Rheumatoid arthritis is characterized by chronic local inflammation in synovial tissue. The cells that comprise the mononuclear infiltrate are, for the most part, immunoreactive cells that engage in multiple interactions leading to the proliferation of the synovial tissue and the synthesis of immunoglobulins (1–5) and mediators of cellular immunity (4, 5). Because lymphocytes, macrophages, and dendritic cells play an important role in both cellular and humoral responses, a number of investigations of the distribution and interrelationships of these cells have been carried out. The presence of large numbers of T lymphocytes as compared with B lymphocytes, both in synovial fluid (6–8) and synovial tissue (9–12), has emphasized the important role of the T lymphocyte and the cellular immune response in the rheumatoid chronic inflammatory reaction.

Recently, a series of monoclonal antibodies reactive with cell-surface determinants of human T lymphocytes has been developed. These antibodies, which selectively react with human T cells, provide an opportunity to differentiate T cell subpopulations in patients with autoimmune diseases. One group of such antibodies is the OKT group in which OKT3 antibody reacts with T cells (13), OKT4 reacts with helper/inducer T cells (14, 15), and OKT8 reacts with suppressor/cytotoxic T cells (14, 16). An increased ratio of T4 to T8 cells in the peripheral blood of rheumatoid patients has been reported by several groups (17–20). By contrast, decreased ratios of T4 to T8 cells, compared to peripheral blood, have been measured in rheumatoid synovial fluid (19–22). In synovial tissue, markedly increased numbers of T4 cells have been described by three laboratories utilizing either immunofluorescence (23, 24) or immunohistochemical staining techniques (25, 26). Most of these T4 cells were in close contact with HLA-DR-expressing macrophage-like cells. On the other hand, a predominance of suppressor/cytotoxic cells in intact rheumatoid synovial tissue (27) and both normal (28) and decreased (29) ratios of T4 to T8 cells in synovial tissue digestes have also been described. This variation in the observations reported by different investigators has indicated to us the desirability of careful identification of the T cell subsets and other immunoreactive cells of the rheumatoid synovial membrane in different areas of tissue specimens and of establishing the pattern
in which these cells are distributed in relation to each other.

In the present study, an immunoelectron microscopic technique using monoclonal antibodies against the subsets of T lymphocytes has been used to identify these cells and establish their pattern of distribution in relation to other immunoreactive cells, especially the macrophage-like cells.

Materials and Methods

Rheumatoid Synovial Tissue. Samples of synovial membranes were obtained during reconstructive knee surgery from six patients with definite or classical rheumatoid arthritis (30). All patients were considered to have moderately to severely active synovitis at the time of the surgery. Tissue specimens were cut into small pieces, ~5 mm³, and immediately fixed in 2% or 4% paraformaldehyde, 0.075 M lysine, 0.01 M sodium-m-periodate solution, for 4 h at 4°C as described by McLean and Nakane (31). The fixative was changed two to three times. They were then washed two times and then overnight with PBS (0.01 M phosphate buffer containing 0.15 M sodium chloride, pH 7.6) containing 4 × 10⁻⁴ M digitonin and 10% sucrose, and then washed for 3 h each with phosphate-buffered saline (PBS) containing 15% sucrose and PBS containing 20% sucrose (32). The tissues were then transferred to a solution of PBS containing 5% glycerol and 25% sucrose for 3 h or longer, then were embedded in OCT compound (Lab-Tek Products, Naperville, IL) in polyethylene vials (Ernest F. Fullam, Inc., Schenectady, NY) and snap frozen in liquid nitrogen. Specimens were stored in a ~80°C freezer until used.

Antisera and Other Reagents. Mouse monoclonal antibodies with well-defined specificities denoted OKT3, OKT4, and OKT8 were purchased from Ortho Pharmaceutical Corp. (Raritan, NJ). Normal horse serum, biotinylated horse anti-mouse IgG, avidin DH, and biotinylated peroxidase (ABC-kit) were obtained from Vector Laboratories (Burlingame, CA). Digitonin and 3,3′-diaminobenzidine were purchased from Sigma Chemical Co. (St. Louis, MO).

Immunohistochemical Staining and Immunoelectron Microscopic Studies. Frozen sections, 6-10-μm thick, were cut in a cryostat at ~30°C and mounted on gelatin and egg albumin-coated slides (33). After drying for 30 min in room air, the sections were washed with chilled PBS. The following incubations were carried out sequentially at room temperature in a humid chamber, and the sections washed for 20 min or longer with three changes of PBS between each step. Normal horse serum diluted 1:250 was first applied to the sections for 30 min to reduce background staining. After blotting excess serum from the sections, they were incubated with an appropriate volume (100-200 μl) of diluted mouse monoclonal antibody (1:10 to 1:40 dilution) for 60 min. Control tissue sections were incubated in identical fashion with normal mouse IgG (Cappel Laboratories, Cochranville, PA) or PBS alone. After washing, biotinylated horse anti-mouse IgG antibody was permitted to react with the sections for 60 min. Finally, the sections were incubated with a complex of biotinylated peroxidase and avidin DH (Vectastain ABC Reagent; Vector Laboratories, Burlingame, CA) for 90 min (34). The slides were then immersed in incomplete Karnovsky’s solution (0.2 mg diaminobenzidine/ml of 0.05 M Tris-HCl, pH 7.6) without hydrogen peroxide for 30 min. They were then transferred to Karnovsky’s solution containing 0.005% hydrogen peroxide for 2-10 min, depending upon the intensity of staining, and then washed in PBS. Sections were then fixed with 1% OsO4 for 1 h and washed in PBS. They were then covered temporarily with a glass coverslip over 90% glycerin in PBS and photographed under the light microscope (35). After removing the coverslip, sections were washed in PBS and dehydrated in graded alcohol to 100%. While the sections were still wet, plastic capsules filled with Epon 812 were inverted over the sections. After polymerization of the Epon 812, the slides were heated on a hot plate and the sections removed from the slides. Fields were chosen for electron microscopic study by examining the light microscopic pictures on a dissecting microscope. Sections 100-nm
thick were cut perpendicularly to the surface of the specimen with an LKB microtome and examined for peroxidase staining in a Phillips 300 electron microscope with and without counterstaining. The unstained sections were counterstained with uranyl acetate and lead nitrate.

Scoring of Immunoreactive Cells. In each of five surgical specimens, five to seven fields containing >80 cells each were chosen for each monoclonal antibody and identifiable immunoreactive cells in each field were scored by the immunoelectron microscopic technique. Pictures of intermediate magnification (× 5,200) as well as low magnification (× 2,400) were taken to establish the extent of cellular infiltration in each field and the types of cells present. Lymphocytes, both stained and unstained, plasma cells, and macrophage-like cells were identified and counted.

In one of the six synovia examined, almost all of the mononuclear cell collections consisted mainly of plasma cells. Because the findings in this synovium were not characteristic of those in other synovia, they were not included in the differential counts of the perivascular infiltrates that are reported here.

Results

Light Microscope Findings. The perivascular mononuclear cell collections, as seen in the light microscope, tended to vary in size and in density of lymphocytic infiltration. In areas of extensive infiltration, dense perivascular collections of T3 staining cells were observed. Staining of adjacent sections showed that a majority of these T cells were of the T4 helper/inducer variety (Fig. 1, A). In these areas, T8 cells were usually decreased in number and tended to be scattered through the lymphocyte collections (Fig. 1, B). In less densely infiltrated areas, it was difficult to estimate the amounts of T4 and T8 cells. Moreover, it was not possible in the light microscope to establish the relationship of the T lymphocyte subsets to the other cell types in the cellular infiltrates and to each other. In order to determine the percentage of T4 and T8 cells in varying types of cellular infiltrates and to establish the pattern of their relationship to the other cells in the mononuclear cell infiltrates, immunoelectron microscopic studies were carried out.

Immunoelectron Microscopic Identification of Positively Staining Lymphocytes. In Fig. 2, A, a portion of the field stained with OKT4 antibody, viewed in Fig. 1, A at the light microscopic level, is shown as it appears in the electron microscope without counterstaining. It is seen that the cell membranes have been stained permitting positively staining cells to be identified by the specificity of their electron-dense rim staining. Most of the positive cells showed continuous staining of their cell membranes and frequently the stained membrane had an irregular and villous appearance. No intracellular staining was observed except for occasional endogenous peroxidase staining observed in macrophage-like cells that did not exhibit membrane staining.

Intracellular detail was established in the same cells by counterstaining of adjacent ultrathin sections with uranyl acetate and lead nitrate (Fig. 2, B). Although the cellular detail in such sections was not optimal because of the prior immunoperoxidase staining steps, it was possible to establish the characteristics of the cells without difficulty. Scoring of positive cells, therefore, as well as identification of cell types (lymphocytes, plasma cells, or macrophage-like cells) were done on counterstained sections. When membrane staining was doubtful, non-counterstained sections were examined to check the staining. Penetration of
Figure 1. (A) Perivascular lymphocyte collection stained with OKT4 antibody. The majority of the cells are positively stained. v, vessel. Original magnification, ×250. (B) Adjacent section stained with OKT8 antibody. Positively staining cells are scattered and less in number than the T4 staining cells. Note the same vessels observed in A (V1 and V2) are shown. Original magnification, ×250.
FIGURE 2. (A) Part of the field in Fig. 1, A, stained with OKT4 antibody and observed without counterstaining in the electron microscope. Membrane staining is present (arrow) and positively staining cells are numbered (X 5,200). Most of the positive cells show continuous staining and frequently the membrane appears ruffled and villous in appearance. (B) The identical field to A is counterstained with uranyl acetate and lead nitrate. Cells that have the same numbers in A coincide with each other. Note the positive membrane staining (arrow). X 5,200.
antibodies into the synovium was deep enough to stain all layers of the specimen as indicated by examining sections taken from the under surface of the tissue mounted on a glass slide. Control sections treated with normal mouse IgG or PBS instead of mouse monoclonal antibodies were always negative (Fig. 3).

**Lymphocyte-rich Areas.** The density of lymphocytic infiltration varied widely in different areas. As previously reported (36, 37), some cellular accumulations were composed mainly of lymphocytes (lymphocyte-rich areas), and others had mixtures of lymphocytes, macrophage-like cells, and plasma cells (transitional areas). Fig. 4 shows a hemotoxylin-eosin stained section showing a lymphocyte-rich area and a contiguous transitional area. In occasional areas, most of the cells were plasma cells. Such plasma cell-rich areas contained few lymphocytes and the lymphocytes in these areas were not included in the lymphocyte counts described below.

Lymphocyte-rich areas, made up almost entirely of small lymphocytes, were the most common type of perivascular cellular collection observed. When such areas were stained with OKT3 antibody, ~80% of the lymphocytes stained positively, indicating that they were T cells (Fig. 5). When such areas were stained with OKT4 antibody (Fig. 6), the majority of the lymphocytes were found to be T4 (helper/inducer) cells. Finally, when the lymphocyte-rich areas were stained with OKT8 antibody, it was observed that the T8 (suppressor/cytotoxic) cells were much less frequent than the T4 cells and were distributed in a scattered manner in the areas stained (Fig. 7). Although the T4 cells tended to form clusters, most of the T8+ cells appeared isolated. The majority of lymphocytes

**FIGURE 3.** Control section stained in identical fashion as in Fig. 2, A with normal mouse IgG and observed without counterstaining. Note the absence of specific staining. × 6,900.
FIGURE 4. Hematoxylin-eosin stained section showing a lymphocyte-rich area contiguous to a transitional area. The lymphocyte-rich area (L) consists almost entirely of small lymphocytes. The transitional area (T) contains lymphocytes, large mononuclear cells, and plasma cells. X 250.

of both types were small in size and the incidence of typical blastic cells in the lymphocyte-rich area was very low. A small number of macrophage-like cells and a few plasma cells were found in the lymphocyte-rich collections, but there appeared to be no specific pattern relating the distribution of the T4 or T8 lymphocytes to the macrophage-like cells.

Transitional Areas. Transitional areas were made up of lymphocytes, plasma cells, and macrophage-like cells as previously described (36, 37). In such areas the fraction of T4 cells in the lymphocytes present was significantly reduced to about one-third of the total (Fig. 8), while the fraction of T8 cells rose, usually to exceed that of the T4 cells (Fig. 9). Not only was there a variety of cells in the transitional areas, but the cells were generally large in size and more loosely packed than in the lymphocyte-rich areas. Fully developed blast cells, which attained a diameter of more than 10 μm and contained a large amount of cytoplasm, stained more frequently as T8 cells than T4 cells (Figs. 9 and 10). The macrophage-like cells, which were abundant in this type of area, were frequently in close contact with lymphocytes.

The macrophage-like cells in both the lymphocyte-rich and transitional areas had a variable appearance. Their surface membrane was irregular in contour, showing numerous indentations. In some cells, the membrane was ruffled, but in others, it was not. The nuclei had a thick rim of heterochromatin and small nucleoli. Mitochondria were abundant, but rough endoplasmic reticulum was
not prominent. Lysosomes and phagolysosomes were frequently observed, but were not present in all cells. In preliminary experiments, not reported here, the macrophage-like cells showed variable staining characteristics. When present in interstitial areas of the synovium that were free of lymphoid infiltration, they stained strongly with anti-HLA-DR antibodies (OK-la, anti-HLA-DR) and with two different anti-monocyte monoclonal antibodies (63D3, 61D3). In transitional areas, the macrophage-like cells took a diffuse stain with anti-HLA-DR antibody at the light microscopic level; however, in the electron microscope, the stained material appeared to be shed between the cells. With anti-monocyte antibodies, the macrophage-like cells in the transitional areas showed weak staining at the light microscopic level; in the electron microscope, some but not all of these cells showed definite membrane staining. Lining layer macrophage-like cells stained strongly with anti-HLA-DR and anti-monocyte antibodies.

Relation of T4 and T8 Cells to the Percentage of Lymphocytes in Mononuclear Cell Infiltrates. The percentage of lymphocytes varied widely in different areas of mononuclear cell infiltration, approaching 90% in lymphocyte-rich areas and falling as low as 20% in transitional areas. However, the percentage of total lymphocytes that stained as T3 lymphocytes in any given area remained fairly constant at ~80% regardless of the numbers of other cell types present (Fig. 11), indicating that the dominant lymphocyte in all areas was the T lymphocyte.

To ascertain the relationship of T4 to T8 cells in different areas of the
synovium containing varying concentrations of lymphocytes, the percentages of T4 and T8 cells (expressed as percentage of total lymphocytes) were plotted against the percentage of lymphocytes present in the cell populations of the different areas examined. It can be seen in Fig. 12 that the percentages of both T4 and T8 cells were closely related to the percentage of total lymphocytes present. The percentage of T4 cells rose with the percentage of lymphocytes in the area ($r = 0.80, P < 0.001$) and the percentage of T8 cells fell ($r = 0.70, P < 0.001$). Thus, the T4/T8 ratio varied in a highly significant manner with the percentage of lymphocytes in a given area: the higher the lymphocyte density, the greater the ratio.

The above observation is further analyzed in Table I. In this table, mean values of the percentages of T4 and T8 cells, expressed as percentage of total lymphocytes, are given for two different kinds of areas: (a) areas in which the percentage of lymphocytes was $>60\%$ and (b) areas in which this percentage was $<60\%$. It is seen that the T4/T8 ratio in the areas containing over $60\%$ lymphocytes was 2.9 and in the areas containing $<60\%$ lymphocytes, it was 0.80.

Relation Between T4 and T8 Cells and Macrophage-like Cells. Macrophage-like cells, which interact with lymphocytes, play an important part in the immune responses of these cells. Although present only in small numbers in the lymphocyte-rich areas, macrophage-like cells were seen in increased numbers in the

FIGURE 6. Perivascular lymphocyte-rich cellular collection (vessel not shown) consisting mainly of lymphocytes staining positively with OKT4 antibody. The majority of the lymphocytes, labeled 4, are T4 cells. $\times 2,400$. 
transitional areas. It is of interest that, as the percentage of macrophage-like cells increased (Fig. 13), the percentage of T4 cells in the lymphocyte population fell ($r = -0.71, P<0.001$) and the percentage of T8 cells rose ($r = 0.68, P<0.001$). Fields in which <30% of the cells were macrophage-like cells were relatively rich in T4 cells (Table II) and poor in T8 cells (mean T4/T8 = 3.0). On the other hand, in fields where >30% of the cells were macrophage-like cells, i.e., the transitional areas, T8 cells were more plentiful than T4 cells (mean T4/T8 = 0.88).

**Discussion**

In this study we have used an immunoelectron microscopic technique to identify the T cells and T cell subsets in the rheumatoid synovial membrane. To correlate light and electron microscopic observations, adjacent sections were examined in the light and electron microscopes. Immunoelectron microscopic staining of the membranes of the lymphocyte subsets using monoclonal antibodies made it possible not only to quantitate the separate T cell populations in the cellular infiltrates, but also to examine their ultrastructure and their relationship to the macrophage-like cells and plasma cells of the synovial infiltrates.

Positively staining T lymphocytes were characterized by continuous membrane staining, which frequently had a villous or irregular surface appearance. In
FIGURE 8. Perivascular transitional area stained with OKT4 antibody (vessel not shown). A mixture of lymphocytes (L), plasma cells (P), and macrophage-like cells (M) is seen. There is a decreased percentage of lymphocytes in comparison with the lymphocyte-rich areas. T4 cells are decreased in this field. X 2,800.

lymphocyte-rich areas around the post-capillary venule (36, 37), made up mainly of dense collections of small lymphocytes, the cells were predominantly stained by OKT3 antibody, indicating that they were mainly T cells. Staining of adjacent sections showed abundant T4 cells in such areas (63 ± 1.8% of the lymphocytes), while T8 cells were present in lesser numbers (22 ± 3.2%). In this respect the T lymphocytes of the lymphocyte-rich areas had a T cell subset composition that resembled that of the blood. Further electron microscopic examination of the varying areas of the synovium demonstrated that the percentage of T4 cells in any given area was proportional to the percentage of lymphocytes in that area, the lymphocyte-rich areas having the highest concentration of T4 cells. In view of the fact that the T4:T8 ratio is elevated in the peripheral blood of rheumatoid patients (17–19), the perivascular lymphocyte-rich collections may consist of an unselected population of T lymphocytes that have emigrated from the post-capillary venule.

When the relation of the macrophage to the distribution of the T cell subsets was examined, it was observed that the percentage of T8 cells in any given area was proportional to the percentage of macrophage-like cells in that area. In transitional areas (36, 37), in which not only lymphocytes but also macrophage-like cells, plasmablasts, plasma cells, and occasional lymphoblasts are found, T8 cells (44 ± 3.2%) predominated over T4 cells (35 ± 3.0%). The presence of a
variety of cell types in the transitional areas suggests that these are areas of immunological reactivity and it is of interest, therefore, that T8 cells tended to be more highly concentrated in such areas. It is of interest also that the T8 cells tended to be large in size and frequently had the appearance of blast cells, suggesting that they may proliferate in immunologically active areas.

The differing T4:T8 ratios in the lymphocyte-rich and transitional areas may explain the divergent results obtained by different investigators in staining intact synovial tissue for T4 and T8 cells. High ratios of T4:T8 cells (23-26) may represent staining of predominantly lymphocyte-rich areas and lower ratios (27) may represent staining of predominantly transitional areas. The relatively decreased ratio found on staining synovial tissue digests (28, 29) may represent an average value of specimens in which transitional areas predominated over lymphocyte-rich areas.

It is noteworthy that almost all studies in which the synovial fluid lymphocytes have been examined by monoclonal antibody staining (19-22, 29) have reported decreased synovial fluid T4:T8 ratios in comparison with the synovial membrane. One possible explanation for this difference is that the T8 cells of the transitional areas may migrate into the synovial fluid more freely than the T4 cells of the lymphocyte-rich areas. This would be compatible with the relatively loose packing of the lymphocytes in the transitional areas in comparison with the dense packing of the lymphocytes in the lymphocyte-rich areas.
FIGURE 10. Macrophage-like cells (M) and a T8 cell in close contact in a transitional area. The positively staining T8 cell is large in size and contains a large amount of cytoplasm. x 8,800.

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FIGURE 11. Relationship of T3 cells to total lymphocytes. Percentage of T3 cells is fairly constant in relation to per cent total lymphocytes in any given area. Each point represents a field in which >80 cells were counted. \( y = 0.076x + 73.5, \ r = 0.18 \).

The presence in the transitional areas of increased numbers of plasma cells and of macrophage-like cells in close contact with T8 cells, frequently in a blastic state, is suggestive of the type of cellular interaction which occurs in the autologous mixed lymphocyte reaction (AMLR) (38). This reaction develops in
DISTRIBUTION OF T CELL SUBSETS IN RHEUMATOID SYNOVIM

FIGURE 12. Relation of T4 (○) and T8 (●) cells to total lymphocytes. The per cent lymphocytes as T4 cells is proportional to the per cent of total lymphocytes, whereas the per cent lymphocytes as T8 cells is inversely proportional to the per cent of lymphocytes. T4 (○): y = 0.58x + 15.7, r = 0.80, P<0.001. T8 (●): y = -0.59x + 67.8, r = -0.70, P<0.001.

TABLE 1  
Relation of T4+ and T8+ Cells to Total Lymphocytes in Synovial Infiltrates

| Lymphocytes as % total cells | <60  | >60  |
|-----------------------------|------|------|
| T4 as % lymphocytes         | 35 ± 3.0* | 63 ± 1.8 (P<0.001) |
| (n = 14)                    | (n = 15) |
| T8 as % lymphocytes         | 44 ± 3.2 | 22 ± 3.2 (P<0.001) |
| (n = 17)                    | (n = 16) |
| T4/T8                       | 0.80   | 2.9   |

* Mean SEM.

n, Number of the fields selected from five patients.

vitro when the ratio of autologous non-T cells to T cells is sufficiently high to stimulate a T cell proliferative response. In this response, T8 cells increase in number (39). It is of interest that in human graft-vs.-host disease, an in vivo mixed lymphocyte reaction, the lymphocytic infiltrates in the skin were exclusively of T8 variety (40); and in human kidney grafts undergoing rejection the T8 to T4 ratio in the rejection tissue was very significantly elevated (41). The above observations suggest the possibility that the transitional areas are areas in which an AMLR is taking place as a result of the increased concentration of macrophage-like cells in these areas.

It is of interest that T4 lymphocyte-rich areas, in contrast to the transitional areas, have a quiescent appearance, as indicated by the absence of plasma cells and blastic cells and a low frequency of macrophage-like cells. The association of a paucity of macrophage-like cells with a histologic picture of apparent cellular inactivity suggests the possibility that the ratio of macrophage-like cells to lymphocytes which emigrate from the post-capillary venule may determine whether T cell stimulation occurs or whether a quiescent nodule of small lymphocytes develops. In this connection it is noteworthy that the presence of dense, nodular collections of lymphocytes in the synovial membrane has been
Figure 13. Relation of percentage T4 (○) and T8 (●) cells in the lymphocyte population to percentage of macrophage-like cells in the cellular infiltrates. T4(○): y = -0.92x + 77.0, r = -0.71 (P<0.001). T8(●): y = 0.88x + 4.9, r = 0.68 (P<0.001).

Table II
Relation of T4+ and T8+ Cells to Total Macrophage-like Cells in Synovial Infiltrates

| Macrophage-like cells as % total cells | <30     | >30     |
|---------------------------------------|---------|---------|
| T4 as % lymphocytes                   | 63 ± 2.1* (n = 13) | 38 ± 3.4 (n = 16) (P<0.001) |
| T8 as % lymphocytes                   | 21 ± 3.7 (n = 14) | 43 ± 3.1 (n = 19) (P<0.001) |
| T4/T8                                 | 3.0     | 0.88    |

* Mean SEM.

n. Number of the fields selected from five patients.

reported to be associated with a less destructive variety of synovitis (42).

It has recently been shown that macrophage-like cells are heterogeneous in structure and function. Dendritic cells, distinct from classic mononuclear phagocytes, have been reported to express large amounts of HLA-DR antigens on their surface, to act as potent stimulators of mixed lymphocyte reactions (43–46) and to serve as accessory cells for immune responses (47–50). In rheumatoid synovium, close contact between T lymphocytes and macrophage-like cells has
been observed (37) and staining of light microscopic sections with monoclonal antibodies has demonstrated close contact between T4 helper/inducer cells and HLA-DR positive interdigitating cells (23–26). The interpretation has been made that these macrophage-like cells are dendritic cells. Since the accessory T lymphocyte stimulating cell is thought to be the dendritic cell (51), it would be helpful to be able to determine whether the macrophage-like cells observed in the transitional areas are dendritic cells. Criteria for the electron microscopic appearance of dendritic cells have been described (51). However, it has not been possible in the present experiments to identify the macrophage-like cells observed as dendritic cells on the basis of their appearance in the electron microscope.

An interesting difference in the distribution of T4 and T8 cells in different types of chronic inflammatory tissue, recently described by Van Voorhis et al. (52), is pertinent to the present findings. They noted that in the cutaneous lesions of lepromatous leprosy, which are dispersed, unorganized and rich in macrophages and plasma cells, most of the T cells were of the T8 variety. On the other hand, in the cutaneous lesions of tuberculoid leprosy, which are lymphocyte-rich and highly organized, the majority of the lymphocytes were T4 lymphocytes. In leprosy therefore, active lesions were rich in T8 cells and less active ones were rich in T4 cells. In view of these observations, it is possible that the lymphocyte-rich areas of the rheumatoid synovium resemble the tuberculoid type of lesion and the transitional areas the lepromatous type. In both leprosy and rheumatoid arthritis, the apparent proliferation of T8 cells in transitional areas may represent an attempt on the part of the host to modulate the immune response that appears to take place in such areas.

Summary

The perivascular mononuclear cell collections of the rheumatoid synovium were examined both at the light and electron microscopic level by an immunoperoxidase staining technique using monoclonal antibodies directed against T cell subsets. These accumulations were variable in composition and size, not only in specimens from different patients but in the same specimen. Some areas (lymphocyte-rich areas) contained mainly small lymphocytes in clusters and others (transitional areas) contained blast cells, macrophages, and plasma cells in addition to lymphocytes. The percentage of T4 staining cells correlated positively and the percentage of T8 staining cells correlated negatively with the percentage of lymphocytes in any given area. In contrast, the percentage of T4 cells correlated negatively and the percentage of T8 cells correlated positively with the percentage of macrophage-like cells in these areas. 

~80% of the total lymphocytes, both in the lymphocyte-rich areas and transitional areas, were T lymphocytes (OKT3 staining). In lymphocyte-rich areas, helper/inducer T lymphocytes (OKT4 staining) were predominant over suppressor/cytotoxic lymphocytes (OKT8 staining), and in such areas the mean T4:T8 ratio was 2.9. Macrophage-like cells were seen only in small numbers in this type of area. In the transitional areas, suppressor/cytotoxic lymphocytes (OKT8 staining) predominated over helper/inducer lymphocytes (OKT4 staining). In such areas the mean T4:T8 ratio was 0.8. The T8 cells in the transitional areas tended to be large in size and often had a blastic appearance, and the
abundant macrophage-like cells infiltrating these areas were frequently in close contact with T8 lymphocytes.

These findings indicate that the ratio of T4 to T8 lymphocytes in rheumatoid synovium varies with the type of area examined. In lymphocyte-rich collections, made up largely of quiescent small lymphocytes, T4 cells are predominant. In areas of apparent immunological reactivity, T8 cells are predominant. It is suggested that T8 cells proliferate in immunologically active areas of the synovium as a result of local stimulation of a T cell-mediated immune response.

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