The Pathogenesis of Experimentally Induced Trypanosoma brucei *Infection in the Dog*

II. Changes in the Lymphoid Organs

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Marked changes were found in the spleen and lymph nodes of dogs experimentally infected with *Trypanosoma brucei*. These changes were considered to occur in two phases. First, in animals examined on Days 8 and 16 after inoculation, there was an intense proliferative response; this involved particularly the B-dependent follicular areas and was accompanied by a dramatic increase in the number of plasma cells in the splenic red pulp and medullary cords of the lymph nodes. Although numerous large proliferating lymphoblasts were found in the periarteriolar regions of the spleen and in the peripheral part of the paracortical areas of the lymph nodes, the presence of cells containing Ig in these regions made it difficult to evaluate the degree of involvement of thymus-derived cells. The plasma cell response involved both IgM and IgG, although the increase in IgM-containing cells was most striking. During this initial phase there was focal hemorrhage, deposition of fibrin, necrosis, and infiltration of polymorphonuclear leukocytes in the spleen, these changes being most severe in the peripheral follicular areas. Following the initial proliferative phase and prior to the death of the host during the fourth week of the infection, the spleen and lymph nodes became less reactive, and there was marked disorganization and disruption of their architecture. Compared with characteristics earlier in the infection there was greatly decreased proliferative activity and a reduction in size of the splenic white pulp and lymph node cortices. Germinal centers were reduced in number, size, and activity, had a disorganized appearance, and, by immunofluorescence, showed a reduction of immunoglobulin on the dendritic reticular cells. The lymph node sinuses exhibited a decrease in content of lymphocytes and contained massive numbers of macrophages, including numerous multinucleated giant cells. There was also severe disruption of the reticulum cell network of the sinuses; and accumulations of polymorphonuclear leukocytes, along with extensive deposition of fibrin, were commonly found in the subcapsular sinuses. During this period, foci of erythropoietic cells were present throughout the red pulp of the spleen. (Am J Pathol 1981, 102:182–194)

THE ABILITY of *Trypanosoma brucei* to change the antigenic nature of its surface coat glycoprotein is an important factor in enabling the parasite to persist within the host and produce disease. The resultant sequence of parasitemic waves leads to a sustained high level of antigenic challenge to the immune system of the host. In laboratory animals infected with *T brucei* there is profound proliferative activity in the lymphoid organs, accompanied by expansion and activation of the mononuclear phagocytic system.[1,2] Both morphologic studies and examination of lymphocyte surface markers show that a major component of the proliferative response involves B lymphocytes, ie, surface-Ig-positive, bone-marrow-derived cells.[2-4] Large numbers of plasma cells are present in the spleen and lymph nodes of infected animals, and there is marked hypergammaglobulinemia. However, in the face of this apparent high level of immunologic reactivity, there is evidence that infected mice and rats are immunosuppressed.[2,5,6] Furthermore, in longstanding infections with *T brucei* in rats and mice there is depletion of the lymphoid organs.[1,7] However, the extent to which changes in the lymphoid system of trypanosome-infected laboratory an-

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imals resemble those occurring in other species remains to be determined.

In dogs, infection with *T brucei* results in an acute fatal disease characterized by high parasitemia and invasion of the tissues by large numbers of trypanosomes. In a previous paper we have described the tissue lesions found in dogs experimentally infected with *T brucei*.[8] The present paper describes the sequential changes in the lymphoid organs of infected dogs as evaluated by histologic and immunofluorescence techniques, and consideration is given as to how these changes relate to possible functional defects in the host's immune response.

Materials and Methods

Experimental Animals

Fifteen crossbred dogs, of mixed sexes, aged between 1.5 and 3 years, were used for the study. The origin and disease-free status of these animals has been described previously.[8]

Experimental Design

Eleven dogs were infected with *T brucei* (TREU 667), each animal receiving 10^3 motile trypanosomes by intravenous injection. The remaining 4 dogs were maintained as uninfected controls. Two infected dogs were killed on Day 8 after inoculation, and a further 3 infected dogs were killed on Day 16; the remaining 6 infected dogs were killed in the terminal stages of the disease on Days 21–25.

Histologic Procedures

All dogs were exsanguinated under general anesthesia induced by sodium pentabarbitone. The spleen and lymph nodes, consisting of submaxillary, prescapular, axillary, external inguinal, and popliteal nodes, were immediately removed and weighed. Small pieces of lymph node and spleen were fixed in 10% neutral buffered formalin and postfixed in mercuric chloride-formal; duplicate samples were fixed in Carnoy's solution. Tissue blocks were embedded in paraffin wax, and sections were cut at 6 μ and stained with Mayer's hematoxylin and eosin. Selected sections were also stained with Martius scarlet blue, Gordon and Sweets's reticulin stain, and methyl green pyronin.

Immunofluorescence Procedures

Small pieces of spleen and lymph node (submaxillary, prescapular and popliteal nodes) approximately 5 × 5 × 2 mm were processed for immunofluorescence as described by Eidelman and Berschauer.[9] Briefly, the blocks were fixed for 4 hours at 4 C in a solution of 10% formalin in 0.15 M PBS, pH 7.3, and washed in 30% sucrose at 4 C for 12–24 hours. The blocks were then snap-frozen in liquid nitrogen and either stored individually at −20 C in sealed plastic containers or immediately sectioned in a cryostat. Sections cut at 4 μ in thickness were air-dried and stained either for IgG or IgM. After staining for 30 minutes, the sections were washed for 20 minutes in PBS, pH 7.3, and then mounted in a (9:1) mixture of glycerol and barbital buffer, 0.1 M, pH 8.6. Stained sections were examined with a Leitz Orthoplan microscope equipped with incident light illumination. Fluorescein-isothiocyanate (FITC)-labeled antidog IgG was obtained from Microbiological Associates (Bethesda, Md) and FITC-labeled goat antidog IgM from Cappel Laboratories (Downington, Pa). Each conjugate was checked for specificity by immunoelectrophoresis and by the ability of unconjugated anti-IgG and anti-IgM serums to block the appropriate fluorescence staining.

Results

Macroscopic Findings

The spleens of all dogs examined on Days 8 and 16 of infection were markedly enlarged (Table 1). On cut section the white pulp follicles were prominent, and the red pulp was pinkish red in color, as opposed to the dark red color of control spleens. In animals examined on Days 21–25, the degree of splenomeg-

Table 1—Weight of Spleen and Lymph Nodes in Dogs Infected With *Trypanosoma brucei*

Dog	Days after inoculation	Spleen (g)	Lymph nodes* (g)
1	Control	62.6	17.5
2	Control	55.7	15.0
3	Control	41.0	10.1
4	Control	65.3	11.5
5	8	94.2	10.1
6	8	149.1	15.3
7	16	131.4	49.8
8	16	137.2	120.0
9	16	167.3	83.9
10	21	141.3	50.9
11	22	119.3	30.6
12	23	74.9	40.9
13	24	73.7	47.1
14	25	117.4	43.8
15	25	78.9	65.4

* Combined weight of submaxillary, prescapular, axillary, external inguinal, and popliteal lymph nodes.

aly, in general, was less pronounced, although there was considerable variation from one animal to another (Table 1). On cut section the spleen was darker red in color, and the white pulp follicles appeared to be reduced in size.

Marked lymph node enlargement was found in all dogs examined from day 16 of infection onward (Table 1). The degree of enlargement was greater at Day 16 than in terminal cases. On cut section the lymph nodes were extremely moist, and copious gelatinous lymph exuded from their cut surfaces. At Day 16, lymph node enlargement involved expansion of the cortical and medullary regions; however, in terminal cases, much of the enlargement appeared to be due to expansion of the medullary areas, which at this time were often yellowish-brown in color. Petechial hemorrhages and sometimes more extensive areas of hemorrhage were found in some of the lymph nodes of all infected dogs from Day 16 onward.

Historical and Immunofluorescence Findings

The Normal Spleen

The white pulp of the normal dog spleen envelopes part of the arterial system after the arteries emerge from connective tissue trabeculas. In these regions the arteries are surrounded by a cuff of small lymphocytes, known as the periarteriolar region. At intervals, lymphoid follicles are found outside the periarteriolar region. In the control dogs in the present study the center of these follicles was composed of small lymphocytes with densely staining nuclei similar to those found in the periarteriolar region. Surrounding this central area of small lymphocytes (central follicular area) was a zone, approximately 8–12 cells in width, composed of larger lymphocytes with paler staining nuclei and often prominent nucleoli (peripheral follicular area). A prominent network of coarse reticulin fibers was present in the periarteriolar region and in the peripheral follicular area (Figure 1). Germinal centers were found in some of these follicles and when present were always within the central follicular area. There was often a distinct arrangement within the germinal centers. The pole adjacent to the central arteriole contained densely packed large lymphoblasts, while the opposite pole was slightly more loosely arranged and contained more small lymphocytes and tingible-body macrophages. The marginal zone surrounding both the periarteriolar region and the follicles contained numerous medium-sized lymphocytes, some large lymphocytes and macrophages, and a few polymorphonuclear leukocytes.

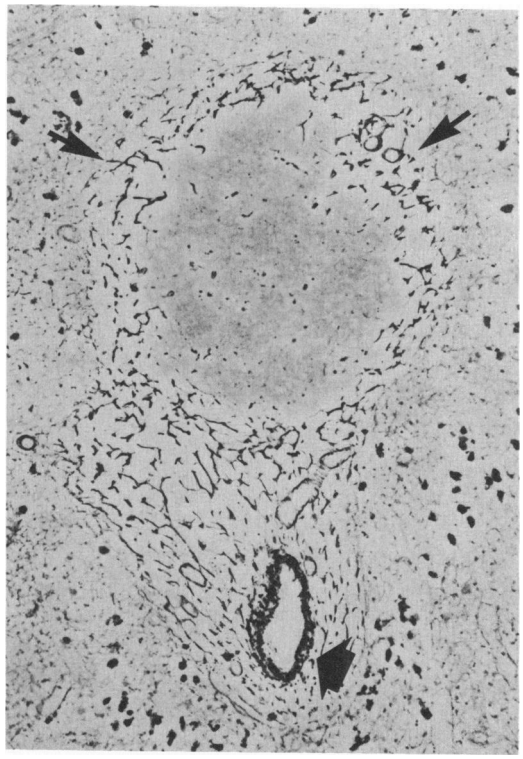


Figure 1—Spleen from a control dog stained for reticulin. Coarse reticulin fibers are present in the periarteriolar region and in the peripheral follicular area (*small arrows*) of the white pulp (*large arrow*, central arteriole). (Reticulin stain, ×80)

By immunofluorescence, spleens from control animals were found to have more cells containing IgG than those containing IgM (Table 2). With both classes of antibody the majority of the cells were distributed fairly uniformly throughout the red pulp cords. Within some of the white pulp follicles, granular deposits of IgM and IgG were found on the dendritic reticular cells of germinal centers; sometimes about a third of the germinal center at one pole was negative for immunoglobulin deposits. Very occasional cells containing IgM or IgG were found within the germinal centers, and occasional IgM-containing cells were present in the periarteriolar region.

The Spleen of Dogs Infected With T brucei

Day 8 of Infection: In the animals examined 8 days after inoculation, there was marked proliferative activity within the splenic white pulp. The periarteriolar region contained numerous lymphoblasts, some plasma cells, and relatively few small lymphocytes. By immunofluorescence, small numbers of cells in the periarteriolar region were positive for IgM and IgG. Large, active germinal centers containing heavy granular deposits of IgG and IgM were found in the majority of follicles; these showed high mitotic activ-

ity with numerous tingible-body macrophages and a prominent mantle layer of small lymphocytes. The peripheral follicular area also showed high mitotic activity and contained numerous large lymphoblasts. A common and striking finding within this area was the disruption of the lymphoid cellular arrangement due to accumulation of red blood cells; this disruption was often accompanied by localized necrosis of the lymphoid cells, deposition of fibrin, and infiltration by polymorphonuclear leukocytes (Figure 2). These lesions sometimes extended into the periarteriolar regions and occasionally the central follicular areas. Similar foci of necrosis and deposits of fibrin were observed in the adjacent red pulp. The marginal zone of the white pulp was densely populated by medium-sized lymphocytes, large lymphoblasts, and a few polymorphonuclear leukocytes.

In the red pulp, there was a marked increase in the number of plasma cells and large lymphocytes, which showed a high rate of mitotic activity. These were found mainly within the cords of Billroth, but also in smaller numbers in the sinuses. Immunofluorescence confirmed the massive increase in plasma cells; although this increase involved both IgM and IgG, the increase in cells containing IgM was most pronounced (Table 2).

Day 16 of Infection: By Day 16 after inoculation there was intense proliferative activity involving particularly the white pulp follicles. The periarteriolar region showed reactivity and cell content similar to that observed on Day 8. An extremely high rate of mitotic activity was observed in most germinal centers and in the peripheral follicular areas. The latter contained large numbers of lymphoblasts. By immunofluorescence, small numbers of Ig-containing cells, mainly of the IgM class, were found in the periarteriolar region and peripheral follicular areas. However, the germinal centers no longer showed a distinct arrangement, and the mantle zone of small lymphocytes was often reduced or virtually absent. A few of the germinal centers at this time showed much less proliferative activity, had a rather loose cellular arrangement, and contained fewer lymphoblasts. In addition, although granular deposits of Ig were still present in the germinal centers, they were less prominent than on Day 8. Fibrin deposits and occasional foci of necrosis were sometimes observed within the peripheral follicular and periarteriolar areas of the white pulp and within the sinuses of the red pulp. The marginal zone was extremely cellular and contained predominantly medium and large lymphocytes.

Table 2—Comparative Prevalence of Cells Staining for IgM and IgG in the Spleen and Lymph Nodes of Dogs at Different Times After Infection With Trypanosoma brucei

Duration of infection	Spleen		Lymph nodes	
	IgM	IgG	IgM	IgG
Control	+	+ + +	+	+ +
Day 8	+ + +	+ + + +	+ +	+ + +
Day 16	+ + + + +	+ + + + +	+ + + +	+ + + +
Days 21–25	+ + +	+ + + + +	+ + + +	+ + + +

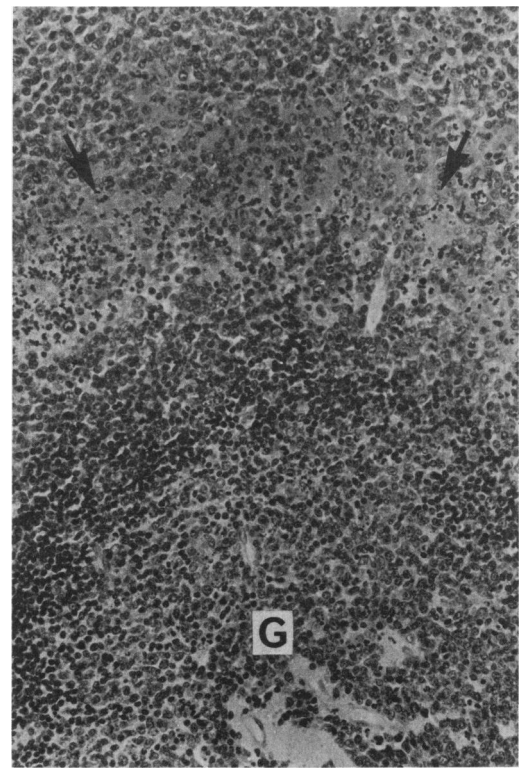


Figure 2—Spleen of a dog examined on Day 8 of infection. There is fibrin deposition, cellular necrosis, and infiltration by polymorphonuclear leukocytes in the peripheral follicular area (*arrows*). (G, germinal center). (H&E, × 140)

On Day 16, the red pulp contained even greater numbers of plasma cells. The mitotic activity was somewhat less than observed on Day 8. The increase in plasma cells again involved cells of both Ig classes, with the increase in those containing IgM being particularly dramatic, so that these slightly outnumbered the cells containing IgG (Table 2; Figures 3 and 4). There was a striking increase in macrophages in the red pulp sinuses; many of these showed abundant vacuolated cytoplasm, sometimes containing phagocytic granules. A few megakaryocytes, occasionally accompanied by small foci of erythropoietic cells, were also found.

Terminal Stages of the Disease: In dogs examined on days 21–25, the spleen was much less reactive than on Day 16. Although the periarteriolar regions showed considerable variation from one animal to

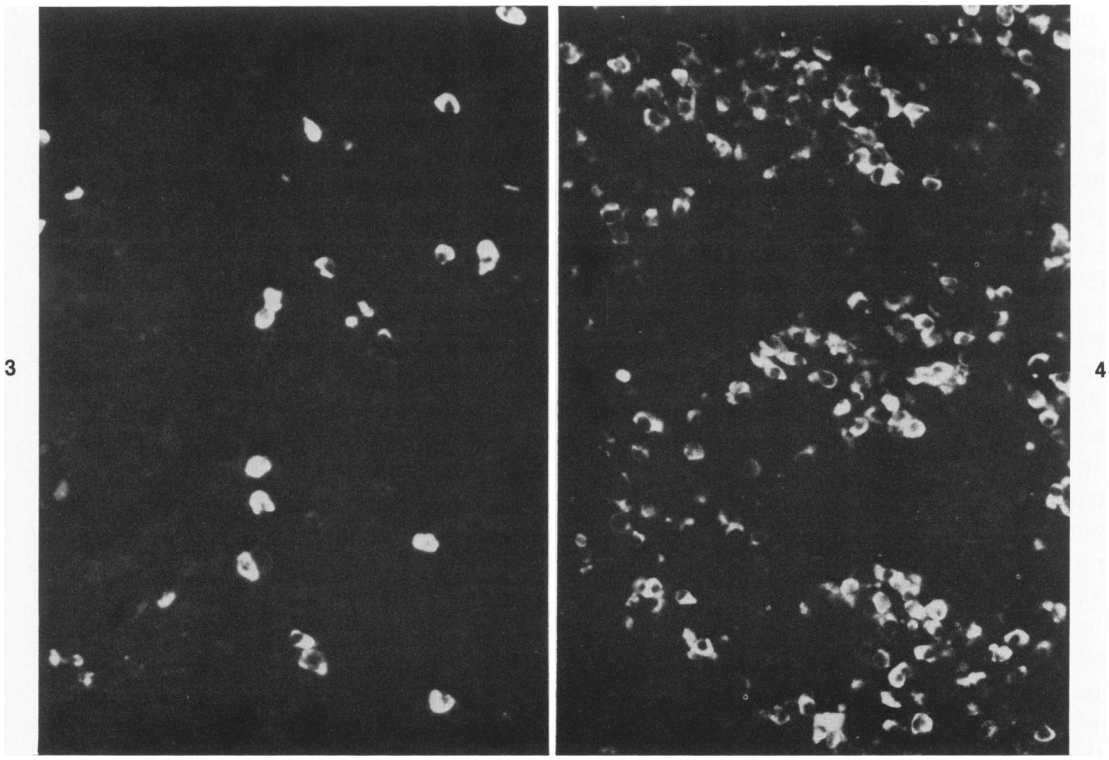


Figure 3—Spleen from a control dog stained for IgM. The red pulp contains small numbers of cells containing IgM. (Immunofluorescence, ×200) **Figure 4**—Spleen of a dog examined on Day 8 of infection stained for IgM. There is a marked increase in the number of cells containing IgM in the red pulp. (Immunofluorescence, ×180)

another, in general they were relatively less active and showed a higher content of small lymphocytes. The follicular areas appeared very disorganized, and discrete active germinal centers were uncommon. Sometimes the central follicular area was largely depleted of lymphocytes and contained numerous macrophages; this appeared to be the remains of a previously active germinal center. By immunofluorescence, many of the follicles showed no granular deposits of Ig on the dendritic reticular cells, and those follicles that were positive stained with low intensity. At this time, the peripheral follicular area made up a large part of the follicle, and this area was still relatively active, containing numerous large lymphocytes (Figure 5). In many animals the marginal zone was much less cellular than it was earlier in the infection, although the cell types were similar.

In the red pulp the content of plasma cells, although obviously still increased, was less marked than on Day 16. By immunofluorescence, this was reflected as a marked decrease in the numbers of cells containing IgM compared to earlier in the infection (Table 2). Occasional Russell body-containing plasma cells were found. The sinuses and cords contained numerous active macrophages (Figure 6) many of which were involved in erythrophagocytosis. Small focal deposits of fibrin were found in the red pulp sinuses and thrombi were occasionally observed in branches of the splenic vein. An additional striking feature at this stage of the infection was the widespread presence of megakaryocytes and foci of erythropoietic cells in the red pulp sinuses and cords (Figure 7) and in the marginal zone.

The Normal Lymph Node

The normal canine lymph node shows a histologic structure similar to that of lymph nodes of other mammalian species. The cortex is composed of an outer follicular area containing primary and secondary follicles and an inner paracortical zone within which the postcapillary venules are found. As compared with the lymph nodes of rats and mice, the ratio of cortex to medulla tends to be lower. Indeed, in the control animals in the present study, the cortex was often extremely variable in width from one area to another within the same lymph node. Another feature was infolding of the follicular cortex around connective tissue trabecula extending inward from the capsule. Moderate numbers of germinal centers were found in the outer cortex; these were surrounded by a prominent mantle of small lymphocytes.

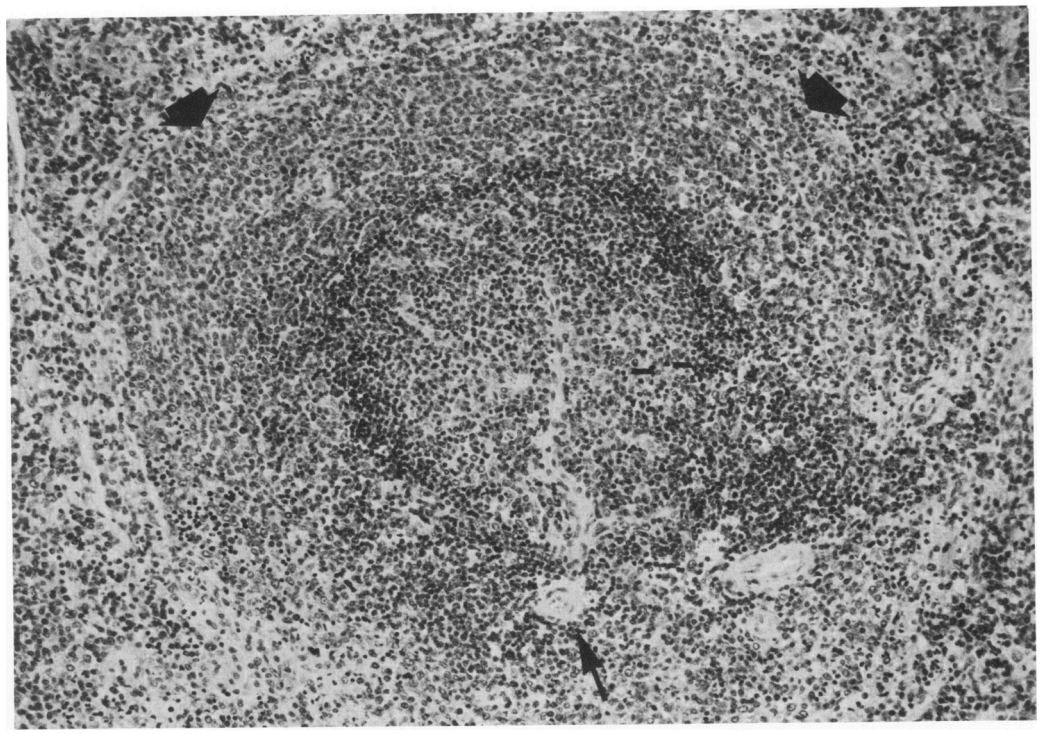
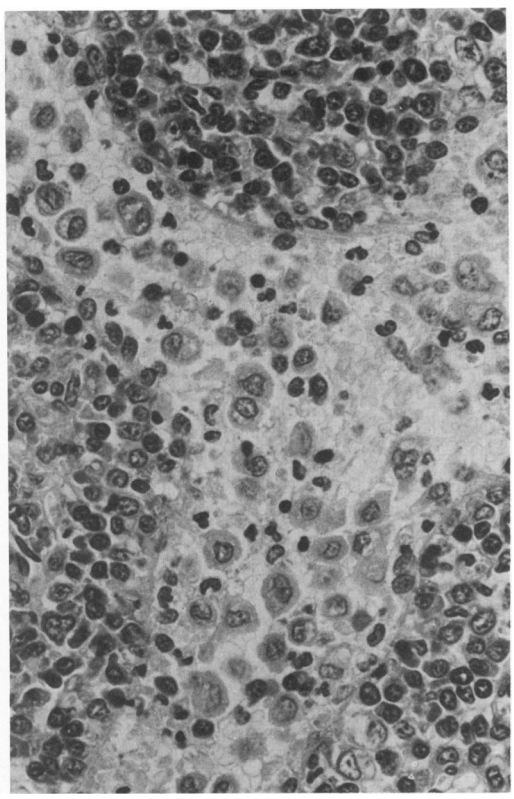


Figure 5—Spleen of a dog examined on Day 22 of infection, showing an area of white pulp. There is marked expansion of the peripheral follicular area (*large arrows*) (*small arrow*, central arteriole). (H&E, ×90)



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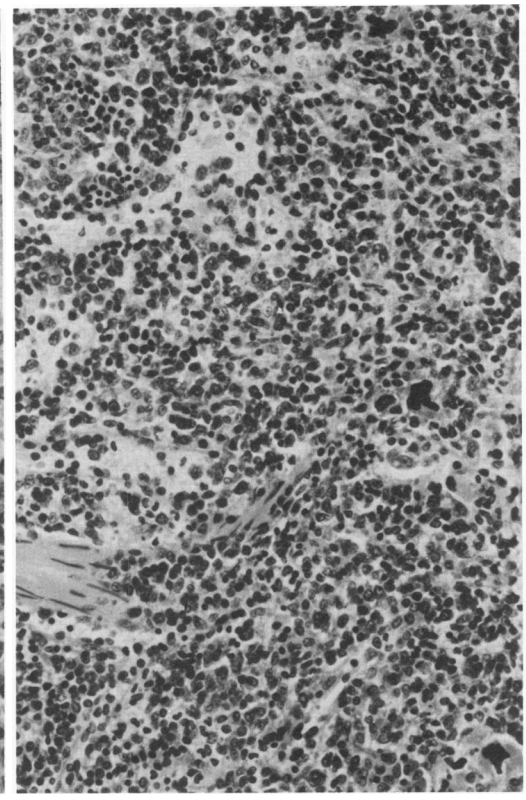

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Figure 6—Spleen of a dog examined on Day 22 of infection. A red pulp sinus is shown containing large numbers of macrophages. Numerous plasma cells are present in the adjacent cords of Billroth. (H&E, ×350) **Figure 7**—Spleen of a dog examined on Day 25 of infection, showing an area of red pulp. Foci of erythropoietic cells and small numbers of megakaryocytes are present throughout the red pulp. (H&E, ×250)

The medullary cords were found to contain a mixture of lymphocytes and plasma cells. As in other species, the sinuses were lined by reticulum cells, and similar reticulum cells formed a network traversing the sinus lumens. Within this network was found a scanty cell population composed mainly of macrophages and small lymphocytes.

By immunofluorescence, the content of immunoglobulin-positive cells in the control lymph nodes was found to vary between animals and from one node to another in the same animal. However, the distribution within the lymph nodes was fairly constant. There was always a greater number of IgG- than IgM-positive cells (Table 2). The majority of cells containing both immunoglobulin classes were found in the medullary cords. Very small numbers of positive cells of both classes were found in the paracortex. In addition, small clusters of IgM-containing cells were often observed in the outer cortex around the follicular areas. Granular deposits of IgM and IgG were found within those follicles that contained germinal centers; in many of these, a portion of the follicle at the medullary pole was negative for immunoglobulin.

The Lymph Nodes of Dogs Infected With T brucei

Day 8 of Infection: By Day 8 of infection with *T brucei* the lymph nodes showed no obvious enlargement. However, there was marked germinal center activity (Figure 8), and foci of large proliferating lymphocytes were found in the areas of the paracortex adjacent to the follicular areas. In the medulla, there was a moderate increase in large lymphocytes and plasma cells found in the medullary cords. There was also an increase in the cell content of the sinuses, involving mainly small and large lymphocytes. A few trypanosomes were also observed in the sinuses. By immunofluorescence there was a slight increase in the numbers of cells of both Ig classes (Table 2), but the distribution of positive cells was similar to the controls. Most follicles contained prominent granular deposits of Ig.

Day 16 of Infection: At Day 16, the lymph nodes were extremely swollen. There was marked expansion of the cortex (Figure 9). The outer paracortex and the areas immediately surrounding the follicles contained numerous large lymphocytes with prominent nucleoli and exhibiting an extremely high rate of mitotic activity. However, in the central parts of the paracortex there were areas, usually around the postcapillary venules, that were much less active and were still populated predominantly by small lymphocytes (Figure 10). By immunofluorescence, numerous cells containing Ig, particularly IgM, were found in the areas

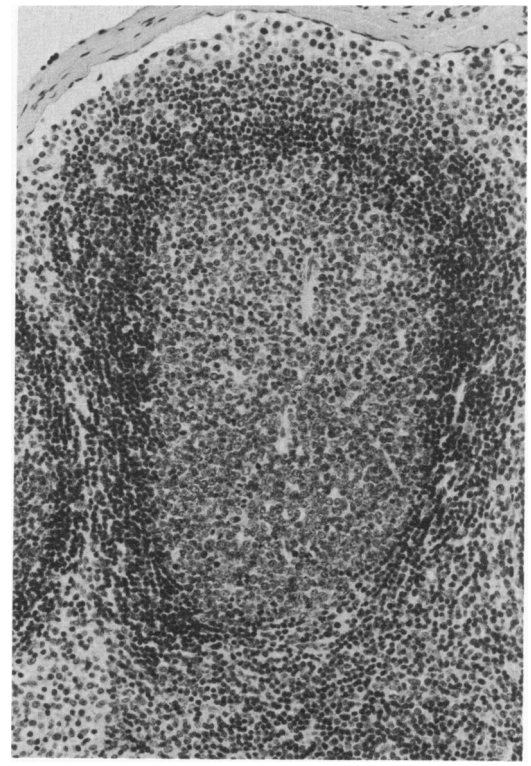
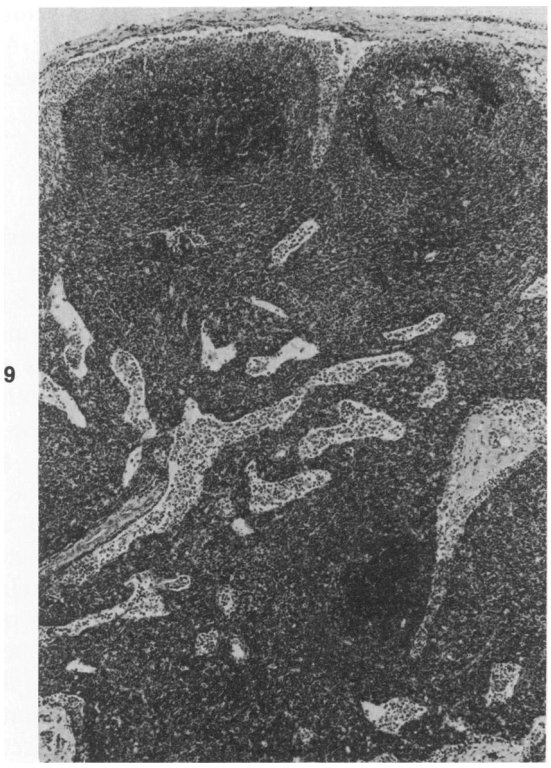


Figure 8—Lymph node of a dog examined on Day 8 of infection, showing an active germinal center. (H&E, × 120)

around the follicles, and a few cells of both classes were found within the follicles. Germinal centers, although no more numerous than in control dogs, were extremely active. The surrounding mantle zone of small lymphocytes was often markedly reduced. Granular deposits of Ig were observed in the germinal centers but the intensity of staining for Ig was less than that in Day 8 lymph nodes.

The medullary cords were markedly distended and contained predominantly a mixture of large pyroninophilic lymphocytes and plasma cells. Similar cell types were found in large areas of the inner paracortex adjacent to the medullary cords, and in both regions there was intense mitotic activity. By immunofluorescence, a marked increase in Ig-positive cells of both classes was observed in these areas (Table 2; Figure 11). An increase in macrophages, showing abundant vacuolated cytoplasm, was found throughout the cortex and in the medullary cords. Many of these cells, particularly in the medullary cords and inner cortex, contained pyknotic nuclear debris and appeared essentially similar to the tingible body macrophages of the germinal centers.

The lymph node sinuses were distended and extremely edematous and showed a high cellular content (Figure 12). There was a marked increase in the number of macrophages and small and large lympho-



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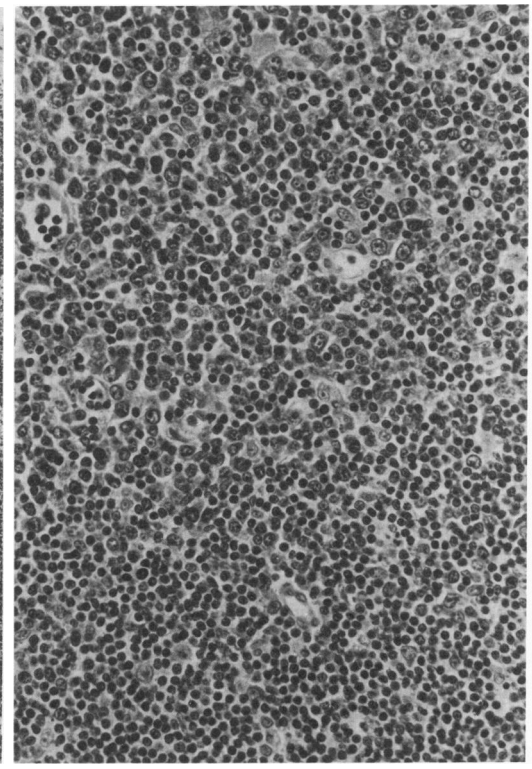

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Figure 9—Lymph node of a dog examined on Day 16 of infection, showing an expanded and highly cellular cortical area. (H&E, ×50) **Figure 10**—Lymph node of a dog examined on Day 16 of infection, showing an area of paracortex. Numerous large lymphocytes with prominent nucleoli are present in the upper part of the figure, which is close to the follicular cortex, while the lower part of the figure (deep paracortex) contains mainly small lymphocytes. (H&E, ×240)

cytes, many of which were actively dividing. Numerous polymorphonuclear leukocytes were found focally both in solid cortical tissue and in the sinuses of some lymph nodes; in these areas, the sinuses often contained fibrin deposits. Trypanosomes were plentiful in the lymph node sinuses (Figure 13), and occasional foci of hemorrhage and erythrophagocytosis were found. By immunofluorescence, cells staining for IgG and IgM were found in the sinuses, being most numerous in the medullary sinuses. Some of these appeared to be lymphoid cells; however, other cells, which were much larger and showed irregular granular cytoplasmic staining, were probably macrophages.

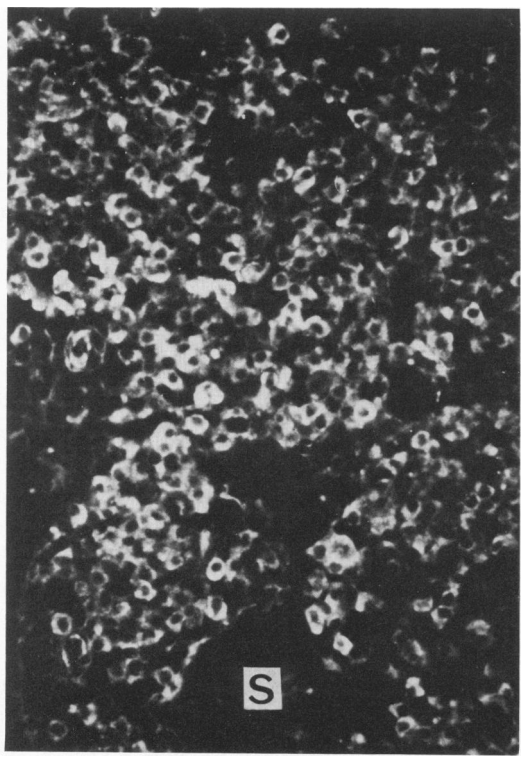
Terminal Stages of the Disease: In animals examined 21–25 days after infection, the lymph nodes were smaller and much less reactive than on Day 16. The cortices were often narrow, giving a low ratio of cortex to medulla, and the paracortex was reduced in many nodes (Figure 14). While mitotic activity and foci of lymphoblasts were found in the outer cortex around the follicles, this area was much less reactive than on Day 16. Numerous large vacuolated macrophages were found throughout the cortex but were particularly numerous in the area adjacent to the subcapsular sinus. By immunofluorescence, numerous cells containing Ig were found scattered throughout this outer cortical area. The numbers of germinal centers varied among animals, but generally they were reduced in number, size, and activity, compared with those observed in the earlier stages of infection. These germinal centers often exhibited a rather loose cellular arrangement, with reduced mitotic activity, and a decreased lymphocytic content (Figure 15). Seen by immunofluorescence, these changes were associated with a marked decrease in the number of follicles containing immunoglobulin deposits and a reduction in intensity of staining of these that were still positive. The medullary cords of lymph nodes from moribund dogs contained large numbers of mature plasma cells with occasional Russell-body-containing cells and numerous large macrophages similar to those observed in the medullary sinuses (Figure 16). A similar cell population was found throughout large areas of the inner paracortex; and in both of these areas there was much less mitotic activity, compared with that earlier in the infection. As on Day 16, both the medullary cords and the inner paracortex contained large numbers of cells positive for IgG and IgM (Table 2).

The lymph node sinuses were distended and contained massive numbers of macrophages, including

many binucleate and mutinucleated giant cells (Figures 16 and 17). These cells were large, often rounded, and sometimes contained phagocytosed cell debris, hemosiderin, and erythrocytes. Trypanosomes were readily observed in the sinuses; however, there was a marked decrease in the content of lymphocytes. Seen by immunofluorescence, the sinuses contained increased numbers of cells containing Ig of each class; the majority of these were large cells showing bright, irregular cytoplasmic fluorescence and presumably correspond to the macrophages observed histologically (Figure 18). A few of these large fluorescent cells were also observed in the medullary cords and in the cortex. In contrast to the structure of sinuses in the normal node, where there is an organized fixed network of reticulum cells, there was marked disorganization of the sinuses in infected lymph nodes. The lining of the sinuses was rather ragged in appearance, and in some areas it appeared to be discontinuous. Furthermore, there was little evidence of a network of reticulum cells traversing the sinuses (Figures 17 and 19). In many lymph nodes, the subcapsular and cortical sinuses were markedly distended by large accumulations of cells and fibrin; these clumps contained numerous macrophages, polymorphonuclear leukocytes, dead and dying cells, and occasional clusters of trypanosomes (Figure 19). Similar material was observed in afferent lymphatics but was rarely seen in the medullary sinuses.

Discussion

There are no definitive studies on the distribution of lymphocyte subpopulations within the normal lymphoid organs of the dog. However, the present study makes it clear that the histologic arrangement of the lymphoid tissue in the lymph nodes and the spleen closely resembles that described for other species. One difference that was noted in the canine spleen as compared with that of laboratory animals was the arrangement of the follicles into a central area of small lymphocytes surrounded by a zone of medium-sized lymphocytes, termed the peripheral follicular area. In active follicles the germinal centers were always present within the central area of small lymphocytes. A similar arrangement has been observed in the bovine and caprine spleen.[10,11] At present the functional basis of this demarcation as regards cellular content and immunologic reactivity is not known.



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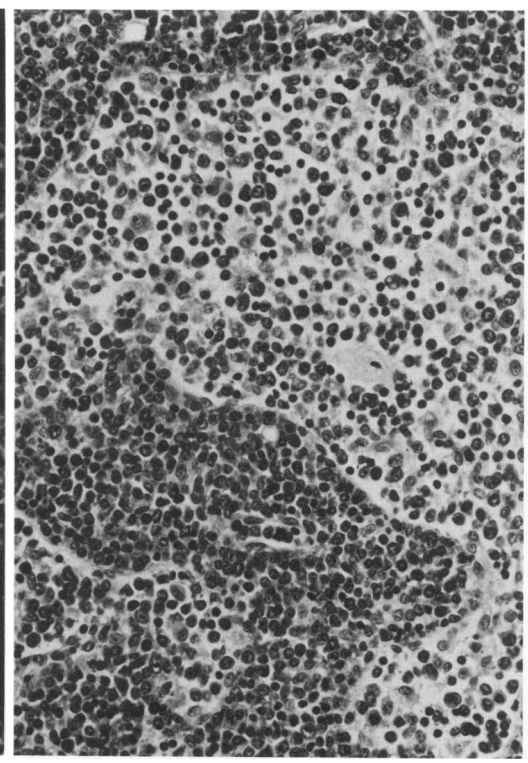

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Figure 11—Lymph node of a dog examined on Day 16 of infection, stained for IgM; an area at the junction of the cortex and medulla is shown, with large numbers of cells containing IgM in the deep cortex and medullary cords (*S*, medullary sinus). (Immunofluorescence, × 250) **Figure 12**—Lymph node of a dog examined on Day 16 of infection showing an area of medulla. There is a marked increase in the content of lymphocytes in the medullary sinuses, and the medullary cords are distended with large lymphocytes and plasma cells. (H&E, × 190)

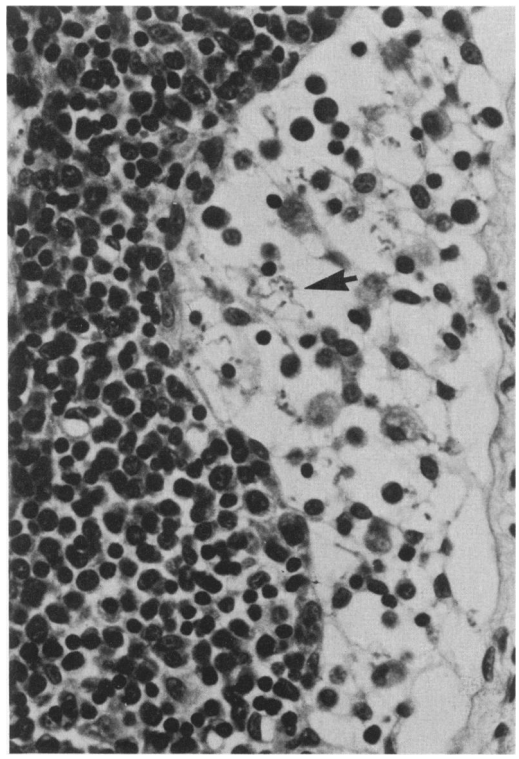

Figure 13—Lymph node of a dog examined on Day 16 of infection. Numerous trypanosomes (*arrow*) can be seen in a medullary sinus. (H&E, ×280)

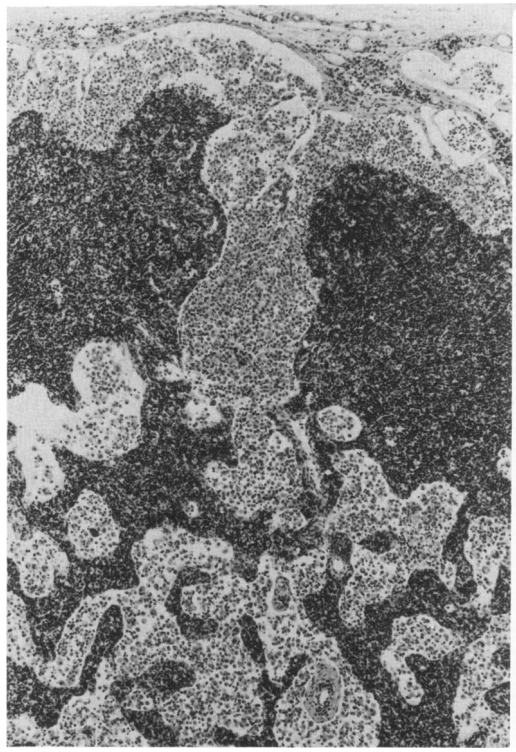

Figure 14—Lymph node of a dog examined on Day 24 of infection. The cortex is extremely narrow, there is little evidence of follicular activity, and the sinuses are distended. (H&E, ×50)

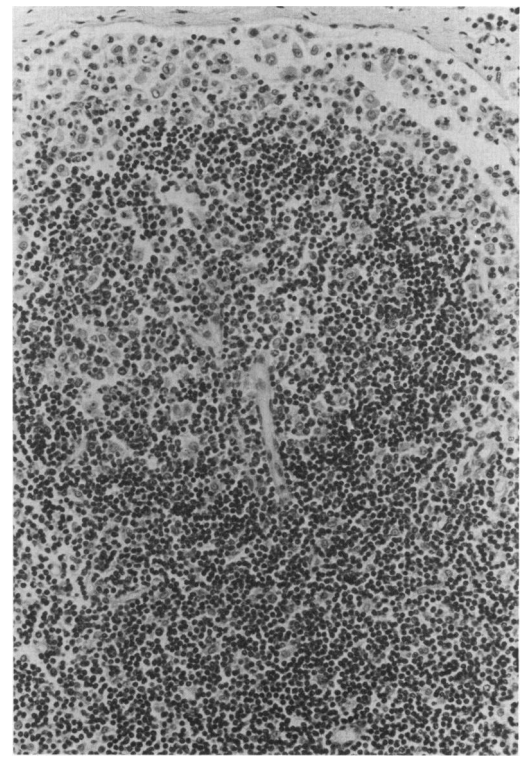

Figure 15—Lymph node of a dog examined on Day 24 of infection, showing a germinal center in the outer cortex. In contrast to that illustrated in Figure 8, the germinal center has a very loose cellular arrangement and contains many fewer large proliferating lymphoid cells. (H&E, ×150)

The spleen and lymph nodes represent, respectively, the principal organs for the trapping of microorganisms and their antigens from the bloodstream and lymphatic system. Within their microenvironments appropriate cell types can interact and respond to the antigens. In the dogs infected with *T brucei*, dramatic proliferative responses were observed in the spleen and lymph nodes. By Day 8, this was marked in the spleen, ie, just after the first wave of parasitemia. In contrast, diffuse proliferative activity was not found in the lymph nodes until Day 16 of infection, when numerous trypanosomes were present in the tissues and in the lymph node sinuses.

Proliferative activity was observed both in areas that are considered, by analogy with other species, to be thymus-dependent, ie, the splenic periarteriolar regions and the lymph node paracortex,[12,13] and in areas concerned with generating antibody-producing cells, ie, the follicular areas. However, in the lymph nodes, proliferative activity in the paracortex was observed mainly in the areas adjacent to the follicles; and both in these sites and in the periarteriolar regions of the spleen B cells were present, as indicated by the detection of cells containing Ig. Therefore it is uncertain to what extent the proliferative response in-

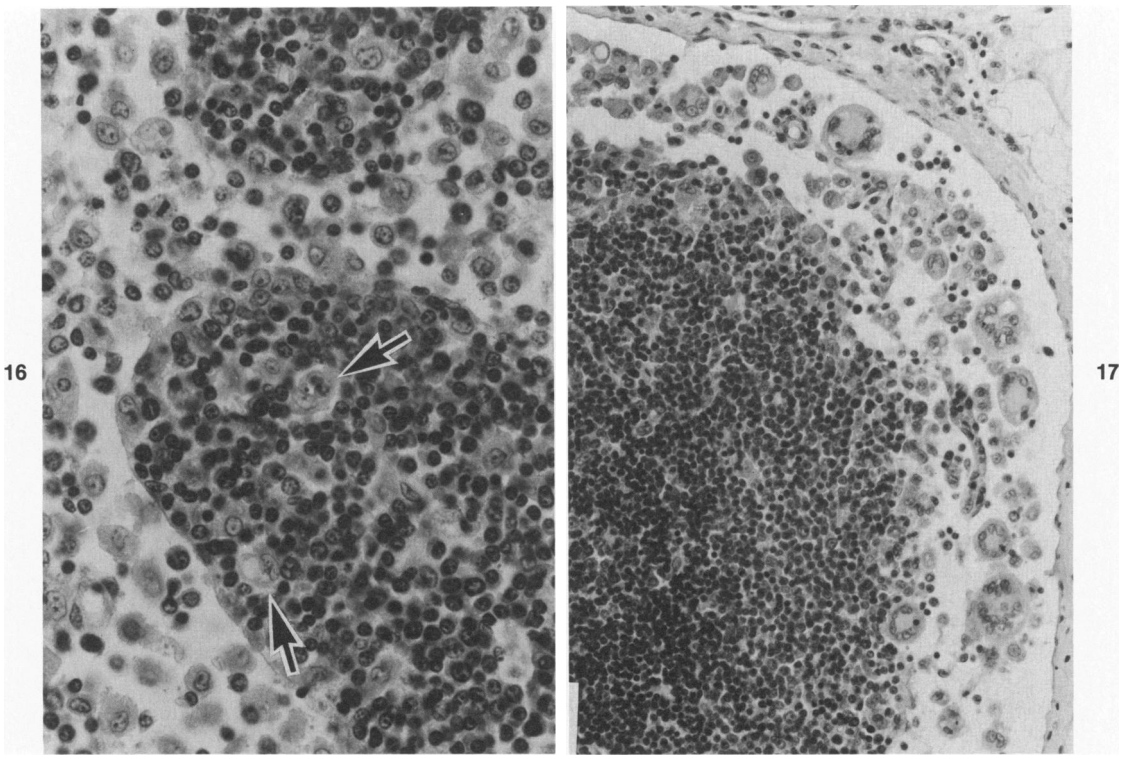

Figure 16—Lymph node of a dog examined on Day 22 of infection, showing an area of medulla. Numerous macrophages are present in the medullary sinuses. Similar cells are also found among the plasma cells in the medullary cords. (*arrows*). (H&E, ×300) **Figure 17**—Lymph node of a dog examined on Day 23 of infection, showing a subcapsular sinus containing many macrophages and multinucleated giant cells. There is disruption of the sinus reticulum cell network, and the inner lining of the sinus appears irregular and discontinuous. (H&E, ×140)

volved thymus-derived cells. It is clear from the numbers of plasma cells that were present that a major component of the initial proliferative response was directed toward antibody production, in particular, IgM. The finding that a significant proportion of the plasma cells contained IgG indicates that the response involved the participation of T cells. However, the intensity of the plasma cell response and the magnitude of the proliferation so early after infection seem excessive to be accounted for entirely by a specific response to the trypanosome. In mice there is evidence that infection with *T brucei* results in a polyclonal type of activation of B cells;[2,14] a similar nonspecific activation may also occur in the dog.

Despite the continued presence of large numbers of trypanosomes in the blood and tissues, the lymph nodes and spleen of dogs examined in the terminal stages of infection showed markedly decreased proliferative activity and cellularity. It would be of interest to know whether this decreased activity was related to a depletion of reactive cells or to an active suppression within the lymphoid organs. The small numbers of lymphoid cells present in the lymph node sinuses and the decrease in cellularity of the splenic marginal zone at this time are suggestive of a decrease in lymphocyte traffic through these organs, in contrast to the large numbers of lymphoid cells found in the lymph node sinuses at Day 16. Therefore, in terminal cases there may be a depletion of recirculating lymphocytes or an alteration in their pattern of migration.

In moribund dogs, there was also a distinct decrease in the numbers of active germinal centers in the spleen and lymph nodes as judged by histologic examination and detection of immunoglobulin on dendritic reticular cells by immunofluorescence. We have observed a similar defect in the spleens of mice infected with *T congolense*.[15] Although the function of germinal centers remains largely unknown, recent evidence suggests that they are involved in the generation of memory cells, dependent on complement-mediated localization of antigen-antibody complexes on the dendritic reticular cells.[16,17] The apparent poor germinal center formation in *T brucei*-infected dogs may therefore be related to inability to localize immune complexes within the germinal centers. This inability might be associated with the absence of cells capable of transporting the immune complexes into the germinal centers, as has been suggested in murine malaria.[18]

A striking feature in the lymph nodes of terminal cases was the presence of massive numbers of macrophages and multinucleated giant cells in the sinuses. Many of these macrophages could also be seen within the cortex and medullary cords. In addition, large thrombi containing numerous polymorphonuclear leukocytes were often observed in the subcapsular sinuses. As evidence that these cells were draining from sites of inflammation within the tissues, tissue lymphatics were often found to have a similar cell content.[8] In mice infected with *T brucei* there is evidence that the immune response to the trypanosomes is involved in the generation of the tissue lesions.[19] During this immune reaction accessory cells such as macrophages and polymorphonuclear leukocytes may be recruited into the tissues, become actively engaged in phagocytosis, and release various enzymes and mediator substances. Such cells, as well as products of the tissue inflammatory responses, will then enter afferent lymphatics and pass to the lymph nodes.[20] In the present study, large amounts of immunoglobulin, presumably in the form of antigen-antibody complexes, were present in the lymph node sinus macrophages. It would be of interest to know whether the large numbers of polymorphonuclear leukocytes and fibrin deposits found in the lymphatics and in lymph node sinuses were related to the presence of immune complexes capable of activating complement and attracting polymorphonuclear leukocytes and initiating clotting by activation of the Hageman factor.[21]

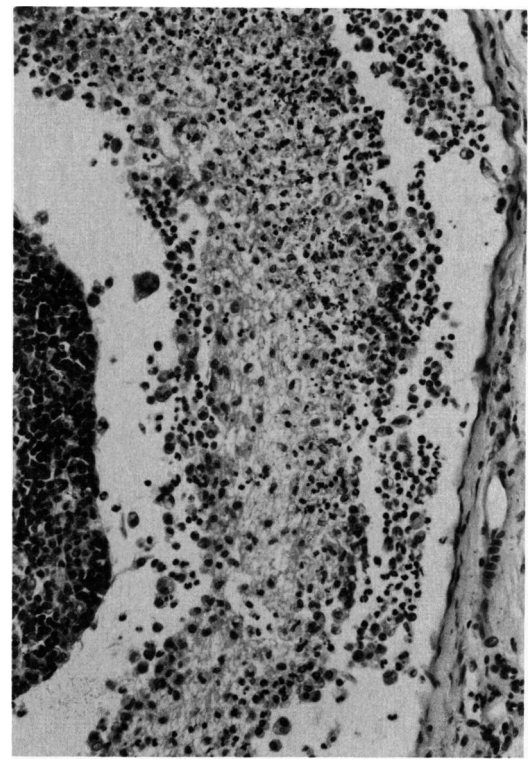

Figure 19—Lymph node of a dog examined on Day 22 of infection, showing a subcapsular sinus that contains a mixture of fibrin, cellular debris, polymorphonuclear leukocytes, and macrophages. There is complete disruption of the reticulum cell network. (H&E, × 140)

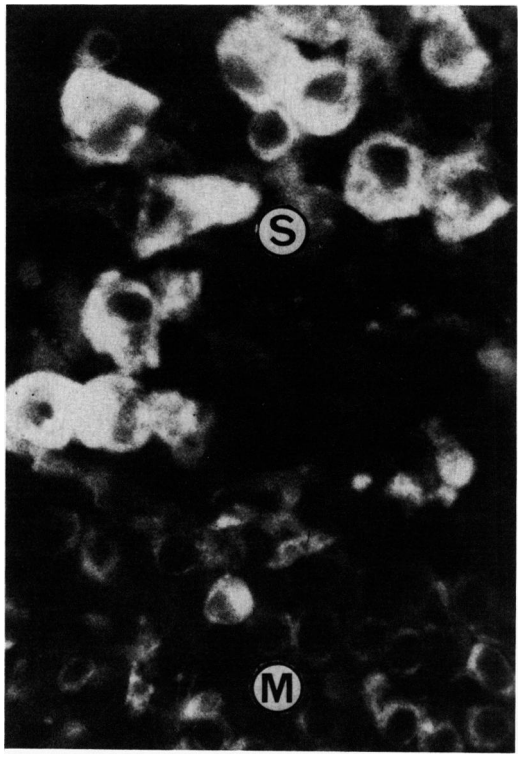

Figure 18—Lymph node of a dog examined on Day 24 of infection, stained for IgG. Large cells containing IgG in their cytoplasm are present in a medullary sinus(*s*). Cells in the adjacent medullary cord (*m*) also stained for IgG. (Immunofluorescence, × 450)

Another prominent feature of the lymph nodes in the terminal cases was a marked disruption of the reticulum cell network in the sinuses. In the normal node these reticulum cells are thought to form a framework upon which macrophages can effectively remove particulate material, including antigens passing through the sinuses.[20,22] Also, macrophages and related cells entering the lymph nodes in afferent lymph are normally retained within the nodes and do not enter efferent lymph.[2] It is thought that antigen-bearing macrophages may migrate from the sinuses into the cortex and, by presentation of antigen to the appropriate cell types, contribute to the initiation of an immune response.[23,24] It is possible that this series of events may be adversely affected by disruption of the sinus reticulum cell network.

In the spleens of dogs examined in the terminal stages of the disease widespread foci of erythropoietic cells were present in the red pulp. This was undoubtedly a response to the anemia that developed

during the infection.[8] Histologic evidence of erythrocyte destruction was seen as erythrophagocytosis by macrophages in the spleen.

A striking feature in the spleens of dogs examined on Day 8 of infection was hemorrhage, necrosis, and fibrin deposition within the peripheral follicular areas and to a lesser extent in the periarteriolar regions and red pulp. We have found similar lesions at the same stage of infection in dogs experimentally infected with *T congolense.*[25] Similar but less severe lesions were also found on Day 16 and in terminal cases. Whether vascular stasis, capillary thrombosis, or hemorrhage was the primary event in the development of these lesions is uncertain. In any case it is difficult to explain why they were restricted to the spleen and were most severe in the peripheral follicular areas so early in the course of the infection.

In conclusion, infection of dogs with *T brucei* results in high levels of parasitemia and the presence of numerous trypanosomes in the tissues and in the afferent lymph. In response, there is intense proliferative activity within the spleen and lymph nodes with the generation of large numbers of immunoglobulin-producing cells. However, in the later stages of infection, there is a marked decrease in reactivity, so that the overall appearance is that of a disorganized, partially depleted, and poorly responding lymphoid system. The effect of these changes on the functional integrity of the immune system now awaits evaluation.

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