Oncologia *17:* 81–88 (1964)

From the U. S. Army Medical Unit, Fort Detrick, Maryland; U. S. Army Medical Research and Development Command, Washington 25, D. C; and Walter Reed Army Medical Center, Washington, D. C.

Antineoplastic Effect of VEE[1] Virus in Mice

The effect of attenuated Venezuelan equine encephalomyelitis virus on mouse leukosarcoma L1210[2, 3]

By C. C. BERDJIS, D. L. HOWIE[4], and W. FRANK[5]

In a preliminary study *Berdjis et al.* [1] established that a similarity existed between the effects of an attenuated strain of Venezuelan equine encephalomyelitis (VEE) virus and total-body x-radiation. It was shown that both exert an elective, injurious effect upon the lymphatic organs and bone marrow. By analogy, the possible oncolytic effect of this virus upon lymphatic tumors was tested.

Tigertt et al. [2]* reported on 8 patients with Hodgkin's disease who had been infected with attenuated VEE virus. Tumor regression was observed in 4 of these patients; in 3 of these 4 this was associated with subjective improvement. There was no clinical or histological evidence of encephalitis in any of the 8 patients.

---

[1] Venezuelan Equine Encephalomyelitis.

[2] The authors wish to thank Dr. *Ira Kline*, Head, Cancer Chemotherapy, Microbiological Associates, Inc., Washington 14, D. C., for his help in providing the tumor-bearing animals.

[3] The Principles of Laboratory Animal Care as promulgated by the National Society for Medical Research were observed in this study.

[4] Present address: Chief, Medical Research Branch, U. S. Army Medical Research and Development Command, Washington 25, D. C.

[5] Chief, Cancer Chemotherapy, Walter Reed General Hospital, Walter Reed Army Medical Center, Washington 12, D. C.

* Antineoplastic effect of virus has been suspected since the initial observation of *Dock* in 1904 [6] who obtained a temporary remission of leukemia in a patient after an attack of influenza. Other authors [7–9] reported similar observations. Well-documented experimental cases in man have been reported by *Southam and Moore* [10, 11] with West Nile virus and by *Newman and Southam* [12] with another virus. Occurrence of viral encephalitis, however, discouraged further use of this virus.

It was therefore of interest to determine the effect of an attenuated virus in a mouse tumor system in an effort to correlate the effect with the observation against human disease.

The purpose of this paper is to set forth the data on the oncolytic effect of attenuated VEE virus on the subcutaneous form of L1210, a highly fatal mouse leukosarcoma.

Materials and Methods

Attenuated VEE virus. VEE virus is a member of the arthropod-borne virus family and immunologically belongs to Casal's group A [3].

The Trinidad strain of VEE virus was attenuated by serial passage in guinea pig heart tissue culture (*Berge et al.*, 1961 [4]). The 80th passage level material, designated TC-80, was used in this investigation.

Animals. Seventy, 2-month old BDF_1, hybrid mice, subline C_{57} BL, with the following parents (C_{57} BL/6 Female x DBA/2 Male) F_1, were used.

Tumors. L1210 is a highly fatal mouse leukosarcoma or transplantable lymphoid leukemia reproduced by mouse to mouse subcutaneous inoculation.

Procedure. The mice were divided into 6 groups (Table I). Groups I through IV were inoculated subcutaneously in the right flank with

TABLE I

Modification of Mouse Leukosarcoma L1210 by Attenuated VEE Virus (TC-80)[1]

Group	Tumor	TC-80	on Day	No. of mice	Last day of death	Animal weight gm		Tumor Size cm
						day 0	at death	
I	+	+	–	12	9	23	25	1.5
II	+	+	0	12	13	23.5	25.2	1.2
III	+	+	2	11	12	24	24.5	1.2
IV	+	+	4	11	11	22.5	24	1.1
V	–	+	0	12	–	22.5	22	–
VI	–	–	–	12	–	22.3	22	–

[1] TC-80 = attenuated Venezuelan Equine Encephalomyelitis (VEE) virus.

leukosarcoma, L1210; Group V received TC-80 alone, and Group VI served as untreated controls.

Each mouse in Groups II, III and IV was inoculated intraperitoneally with approximately 200 mouse intraperitoneal infecting doses of attenuated VEE virus, on days 0, 2 and 4 post-tumor inoculation, respectively.

Mice were kept in cages and fed with standard laboratory diet. They were weighed daily; the day of death was recorded. The growth of the tumor was evaluated and measured daily.

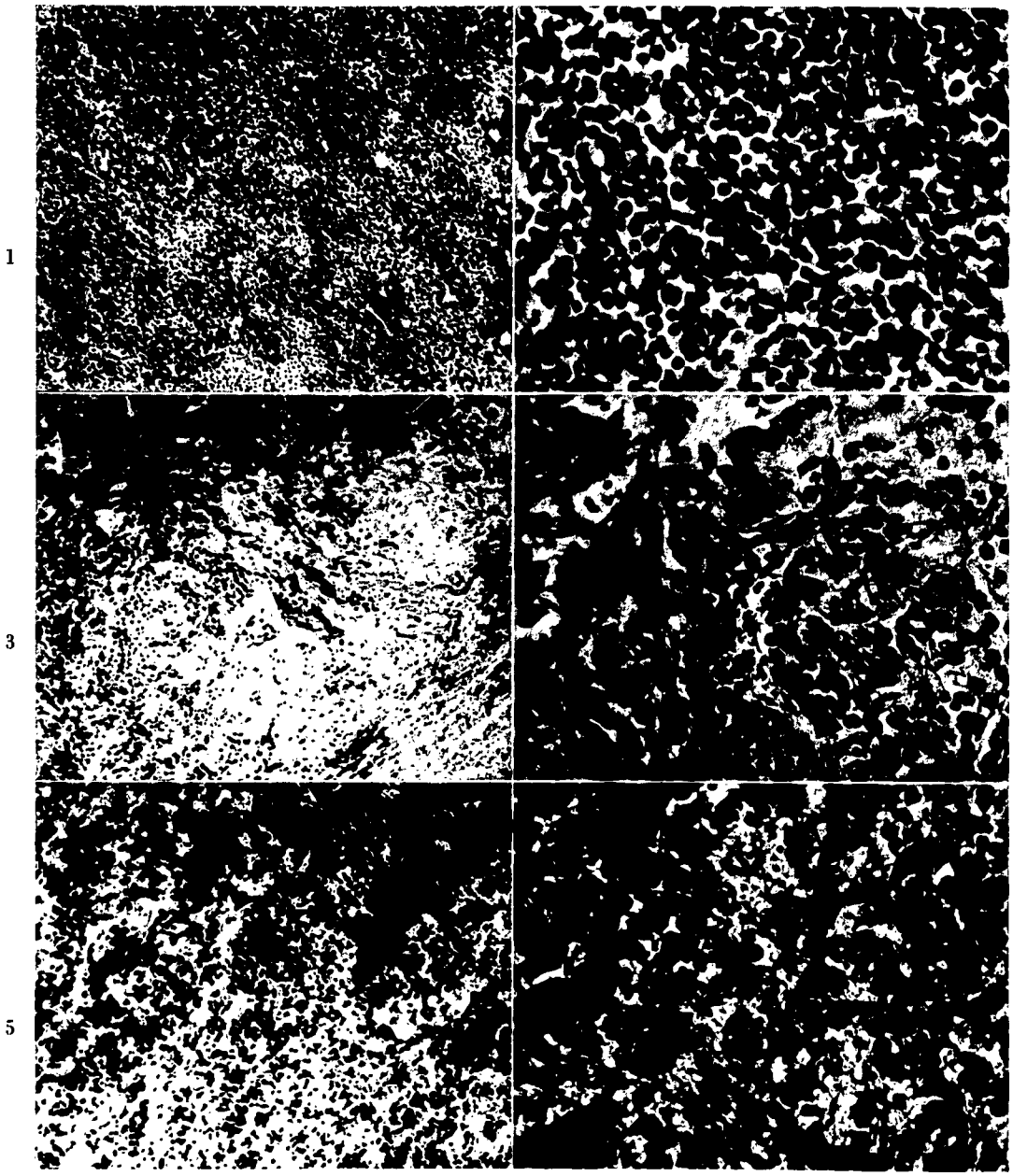
As mice died they were promptly autopsied and representative sections of each tissue including the tumor were fixed in 10% formalin, embedded in paraffin and routinely stained with hematoxylin and eosin. The control mice were sacrificed at appropriate times and similarly processed.

Results

Gross examination (Table I). Variations in the weights of the animals were not significant. The tumors were palpable, and measurable at 6 or 7 days after tumor inoculation at which time there was no observable significant difference between those animals inoculated with VEE virus and the uninoculated groups. By days 8 and 9, however, the tumors began growing rapidly; they attained an average of 1.5 cm in their greatest dimension in the controls, and 1.2 cm in VEE inoculated groups. All mice inoculated with L1210 tumor alone died by day 9. All tumors in animals inoculated with attenuated VEE virus were smaller by day 10. All these mice died between days 11 and 13. Thus, in the virus-inoculated animals there was a delay of 2 to 4 days in the time of death over that noted in the controls. At the time of death, the tumors in VEE inoculated animals were reduced in size and measured 1.1 to 1.2 cm in their greatest dimensions. The tumors in the virus-inoculated animals were softer but well circumscribed. In cross-section, the tumors were pale, grey, or pink, occasionally hemorrhagic, and partly necrotic, whereas in the control group they were reddish grey, firm, and infiltrated the surrounding tissues. Early generalized metastases were grossly detectable in both experimental and control animals but were less prominent in VEE-inoculated mice.

Microscopic Examination – Description of the Tumor

Group I (Figs. 1 and 2). The mouse leukosarcoma (L1210) employed in these experiments was a highly malignant, invasive and infiltrative



Figs. 1 and 2. L1210 leukosarcoma tumor in a control mouse showing the compact structure and cell components. H and E ×101 and ×403.

Figs. 3 and 4. Focal necrosis and fibrin deposition due to the effects of attenuated VEE virus in this tumor. H and E ×101 and ×403.

Figs. 5 and 6. Diffuse necrosis in a tumor treated with VEE virus, showing disturbed architecture, cellular debris and karyorrhexis. H and E ×101 and ×403.

tumor. It was composed of rounded, polyhedral, or ovoid tumor cells with ill-defined or indistinct cell membranes, arranged in closely packed cords, sheets, or coalescent nodules. The cytoplasm was either invisible or faintly basophilic, scanty and dense in the small, rounded cells. Occasionally it was abundant, slightly pink, and appeared to be either granular or foamy, especially in larger cells. The nuclei varied in size, shape, density, and tinctorial affinity. They contained 1 or 2 nucleoli which were either small or prominent. Large, binucleated or multinucleated giant cells were frequently encountered. Mitotic figures were numerous. Metastases occurred in all organs and systems except the central nervous system and skeletal muscle.

Group II. In mice inoculated with VEE on day 0, tumors were smaller and the architectural pattern less compact than controls. The cell components and the histologic structure were essentially similar to controls. Isolated cells, however, had degenerated and there were increased amounts of cellular debris and vacuolated cells. Metastases were small and not widely generalized. They were usually confined to the lymphatic system, bone marrow and liver.

Group III. The tumors in mice inoculated on day 2 were comparable to those of day 0, but the nodules were slightly smaller and appeared to be less compact. Necrosis and/or degeneration of tumor cells occurred either in isolated cells or in clumps of cells. Karyorrhexis with some cellular debris was seen, but neither this change nor decreased mitotic activity were of sufficient magnitude to assess the degree of oncolytic activity at this time.

Group IV (Figs. 3 through 6). Cellular degeneration and necrosis were seen in association with widespread cellular debris, karyorrhexis and karyolysis. This necrosis, at times diffuse and destructive, was not extensive or generalized. The nodules were much smaller, partly necrotic, and exhibited many enlarged and vacuolated cells. The lack of cellular component was evident in each section. Necrosis was associated with or accompanied by hyalinized or filamentous fibrin-like material. Mitotic activity was moderately reduced although numerous mitotic figures were still present.

Group V, TC-80 controls. The mice inoculated with VEE revealed varying degrees of damage to lymphatic system and bone marrow comparable to that previously observed in guinea pigs by ***Berdjis et al.*** [1]. Central nervous system and thymus of these animals were generally unmodified.

Group VI, controls. No significant pathologic changes were seen in the mice of this group.

Discussion

From the analysis of the data presented here it appears that the attenuated virus of VEE exerts an antioncoplastic effect in mouse leukosarcoma L1210 comparable to that described in man by *Tigertt et al.* [2], who reported that in 4 of 8 patients with advanced lymphomas, inoculation of TC-80 produced objective evidence of tumor regression.

In the present study, inoculation of TC-80 produced a similar regression characterized by smaller tumors (1.1 to 1.2 cm versus 1.5 cm, Table 1) in those animals surviving 2 to 4 days longer than controls. This delay may be comparable to the temporary improvement observed by *Tigertt et al.* [2] in man.

No evidence of central nervous system involvement was present in the mice of the present investigation nor in the guinea pigs of the previous study [1]. Similarly, in all 8 patients studied by *Tigertt et al.* [2] no clinical or histological evidence of encephalitis was seen.

Summary

This study was conducted to determine the effect of an attenuated Venezuelan equine encephalomyelitis virus on mouse leukosarcoma L1210.

Groups of mice were inoculated with L1210 tumor and attenuated VEE virus. The virus was given simultaneously with the tumor, or 2 and 4 days after. All were observed to death. They were compared with 3 control groups: one inoculated with VEE alone; one with tumor alone, and the other unmodified.

VEE virus exerted an antineoplastic effect on this tumor comparable to that described in man. This was manifested by objective evidence of tumor regression (smaller tumors), a delay in time to death, and reduced metastatic activity.

In agreement with the previous findings in man, no encephalitis was observed in the virus-infected mice.

Zusammenfassung

In der vorliegenden Arbeit wird die Wirksamkeit eines abgeschwächten venezuelanischen Pferdeencephalitisvirus auf das Leukosarkom L 1210 der Maus untersucht.

Mäusegruppen wurden mit L 1210-Tumor und abgeschwächten VEE-Viren inokuliert. Die Viren wurden gleichzeitig oder 2 resp. 4 Tage nach dem Tumor inokuliert. Als Vergleich dienten 3 Kontrollgruppen: Mit VEE-Virus allein, mit Tumor allein und eine Gruppe ohne jede Inokulation.

VEE-Virus zeigte eine antineoplastische Wirkung auf diesen Tumor ähnlich der beim Menschen beschriebenen. Die Wirkung manifestierte sich in objektivierbarer Tumorrückbildung, Verlängerung der Überlebenszeit und verminderter Metastasierung.

In Übereinstimmung mit früheren Resultaten beim Menschen konnte bei der virusinokulierten Maus keine Encephalitis nachgewiesen werden.

Résumé

Le but de cette étude est de déterminer l'action sur le leucosarcome L 1210 de la souris d'un virus vénézuélien atténué d'encéphalo-myélite équine.

Des groupes de souris furent donc inoculés avec la tumeur L 1210 et le virus VEE atténué. Le virus fut administré en même temps que la tumeur ou bien 2 et 4 jours plus tard. Tous les animaux furent observés jusqu'à leur mort et comparés avec 3 groupes de contrôle: un inoculé seulement avec VEE, l'autre seulement avec la tumeur et le troisième idem.

Le virus VEE manifesta une action antinéoplastique analogue à celle décrite chez l'homme, action qui se manifesta par une régression objective évidente de la diminution des tumeurs, une mortalité plus tardive et une activité métastasique plus réduite.

Comme dans les observations antérieures faites sur l'homme, on n'observe pas d'encéphalite chez les souris inoculées avec le virus.

Riassunto

È stata compiuta una ricerca sull'effetto di un viro attenuato di encefalomielite equina venezuelana sul leucosarcoma L 1210.

Vennero inoculati gruppi di topi con tumore L 1210, e simultaneamente o 2 e 4 giorni più tardi con viro attenuato VEE. Tutti gli animali vennero osservati fino alla morte. Vennero comparati con tre gruppi di controllo, dei quali un gruppo è stato inoculato solamente con VEE, l'altro solamente con tumore e l'ultimo non modificato.

Il viro VEE ebbe un effetto antineoplastico sul tumore comparabile a quello descritto nell'uomo, manifestandosi evidentemente in una regressione tumorale, in un prolungamento della sopravvivenza e nella attività ridotta della metastatizzazione.

In accordo con le esperienze precedenti nell'uomo, nel topo infettato da viro non si è potuto osservare une encefalite.

References

1. *Berdjis, C. C.; Gleiser, C. A.; Gochenour, W. S., Jr.* and *Berge, T. O.:* Virus infection and X-radiation: A comparative study of infection with an attenuated Venezuelan equine encephalomyelitis virus and or X-radiation. J. infect. Dis. *109:* 62–70 (1961).
2. *Tigertt, W. D.; Crosby, W. H.; Berge, T. O.; Howie, D. L.; Kress, S.; Dangerfield, H.; Bass, J.* and *Frank, W.:* The virus of Venezuelan equine encephalomyelitis as an anti-neoplastic agent in man. Cancer *15:* 628–632 (1962).
3. *Casals, J.* and *Brown, L. V.:* Hemagglutination with arthropod-borne viruses. J. exp. Med. *99:* 429–449 (1954).
4. *Berge, T. O.; Banks, I. S.* and *Tigertt, W. D.:* Attenuation of Venezuelan equine encephalomyelitis virus by in vitro cultivation in guinea-pig heart cells. Amer. J. Hyg. *73:* 209–218 (1961).
5. *Tigertt, W. D.:* Studies on the virus of Venezuelan equine encephalomyelitis. Annual Report. Fort Detrick (Maryland), U. S. Army Medical Unit, Parts *I* and *II:* 3–29 (1958).
6. *Dock, G.:* Influence of complicating diseases upon leukaemia. Amer. J. med. Sci. *127:* 563–592 (1904).
7. *Bierman, H. R.; Crile, D. M.; Dod, K. S.; Kelly, K. H.; Petrakis, N. L.; White, L. P.* and *Shimkin, M. B.:* Remissions in leukemia of childhood following acute infectious disease; staphylococcus and streptococcus, varicella, and feline panleukopenia. Cancer *6:* 591–605 (1953).
8. *Paolino, W.* e *Sartoris, S.:* Due casi di leucemia migliorati a seguito di complicazioni infettive. Minerva med. *51:* 3454–3456 (1960).
9. *Einhorn, M.:* Temporary remission in acute leukemia after attack of "acute appendicitis". J. amer. med. Ass. *175:* 1006–1008 (1961).
10. *Southam, C. M.* and *Moore, A. E.:* Clinical studies of viruses as antineoplastic agents, with particular reference to Egypt 101 virus. Cancer *5:* 1025–1034 (1952).
11. *Southam, C. M.* and *Moore, A. E.:* Induced virus infection in man by Egypt isolates of West Nile virus. Amer. J. trop. Med. Hyg. *3:* 19–50 (1954).
12. *Newman, W.* and *Southam, C. M.:* Virus treatment in advanced cancer; pathological study of 57 cases. Cancer *7:* 106–118 (1954).

Authors' address: Lt. Colonel Charles C. Berdjis, M. C.: U.S. Army Medical Unit, *Fort Detrick* Maryland (USA)