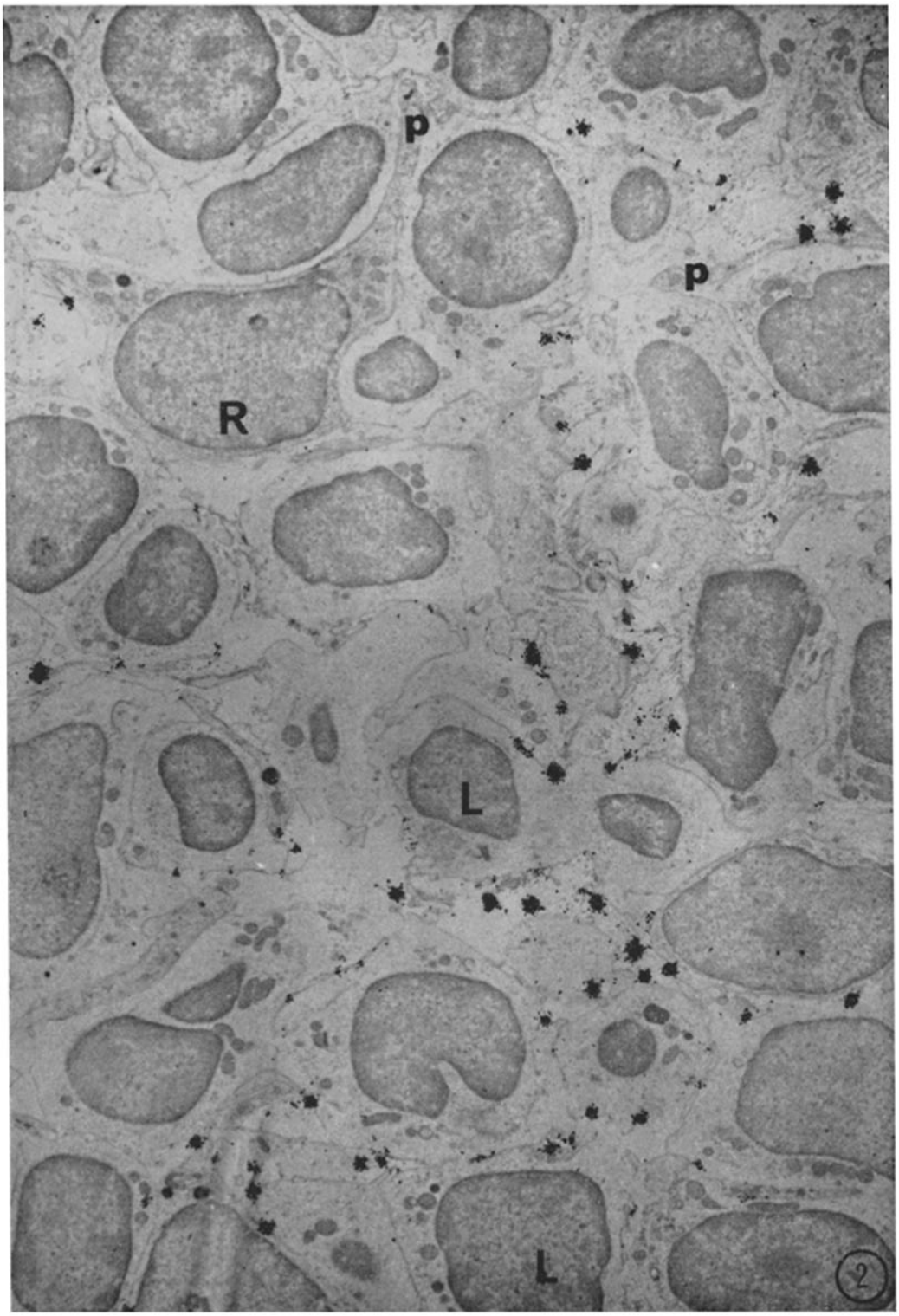
FIG. 1. A low power electron microscopic radioautograph showing the entry of labeled antigen into the superficial lymph node cortex overlying a primary follicle. Note scattered free label in the circular sinus (*S*) and clumps of label in phagocytic cells in the sinus. The inner sinus lining contains several monocyte-type cells and portion of one appears at bottom center. Much antigen has eluded these cells and is percolating between lymphocytes into the underlying follicle. × 5000.



(Nossal et al.: Follicular antigen trapping)

PLATE 44

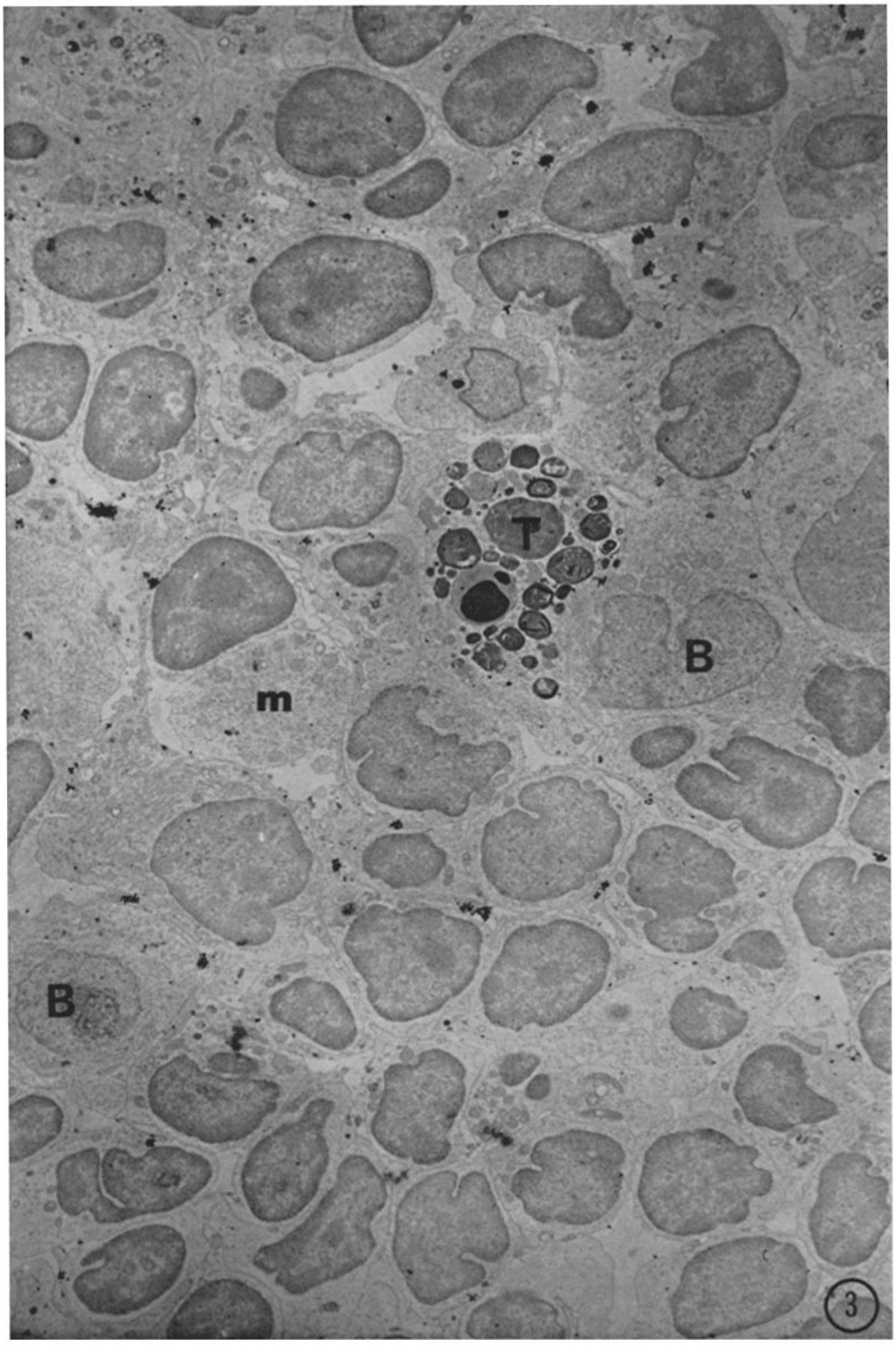
FIG. 2. A typical pattern of localization of antigen in a primary lymphoid follicle. Even at low power, reticular cells (*R*) and their processes (*p*) can be distinguished. Numerous small lymphocytes (*L*) are present, but there are no blasts, mitotic figures, or "tingible body" macrophages. Note that the heavy clumps of label are located between cells and that there is an absence of heavy deposits within lymphocytes. × 5280.



(Nossal et al.: Follicular antigen trapping)

PLATE 45

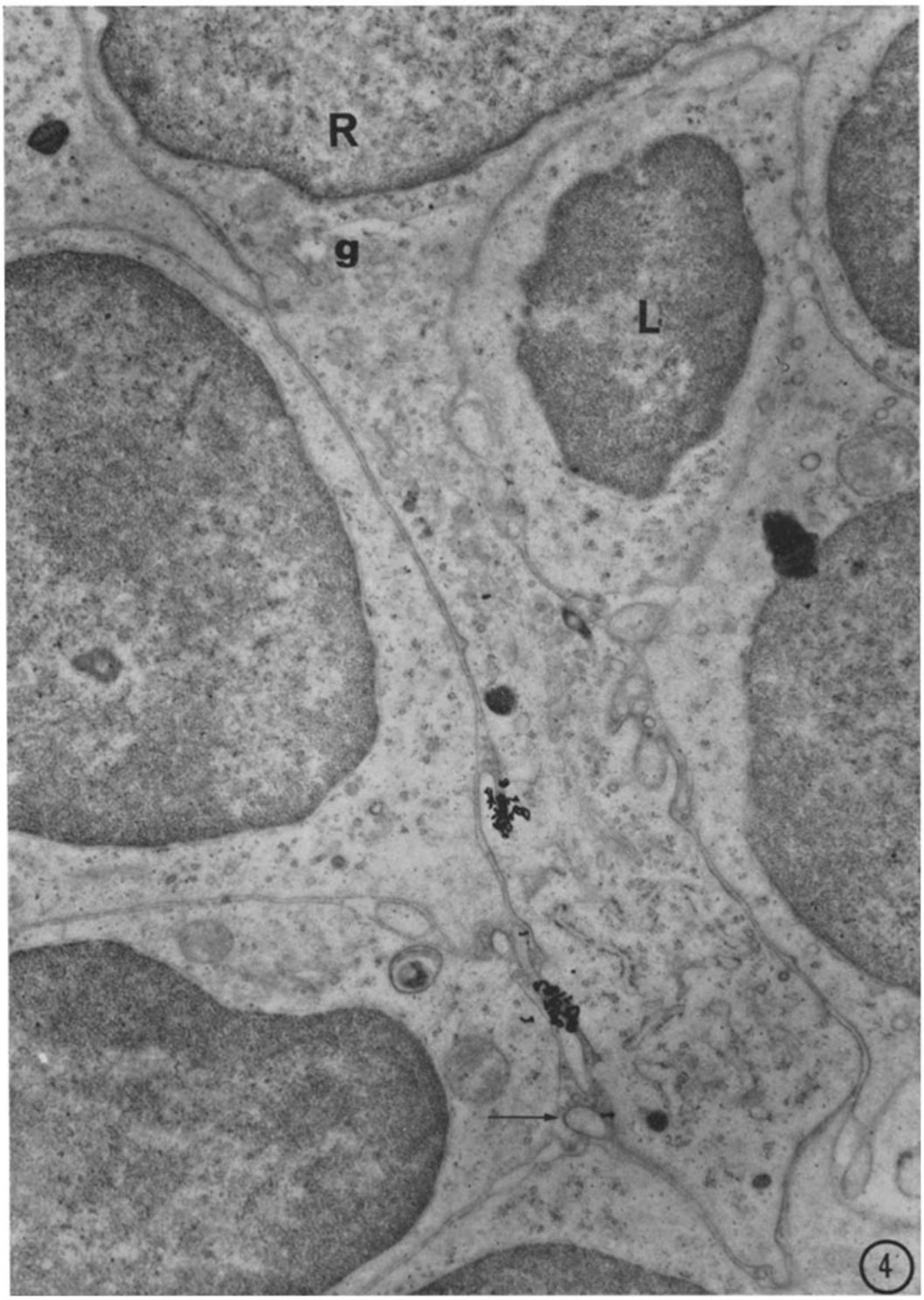
FIG. 3. Low power view of an antigen-retaining secondary follicle. Note the numerous blasts (*B*), the mitotic figure (*m*), and the characteristic tingible body macrophage (*T*) with phagocytozed nuclear debris. Reticular cells and their processes are also present and the labeling pattern still shows many examples of intercellular, membrane-associated silver grains. × 4000.



(Nossal et al.: Follicular antigen trapping)

PLATE 46

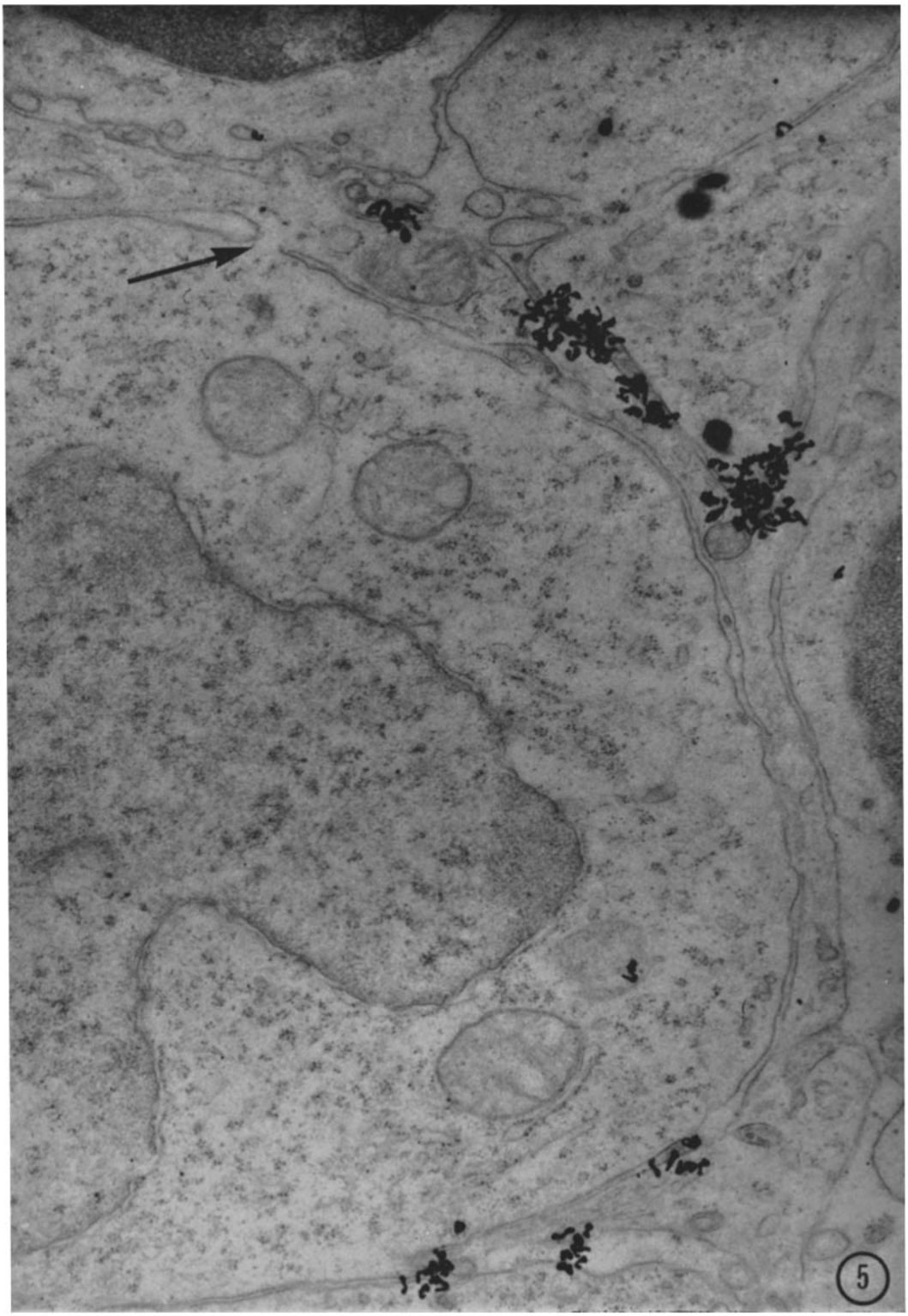
FIG. 4. Higher power view of portion of a primary follicle includes a reticular cell (*R*) and several lymphocytes (*L*). The cytoplasmic process of the reticular cell extends from its source at top, where there is a prominent Golgi region (*g*) in the paranuclear zone, to bottom right where the terminal region displays the characteristic hyaloplasm so frequently observed when the ends of fine processes are viewed in transverse section: several of these fine processes ranging in size down to less than 100 mμ diameter, can be seen (arrow). Note the two heavy clumps of label on or near the plasma membrane at surface invaginations. Even where it is widest, the process contains only occasional dense granules in contrast to medullary macrophages and it makes close contact with at least five lymphocytes in this short extent of its length. × 17,500



(Nossal et al.: Follicular antigen trapping)

PLATE 47

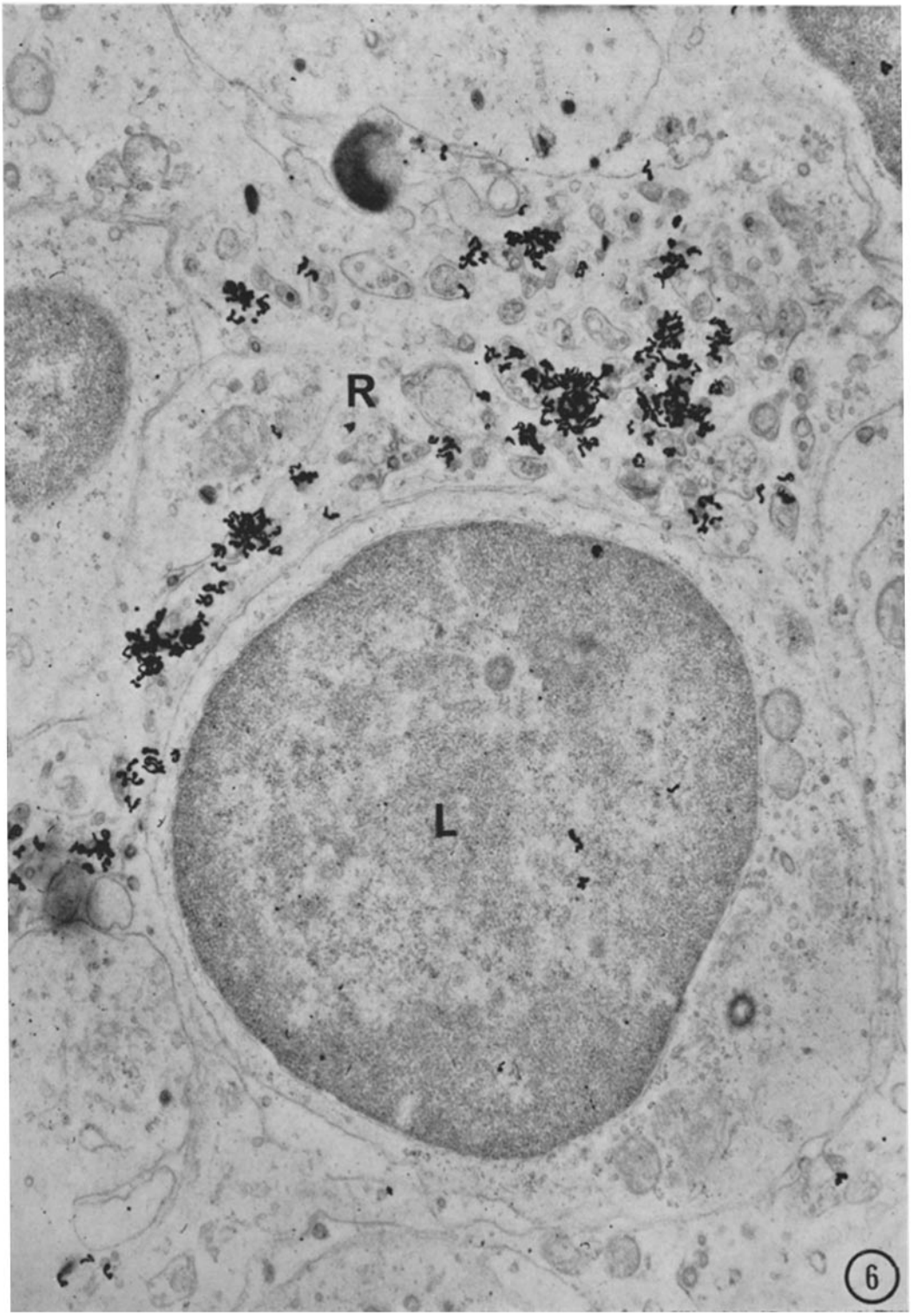
FIG. 5. High power view of a typical region of a secondary follicle. A blast cell with numerous free polyribosomes occupies most of the field. At the arrow, it gives rise to two processes which double back and embrace the cell. These in turn make contact with numerous coarse and fine cell processes at right. Numerous clumps of label are present. Many are at or near the surface of the lymphocyte, and, of course, the surface of adjacent fine cytoplasmic processes. One silver grain overlies the cytoplasm of the lymphocyte. Only serial sections could determine whether this indicated true entry of antigen into the blast cell. × 22,000.



(Nossal et al.: Follicular antigen trapping)

PLATE 48

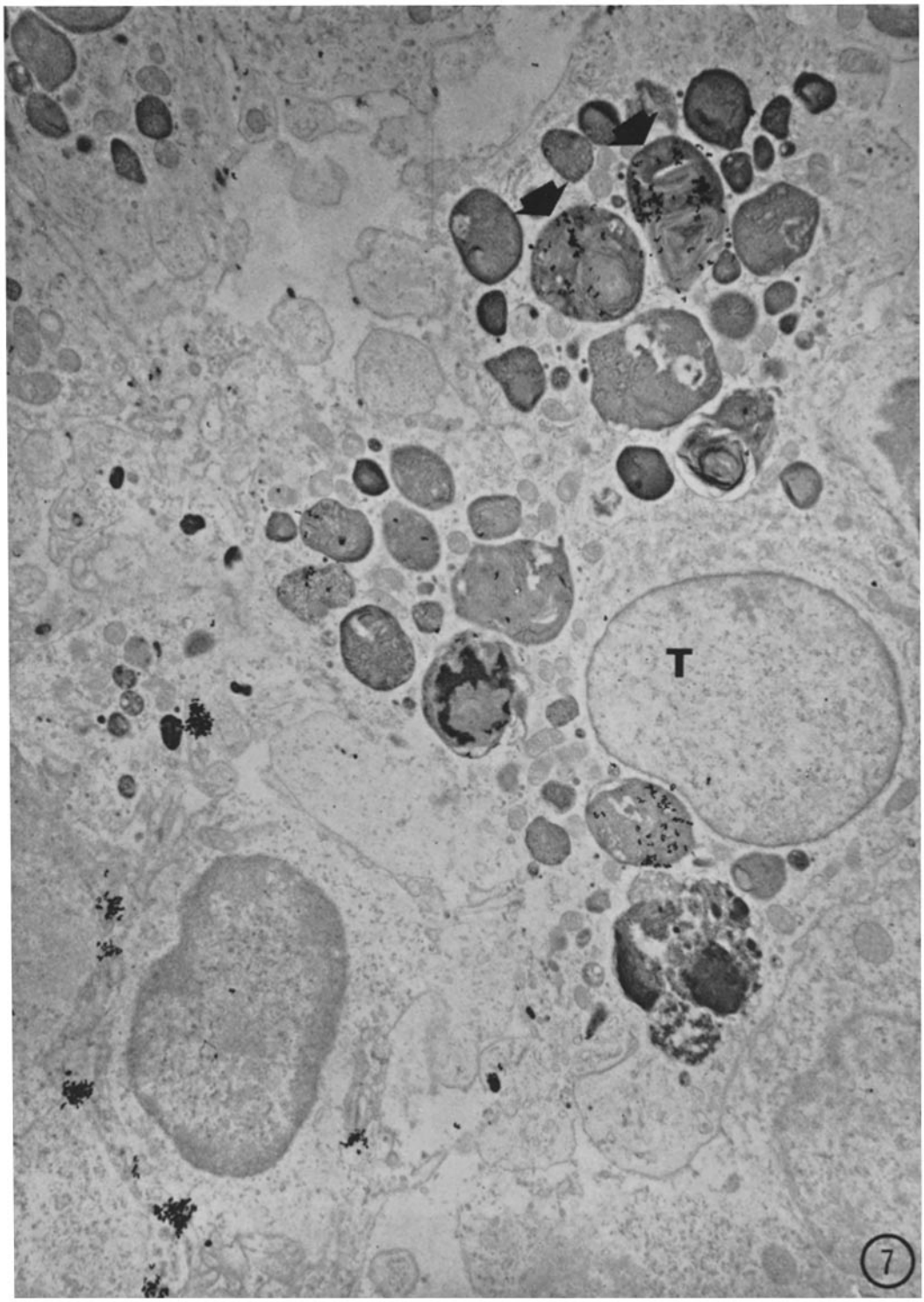
FIG. 6. High power view of portion of a primary lymphoid follicle showing a second type of follicle reticular cell (*R*). The cytoplasm of this cell contains many multivesicular bodies and a considerable amount of label; the nucleus is not in the plane of section. Clearly this cell is neither a typical macrophage nor does it resemble the reticular cell of Fig. 4. This type of cell was encountered rarely and we have no clear picture of how antigen entered it. Note also that the adjacent medium lymphocyte (*L*) has five grains over its nucleus. Serial sections showed that adjacent profiles also had one to three grains over them. The likelihood is that the grains truly represent antigen which has entered the lymphocyte nucleus. × 18,500.



(Nossal et al.: Follicular antigen trapping)

PLATE 49

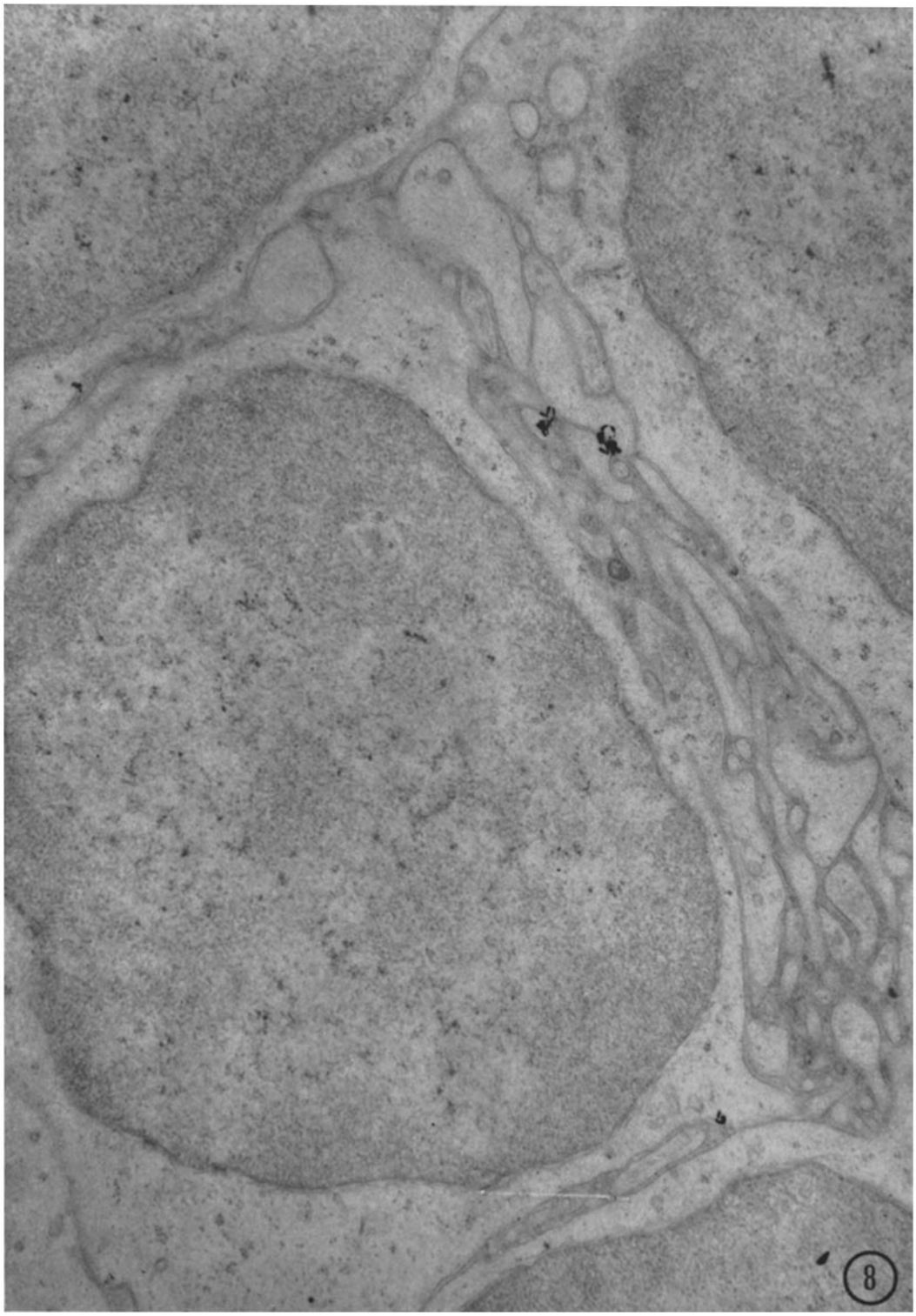
FIG. 7. Portion of an antigen-retaining germinal center. The right half of the picture is occupied by a "tingible body" macrophage (*T*). Note the presence of "moth-eaten" inclusions, myelinic figures, and smaller, more homogeneous dense granules. Two of the myelinic figures (heavy arrows) and several other inclusions show moderately heavy labeling. At left, the usual picture of closely intertwining cell processes and membrane-associated label is seen. × 9100.



(Nossal et al.: Follicular antigen trapping)

PLATE 50

FIG. 8. A view of portion of a secondary lymphoid follicle exhibiting the presence of a homogeneous electron-opaque material between a complex, characteristic pattern of cell processes cut tangentially and in cross-section. This rat had received rat IgG iodinated with ^{125}I and injected into the foot-pad 24 hr before killing. Note two small clumps of label and one single grain close to the surface of various processes. Some free polyribosomes are present in the otherwise clear cytoplasm of adjacent lymphocytes. × 20,000.



(Nossal et al.: Follicular antigen trapping)