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Murine Toxoplasmosis : Development of Bizarre Clusters of Cysts

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Introduction

Avirulent strains of the protozoan parasite *Toxoplasma gondii* are associated with chronic infections, and are characterized by the development of cysts in a wide variety of tissues, particularly the brain and spinal cord. The events leading to the development of the cyst are not known, but some investigators have suggested that the host's immune response may be prerequisite, that cysts will develop only after some form of immunity has been acquired (Frenkel, 1961 ; Nakayama and Matsubayashi, 1961).

The purpose of the present study was to follow the course of infection of the relatively avirulent Beverley strain of *T. gondii* in mice whose ability to respond immunologically was being interfered with by experimental procedures such as administration of the purine antagonist 6-mercaptopurine (Hitchings and Elion, 1963 ; Schwartz, 1963), cortisone treatment (Kass and Finland, 1953 ; Taliaferro, 1957), and splenectomy (Rowley, 1950 ; Sherman *et al*, 1964 ; Taliaferro, 1956) ; to note if any changes would occur either in the pathogenesis of the disease, or in the development and appearance of the cyst.

Methods and Materials

The albino mouse was the animal employed in this study. The techniques used in obtaining the Beverley (BEV) and the virulent RH strains of *T. gondii*, and in infecting mice, were as previously described (Stahl and Akao, 1964). A fresh solution of 6-mercaptopurine (6-MP) was prepared daily by dissolving 6-MP hydrate (6-Mp·H_2O, Nutritional Biochemicals Corporation, Cleveland Ohio) in a mall quantity of 1N NaOH, and then adding saline to a final concentration of 10 mg of 6-MP per ml of solution. One mouse-dose was 1 mg of 6-MP (50 mg per kg of body weight) in 0.1 ml of solution, injected IM every day for 11 days, starting from the first day of the *Toxoplasma* infection. The daily injections were alternated between the outer and the inner aspects of the thigh muscles of the hind legs of the mice. The cortisone (cortisone acetate, Merck and Co., Rahway, New Jersey) was administered to the mice at a rate of 1 mg per 20 gm of body weight, in 0.1 ml of saline solution. The mice received 3 IM injections, given at 48 hour intervals, starting from the day of infection. Splenectomy was performed 3 days prior to the infection with the BEV strain of *T. gondii*. The operation was routine, and recovery of the mice uneventful.

Following the *Toxoplasma* infection, mice were sacrificed at periodic intervals, and peritoneal rinses and various tissues removed for study. In addition, all mice found dead or dying were immediately autopsied. Four weeks after the initial *Toxoplasma* infection, the surviving mice were challenged with the highly virulent RH strain, to test for the presence of the protective immunity to reinfection normally induced by the first infection (Stahl and Akao, 1964).

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Table 1. Effects of inhibiting the immune response of mice to infection with the Beverley strain of *Toxoplasma gondii*

Mice	Trophozoites in peritoneal exudate	Cysts in brain		Mortality after 4 weeks	Immunity to RH challenge*
		First detected	Cyst clusters		
BEV	to day 15	day 10	rare	15%	##
Splex-BEV	to day 18	day 8	abundant	20%	+
Cortisone-BEV	to day 25	day 7	abundant	80%	−
6MP-BEV	to day 23	day 7	sporadic	90%	−

* RH challenge performed 4 weeks after infection with the BEV strain, and immunity graded on the basis of the resultant mortality over a 14-day period of observation. (##) no deaths ; (+) approximately 50 percent mortality ; (−) 100 percent mortality.

Results

It quickly became obvious during the first week of infection that the cortisone-BEV and 6MP-BEV mice were experiencing severe disease, with symptoms such as ruffled fur, diarrhea, shuddering, increasing lethargy, and a precipitous loss of weight. These symptoms progressively worsened, culminating in a rapidly rising mortality rate which, by the end of the fourth week, reached 80 and 90 percent respectively in the 2 groups of mice. In contrast, the BEV and the splex-BEV mice remained relatively symptom-free throughout the experiment, although mortalities did reach 15 and 20 percent, respectively, after 4 weeks of infection. These data, and the results of the tissue examinations are summarized in Table 1. It is interesting to note that in the 3 groups of experimental mice (6MP-BEV ; cortisone-BEV ; splex-BEV), *Toxoplasma* trophozoites were found in peritoneal exudate somewhat longer, and that cysts were detected in the brain somewhat earlier, than in the untreated BEV control mice. The most striking observation however, was that in all 3 groups of experimental mice numbers of cysts were found gathered in clusters (see Fig. 1). Some of the clusters, particularly in the splenectomized and cortisone-treated mice, were very large and bizarre in appearance, with the largest cluster found to contain 54 cysts of varying size. Smaller clusters of from 20 to 40 cysts were seen frequently, as well as large numbers of both single cysts and little knots of 2 to 5 cysts. In respects such as the size and outward appearance of individual cysts, the numbers of trophozoites enclosed within, and infectivity to clean mice, there were no discernable differences between the *Toxoplasma* cysts seen in either the experimental or the control mice.

Information relating to the effectiveness of the various experimental procedures in suppressing the immune response to *Toxoplosma* was provided by the results of the mouse-protection tests, in which all the mice surviving the first *Toxoplasma* infection were challenged with the lethal RH strain, to test for the presence of immunity to reinfection. The BEV control mice easily survived a 14-day period of observation, with no mortalities, thus demonstrating a solid immunity. However, in the 3 groups of experimental animals, 50 percent of the splex-BEV mice, and 100 percent of the 6MP-BEV and cortisone-BEV mice died within 10 days, signifying the absence of an effective immunity.

Discussion

The similarity between the results of the 3 experiments suggests that a mechanism common to all 3 may have been in operation, one indeed involving inhibition or suppression of the immune response. Unfortunately, clearly defined serological data supporting this contention were unobtainable. A series of hemagglutination and agar-gel double diffusion tests were performed, but the crudity of the *Toxoplasma*

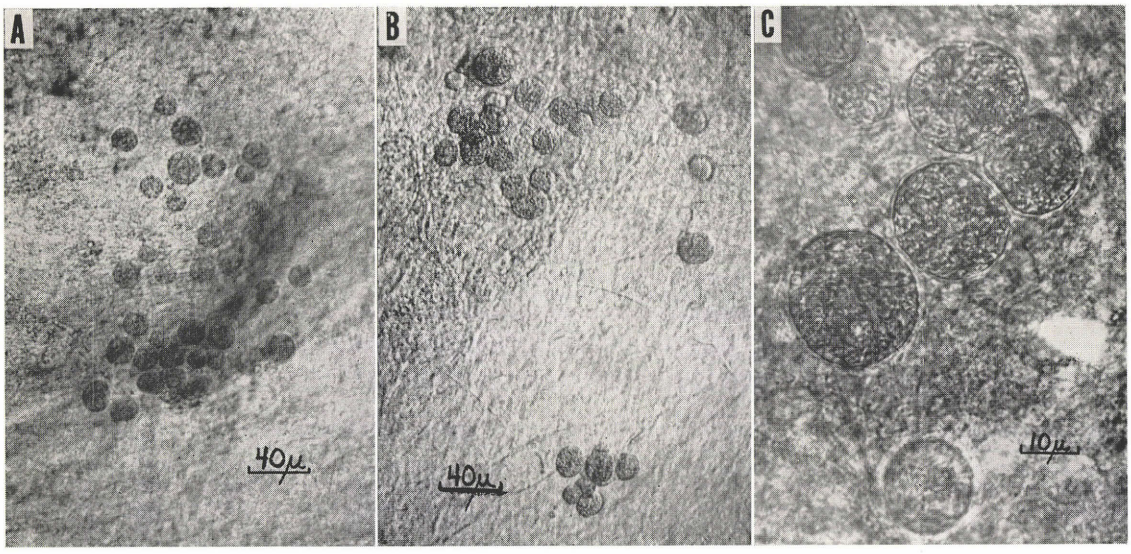

Fig. 1. *Toxoplasma* cyst clusters in unstained press-smears of mouse brain.

A. Cortisone-BEV mouse : large, somewhat diffuse cluster, 27-day infection.
B. 6MP-BEV mouse : 2 clusters containing cysts of varying sizes, 36-day infection.
C. Splex-BEV mouse : compact cluster, 40-day infection.

antigen used, and the variability of the results obtained, do not permit more definite conclusions to be drawn. The most meaningful data were the results of the mouse-protection tests, in which all the experimental mice were challenged with the virulent RH strain after 1 month of infection with the BEV strain. Whereas the BEV control mice demonstrated a strong immunity to reinfection, this protection was not in evidence in the experimental mice. The splenectomized mice did show some prolongation of their survival time as compared to normal mice dying of RH, but the 6-MP and cortisone-treated mice possessed no protection against RH whatever. In addition, distinct changes in the pathogenesis of the primary (BEV) disease were in evidence in the suppressed mice. Higher rates of morbidity and mortality ; longer persistence of trophozoites in peritoneal exudate ; earlier appearance of cysts ; the presence of cysts of many different sizes, both within clusters and scattered throughout the brain ; all are conditions which were in marked contrast to those obtained in mice exhibiting a normal immune response. All these data confirm our impression that an effective suppression of the immune response of the treated mice was achieved.

The unusual, often bizarre, clusters of cysts are believed to be fortuitous gatherings, due mainly to large numbers of trophozoites invading circumscribed regions of the brain through common portals of entry, perhaps weakened or damaged capillaries. In the absence of an effective immune response, trophozoites likely were constantly arriving and adding to the growing clusters. These cyst clusters constituted distinct lesions of the brain, and may well have been the cause of many of the symptoms and deaths in the groups of experimental mice.

It would thus appear that the immune response of the host is not prerequisite to the formation and development of the cyst of *Toxoplasma*, but rather is involved in limiting the proliferation and subsequent invasion of the brain by blood-borne trophozoites from visceral foci such as the liver and spleen.

Summary

Procedures affecting the immune response of the host altered the course of an avirulent *Toxo-*

plasma infection in mice. Mortalities were high, and the mice failed to develop an effective immunity against a subsequent lethal challenge. Cyst production in the brain occurred somewhat earlier and in greater numbers than in normal mice, and was marked by the appearance of large, often bizarre clusters containing as many as 50 cysts. These observations are interpreted as indicating that the immune response of the host is not prerequisite to the formation and development of the cyst of *Toxoplasma.*

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References

1) Frenkel, J. K. (1961): Pathogenesis of toxoplasmosis with a consideration of cyst rupture in *Besnoitia* infection. Survey of Ophthal., 6, (Part II), 799–825.
2) Hitchings, G. H. & G. B. Elion (1963): Chemical suppression of the immune response. Pharmacol. Rev., 15, 365–405.
3) Kass, E. H. & M. Finland (1953): Adrenocortical hormones in infection and immunity. Ann. Rev. Microbiol., 7, 361–388.
4) Nakayama, I. & H. Matsubayashi (1961): Comparative studies on the growth of high and low virulent strains of *Toxoplasma gondii,* with special reference to the production of cyst. Keio J. Med., 10, 43–57.
5) Rowley, D. A. (1950): The effect of splenectomy on the formation of circulating antibody in the adult male albino rat. J. Immunol., 64, 289–295.
6) Schwartz, R. S. (1963): Alteration of immunity by antimetabolites. *in*: Conceptual advances in immunology and oncology. Harper and Row, New York, N. Y., pp. 137–173.
7) Sherman, J. D., M. M. Adner & W. Dameshek (1964): Effect of thymectomy on the golden hamster (*Mesocricetus auratus*). II. Studies of the immune response in thymectomized and splenectomized non-wasted animals. Blood, 23, 375–388.
8) Stahl, W. & S. Akao (1964): Immunity in experimental toxoplasmosis. Keio J. Med., 13, 1–6.
9) Taliaferro, W. H. (1956): Functions of the spleen in immunity. Amer. J. Trop. Med. Hyg., 5, 391–410.
10) Taliaferro, W. H. (1957): Modification of the immune response by radiation and cortisone. Ann. N. Y. Acad. Sci., 69, 745–764.

マウスのトキソプラスマ症，特に脳内における特異なシスト集落の形成について

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本研究は比較的病原性の低い Beverley 株をマウスに感染させ，それによる免疫の形成を種々なる方法で抑制した場合の感染の経過と虫体増殖の模様とを検討したものである。免疫形成の抑制には 6-Mercaptopurine Cortisone の注射，あるいは脾臓の摘出などを行なつた．これ等の操作を施した群では，Beverley 株感染だけの対照群に比してはげしい症状を呈し，死亡率も高くなつた．この現象は 6-Mercaptopurine または Cortisone 注射の場合が，脾臓摘出の場合よりも著明であつた．4週間以上生きのびた動物に強毒性である RH 株を接種すると，対照群のマウスでは全例ともよくその感染にたえて生存したが，実験群では全例または大部分が死亡し，免疫形成の弱いことを示した．

虫体の側から見れば実験群では，マウスの腹腔中に虫体の増殖型が長く残り，脳内にシストの形成が早く認められ，しかもそれらが大きい特異な集落を形成する傾向を示した（第1表および付図）．これらのことは免疫の形成が抑制された場合，虫体の増殖が盛んで，多数の虫体が同じ血管損傷部から遊出し，そこにシストを形成するためと思われる．

以上のことから免疫反応はシスト形成になくてはならないものではないし，一方では虫体の増殖を抑え血中から脳内に虫体が遊出することをさまたげるもののようである．