

## Lymphadenitis in experimental murine toxoplasmosis induced by intramuscular injection of tachyzoites

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**Abstract:** When tachyzoites (RH strain) of *Toxoplasma gondii* are injected intramuscularly, experimental mice survive up to 7 days, 1-2 days longer than those infected intraperitoneally. We observed sequential histopathological changes in inguinal lymph nodes after intramuscular injection of tachyzoites to thighs of specific pathogen free (SPF) mice. Initial findings on 1 or 3 days after the injection were reactive germinal centers, distended sinuses and epithelioid cell clusters in cortical and paracortical regions. Later on 5 days after the injection, however, effacement of nodal structure with depletion of cells and focal necrosis were observed. Necrotizing lymphadenitis in the experimental murine toxoplasmosis suggests the causal relation between *T. gondii* infection and the human disease.

**Key words:** *Toxoplasma gondii*, murine toxoplasmosis, lymph node, lymphadenitis, necrotizing lymphadenitis

In immunocompetent hosts, local lymphadenitis is the most frequent manifestation of human toxoplasmosis together with fever, myalgia, atypical lymphocytosis and hepatosplenomegaly (Ioachim, 1994). The posterior cervical lymph nodes are most frequently involved, but supraclavicular or occipital nodes may be enlarged. Generalized lymphadenopathy is also not infrequent (Jones *et al.*, 1965). Histopathological characteristics of toxoplasmic lymphadenitis have been described by Piringer-Kuchinka *et al.* (1958), Saxen *et al.* (1958) and Dorfman and Remington (1973). Hyperplastic lymph follicles with reactive germinal centers and proliferation of epithelioid cells are basic changes observed in *Toxoplasma* lymphadenitis which are histological manifestations of B cell reactions.

We had experienced a human case, caused

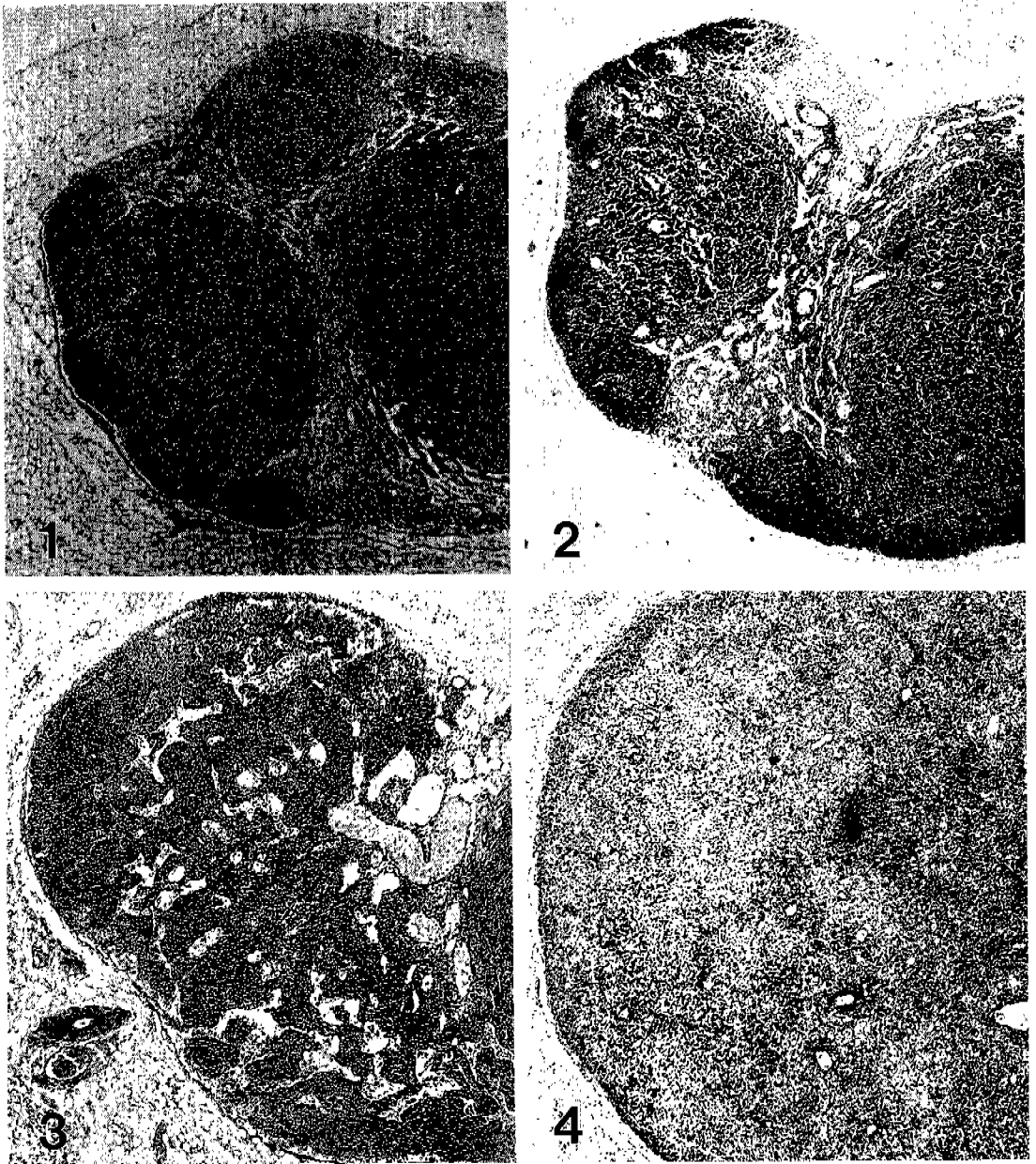
by accidental needle injection of *T. gondii* (RH strain) in a laboratory (Kim *et al.*, 1993). In the case, the only manifestation was enlarged cubital and axillary lymph nodes which showed typical histological findings of *Toxoplasma* lymphadenitis together with elevated serum antibody levels. To observe further the sequential changes in lymph nodes in a similar setting, we designed a simple experiment using mice and tachyzoites of *T. gondii*.

After a preliminary observation, 15 SPF mice (BALB/c strain) were injected with  $10^6$  tachyzoites into right thigh muscle. After the injection, 5 mice were killed on 1, 3 and 5 days, respectively. Inguinal lymph nodes, one at ipsilateral (right) and one at contralateral (left) sides from individual mouse were removed, fixed in 10% neutral formalin and processed in conventional histological techniques. Tissue sections were stained with hematoxylin-eosin.

In the preliminary observation, it was found

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**Figs. 1-4.** Ipsilateral murine inguinal lymph nodes after the intramuscular injection of tachyzoites to thigh. Hematoxylin-eosin stained,  $\times 40$ . **1.** Control lymph node of non-injected specific pathogen free mouse. **2.** Lymph node of one day after the injection. Secondary follicles are well developed in cortical region. **3.** Lymph node of 3 days after the injection. Follicular structures disappeared, and lymph sinuses are tortuously expanded and filled with many monocyte-like cells. **4.** Lymph node of 5 days after the injection. Lymph nodes are enlarged about 1.5-2 times of non-injected controls. Nodal architecture was diffusely damaged with focal necrosis.

that intramuscularly injected mice began to die from the 6th day. All of them died on the 7th day without exception. In inguinal lymph

nodes of control mice, normal architecture such as capsule, primary lymph follicles, sinus and medulla were defined (Fig. 1). In the

**Table 1.** The number of ipsilateral and contralateral inguinal lymph nodes of mice which demonstrate specific histopathological findings of toxoplasmic lymphadenitis. Each group consists of 5 mice

	Secondary follicle	Follicular hyperplasia	Focal necrosis
Control	0 <sup>a)</sup> (0) <sup>b)</sup>	0 (0)	0 (0)
1 day	5 (0)	0 (0)	0 (0)
3 days	1 (5)	4 (0)	0 (0)
5 days	0 (0)	2 (5)	3 (0)

<sup>a)</sup>The numeral indicates the number of ipsilateral inguinal lymph nodes which demonstrate the specific histopathological findings. <sup>b)</sup>The numeral in parenthesis indicates the number of contralateral nodes.

slightly enlarged ipsilateral inguinal lymph nodes, removed on the first day after the injection, revealed secondary follicles with reactive germinal centers, dilated sinuses and focally distributed epithelioid cell clusters at cortical areas (Fig. 2). The nodes removed on the 3rd day were more enlarged. Histologically most follicles disappeared and were replaced by patchy clusters of epithelioid cells at both cortical and paracortical regions. Tingible bodies were not recognized. Lymph sinuses were tortuous, markedly expanded and filled with eosinophilic fluid with many monocyte-like cells (Fig. 3). In the markedly enlarged nodes on the fifth day, nodal architecture was diffusely damaged in all of the experimental mice. Cell numbers were markedly diminished. Areas of focal necrosis were found in 3 of 5 injected mice (Fig. 4). Tingible bodies were found in histiocytes. Tachyzoites had never been observed in the entire specimens.

There were minimal variations of the above described pathological findings between individuals. Almost similar findings were recognized on a certain day after the injection. Pathological findings in nodes of contralateral side developed slower than those observed in the nodes of ipsilateral, such that the findings in the 3rd day at contralateral nodes were comparable to those of the first day at ipsilateral side (Table 1).

By intramuscular injection of *T. gondii* tachyzoites, experimental mice survived 1-2 days longer than those injected peritoneally at the comparable doses. The reason of longer survival is yet to be clarified but the intramuscular site has fewer macrophages which provide a habitat of growth and play roles of transferring *T. gondii* to other tissues. Therefore the intramuscularly injected mice

seem to survive longer than intraperitoneally injected because of the time-lapse in recruiting macrophages.

Basically, the findings of *Toxoplasma* lymphadenitis in this study were not different from the classical descriptions made on the human cases (Ioachim, 1994) especially in the first and 3rd day after the injection. The findings of B cell reaction dominated the picture. A significant finding in this study is the necrotizing lymphadenitis revealed in the 5th day nodes. Because SPF mice were used, their histopathological findings in lymph nodes were not smudged by concomitant infections. In addition, these pathological findings can be concluded to be caused by the protozoon we injected because corresponding lymph nodes of correct lymph drainage were examined after the local injections. In connection with necrotizing lymphadenitis, Ioachim (1994) characterized human *Toxoplasma* lymphadenitis as being neither necrotic nor fibrotic. On the other hand, a human disease entity of necrotizing lymphadenitis (Kikuchi, 1972) has multiple etiologic factors such as typhoid fever, cat-scratch fever or lupus (Cho *et al.*, 1991). But etiology in most cases of the disease is yet to be settled. Possible etiologic relationship between *T. gondii* and the disease has been suggested but not definitely established (Kikuchi *et al.*, 1977). Our findings in the present study support a possible causal relation between them. To confirm the relation, further studies seem to be necessary.

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=초록=

톡소포자충 RH주의 근육내 주입에 의한 마우스 림프절의 조직상

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톡소포자충의 증식형(tachyzoite)인 RH주를 특정무균 마우스(specific pathogen free mouse)의 대퇴부 근육에 주입하면 복강내 계대시 보다 1-2일 더 오래 생존한다. 감염 경과에 따른 서혜부 림프절의 조직병리학적 변화를 관찰하기 위하여 근육내 주입후 1일, 3일 및 5일에 동측(同側) 서혜부 림프절과 반대편 서혜부 림프절을 얻어 hematoxylin-eosin으로 염색하였다. 주입후 1-3일의 동측 림프절 조직은 피질(cortex)과 부피질(paracortex) 영역에서 배종심(germinal center)의 발달, 동(sinus)의 팽창 및 유사피세포괴(cluster of epithelioid cells)의 발달 등이 관찰되었다. 주입후 5일째의 동측 림프절에서는 림프절 구조가 대부분 사라지고, 세포수가 감소되었을 뿐만 아니라 국소 괴사가 관찰되었다. 이 실험에서 관찰한 괴사성 림프절염은 톡소포자충에 의한 림프절염에서 기록되지 않았던 소견으로서 사람의 괴사성 림프절염과 톡소포자충과의 원인적 관계를 시사한다.  
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