REPLICATION OF COXSACKIE B4 VIRUS IN BROWN FAT

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ABSTRACT

Brown fat of adult alcoholic and non-alcoholic mice infected with a heart-adapted strain of coxsackie B4 virus showed the presence of replicating virus up to 8 and 4 post-infection (PI) days, respectively. Immunofluorescent staining (IFAT) of the cryostat sections of brown fat showed virus-specific antigen only as long as the virus could be isolated from the tissue. When brown fat explants from these animals were co-cultivated with vero cells on 21, 45 and 90 PI days, there was no evidence of coxsackie B4 virus remaining latent in the tissue as there was no cytopathic change or virus specific antigen in the co-cultivated vero cells and virus could not be isolated from the culture supernatants of the co-cultivated explants.

INTRODUCTION

Many viruses viz adeno, herpes, measles, rabies viruses, have been shown to remain persistent at different sites and get reactivated under certain conditions. Sulkin and coworkers[1] proposed that brown fat was probably the site at which rabies virus can remain latent in infected bats. This was later proved by Allen *et al*[2,3] by demonstrating the persistence and reactivation of rabies virus in cultured brown fat tissue taken from bats. Brown fat is a very susceptible tissue for the multiplication of coxsackie B viruses (CVB). Grodums and Dempster[4] showed that brown fat remains susceptible to CVB3 infection throughout the life of mice. In certain cases of acute myocarditis recurrent infection with CVB has been noticed[5]. Tsui and Burch[6] suggested that CVB might remain latent at an unknown site and get reactivated at some later stage under unknown conditions. Therefore, the present study was undertaken to see if brown fat of mice provides the appropriate site for multiplication and latency of CVB.

MATERIALS AND METHODS

Animals: Random-bred adult (~ 8-week-old) Swiss albino mice obtained from the animal house of Department of Virology, PGI, were used for this study. Animals were divided into two groups when they were about 4 weeks of age. One group was fed with a 30% solution of ethyl alcohol as the only source of liquid and the other group, with an isocaloric sucrose (40%) solution. After 4 weeks of alcohol/sucrose feeding the animals were inoculated.

Virus: JVB strain of coxsackie B4 virus was used. The passage history of this virus has been described elsewhere[7]. Briefly, the original stock virus which was obtained at 10% mouse carcass was passaged thrice in suckling mice as mouse carcass suspension and 7 times in weanling mice as infected heart suspension by intraperitoneal route. After a total of 10 passages, the 10% suspension of infected heart was passaged once in vero cells to make a stock virus (7.5 Dex. $TCID_{50}$/0.1 ml) for inoculation.

Inoculation of animals and harvesting of interscapular pad of brown fat: Adult Swiss albino mice, both alcoholic and non-alcoholic, were inoculated with 6.5 Dex. $TCID_{50}$/0.1 ml of JVB strain of coxsackie B4 virus by intraperitoneal route. Control animals from both the groups were inoculated with 0.1 ml of similarly processed uninfected vero cell cultures.

On 4, 8, 15, 21, 45 and 90 post infection (PI) days batches of infected and uninfected mice, both alcoholic and non-alcoholic, were sacrificed by bleeding to death from retro-orbital plexus. The interscapular pad of brown fat from each of these animals was harvested asceptically and the tissues from the same batch of animals were pooled. One half of each tissue was processed for cryostat sectioning and the other weighed, washed thoroughly in Hank's balanced salt solution (HBSS) and a 10% suspension (w/v) was prepared in HBSS. On all the PI days mentioned above, virus titration in vero cell culture tubes was carried out using the 10% suspension of brown fat tissue.

The cryostat sections were fixed in acetone at −20°C for 10 min and stained by indirect immunofluorescent method (IFAT) for the demonstration of virus specific antigen in the brown fat tissues.

Explant culture of brown fat tissue and co-cultivation with vero cells: Brown fat tissue from 3 animals each harvested on 21,45 and 90 PI days was used. The tissue was first washed in HBSS thoroughly and cut into small pieces (1 mm^3). Six to 8 such pieces were picked with siliconized Pasteur pipette with bent tip and placed on the coverslip of Leighton's tubes. Two Leighton's tubes were used for each brown fat pad. These tubes were incubated for 2 hr at 37°C for the explants to stick to the coverslips. After the incubation, 1 ml of MEM with 10% goat serum was added to each tube and the tubes were further incubated at 37°C. After 7 days when the explants start showing cellular outgrowth, 10^7 vero cells were added to each Leighton's tube containing growing explants of brown fat. When vero cells formed a monolayer, the coverslips were harvested. The supernatant fluid from each tube was checked for the presence of coxsackie B virus by inoculating into 3 tubes of vero monolayers. The coverslips were washed thrice with HBSS and fixed in cold acetone at −20°C for 15 min and processed for IFAT.

Indirect immunofluorescent staining of brown fat tissue—cryostat sections and explants co-cultivated with vero cells: IFAT was carried out using murine coxsackie B4 antibody and FITC conjugated anti-mouse Ig (Behring Werke, W. Germany) as the first and second antibodies, respectively. The method was essentially the same as described by French *et al*[8] with slight modifications. Briefly, slides were covered with murine coxsackie B4 antibody (1:8 dilution) and incubated at 37°C for 30 min in a humidified atmosphere. After three washes in phosphate-buffered saline (PBS, pH 7.4), 1:8 dilution of FITC-conjugated anti-mouse Ig was added. The slides were again incubated at 37°C for 30 min, washed with 3 changes of PBS, counterstained with 0.1% Evan's blue and mounted in phosphate-buffered glycerol saline.

The slides were examined for fluorescence in a Carl Zeiss fluorescent binocular microscope illuminated with an Osram HBO 200 mercury burner.

RESULTS

Isolation and titration of virus from the brown fat: Table 1 shows the titres of virus in the brown fat of alcoholic and non-alcoholic mice infected with coxsackie B4 virus. The virus titres in the brown fat of alcoholic mice were 3.25 and 2.33 Dex. $TCID_{50}$/0.02 g of tissue on 4 and 8 PI days, respectively. Thereafter, no replicating virus could be detected. In case of non-alcoholic mice, the virus titre in brown fat was 0.5 Dex. $TCID_{50}$/0.02 g tissue on 4 PI days only. Thereafter no replicating virus could be detected on any of the PI days.

Virus specific antigen in the brown fat: Table 1 shows the incidence of virus-specific antigen present in the brown fat of alcoholic and non-alcoholic mice infected with coxsackie B4 virus. In case of alcoholic mice, 100% of brown fat tissue showed presence of virus-specific antigen on 4 and 8 PI days. Thereafter, no antigen-containing cells could be seen in the brown fat by IFAT. In case of non-alcoholic mice, 100% positivity for virus-antigen containing cells could be obtained on 4 PI days only. Thereafter, brown fat tissue become devoid of virus-antigen containing cells.

Throughout the study, brown fat tissue from uninfected control animals was negative for virus as well as virus-specific antigen containing cells.

The results also indicate that the virus-specific antigen in the brown fat was present only as long as there was replicating virus in the tissue (figure 1).

Table 1 *Virus titres and virus-specific antigen in the brown fat of alcoholic and non-alcoholic mice infected with coxsackie B4 virus on different post-infection days*

		Post infection days					
		4	8	15	21	45	90
Alcoholic Mice	Virus titre*	3.25	2.33	–	–	–	–
	Virus ag**	8/8 (100)	8/8 (100)	0/8 (0)	0/8 (0)	0/4 (0)	0/4 (0)
Non-alcoholic Mice	Virus titre*	0 5	–	–	–	–	–
	Virus ag**	8/8 (100)	0/8 (0)	0/4 (0)	0/3 (0)	0/3 (0)	0/3 (0)

* Dex. $TCID_{50}$/0 02 g of tissue.
** No. Pos./No. tested. Figures in the parentheses indicate the percentage positivity.

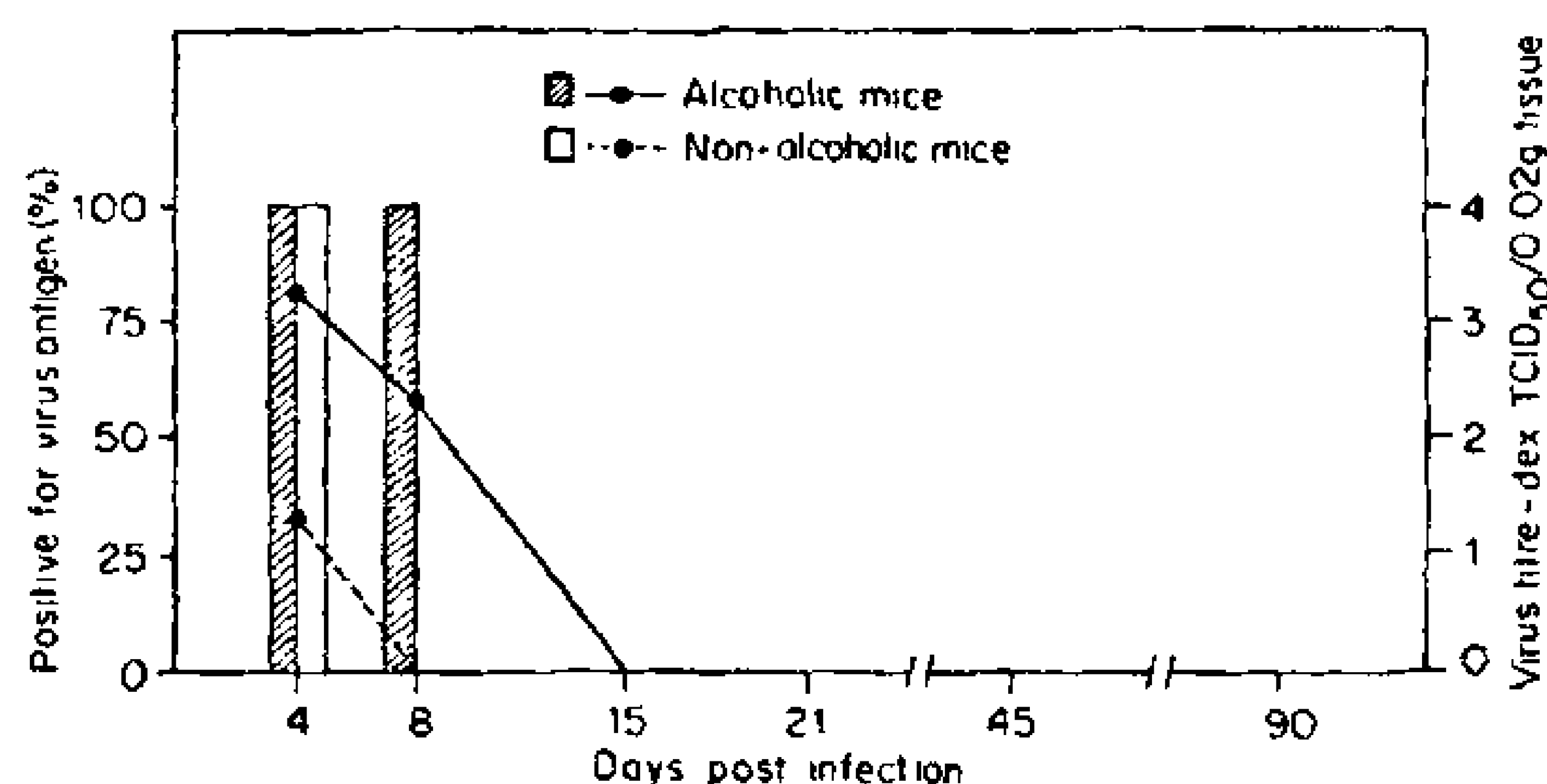

Figure 1. Coxsackie B4 virus titres and virus-specific antigen in the brown fat of infected alcoholic and non-alcoholic mice.

Latency of coxsackie B4 virus in brown fat: Vero cells formed monolayer by about the 7th day after addition to the Leighton's tubes containing growing explants of brown fat tissue. Thin fibroblastic cells from brown fat explants were interspersed with vero cells near the explant area. No CPE suggestive of non-polio enteroviruses could be detected in any of the tubes containing brown fat explants from alcoholic and non-alcoholic infected mice. IFAT of the coverslips containing explants co-cultivated with vero cells did not reveal the presence of virus-specific antigen containing cells. The supernatant fluid, from these Leighton's tubes also failed to yield any virus when vero monolayer tubes were inoculated.

DISCUSSION

Coxsackie B viruses have a marked affinity for brown fat tissue. Grodums and Dempster[9] have shown that brown fat of mice becomes resistant to infection with coxsackie B viruses as the age increases. When adult mice were acutely exposed to cold, coxsackie B3 virus titres increased in the brown fat of infected animals as compared to their counterparts maintained at room temperature[10]. Thus the multiplication of coxsackie B viruses in the brown fat may be enhanced by exposure to cold, a physical immunosuppressive agent. Since cold is known to depress interferon synthesis[11], this might be the mechanism by which coxsackie B viruses multiply to a greater extent. Similar observations have been made in the present study. Coxsackie B4 virus multiplies to a higher titre in the brown fat of alcoholic mice than in their non-alcoholic counterparts. Moreover, in alcoholic mice the virus multiplication is prolonged up to 8 days PI as against 4 days PI in nonalcoholic mice. The increased titre of virus in brown fat of alcoholic mice was probably due to chronic alcohol feeding which is known to depress CMI[12].

Allen and coworkers[2,3] have shown that rabies virus remains latent in cultured brown fat of bats. They could demonstrate rabies antigen containing cells when no replicating virus could be detected. In the present series, however, virus-specific antigen-containing cells could be seen in brown fat of both alcoholic and non-alcoholic mice only as long as the virus was actively multiplying in the tissue.

Studies on amyotropic lateral sclerosis show an epidemiological relationship with past polio-like illness[13]. Tang and coworkers[14] have isolated cox-A9 virus from the muscle tissue of such a case. Though the significance of this isolation is not clear, it might suggest the activation of a latent/persistent enterovirus infection. Tsui and Burch[6] suggested that coxsackie B virus might remain latent and get reactivated under unknown conditions. They feel that this might be the reason of continued histopathological changes in the myocardium of mice infected with coxsackie B4 virus long after the disappearance of replicating virus. In our study, however, we failed to demonstrate latency of coxsackie B virus in brown fat of both alcoholic and non-alcoholic mice. Our results prove that though brown fat provides a very suitable site for coxsackie B viral multiplication during acute stage of infection, it does not act as a location for latency/persistence of this virus.

This paper forms part of the Ph.D. thesis submitted by R. Kaiwar.

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NEWS

DSA AND THE SCIENTIFIC REVOLUTION

..."Many of man's greatest inventions have expanded the capabilities of the human body. The computer has enhanced man's ability to see by making the invisible visible. This new vision lies at the heart of digital subtraction angiography (DSA), an imaging technique that produces clean, clear views of flowing blood or its blockage by narrowed vessels. DSA depends on the injection into the vessels of a contrast agent containing iodine that is opaque to x-rays. The shadow this opacity creates allows doctors to see the flow of blood. Frequently DSA is used to look at blood supply to the heart. Before injection of the contrast substance, an x-ray image is made and stored in a computer. After injection a second image is made highlighting the flowing blood as revealed by the substance. The computer then subtracts image one from image two, leaving a sharp picture of blood vessels such as the coronary arteries, the main suppliers of blood to the heart .... The marriage of the computer and medical imaging devices is already bearing fruit. It holds tremendous promise for the future. 'In medicine, as in our society, we have embarked on a scientific revolution unlike any other in man's history,' said Steven Nissen, a cardiologist at U. Kentucky Medical Sch. A growing number of young, dynamic doctors hold PhDs in physics or computer science along with their MDs. And the burgeoning technology of computer graphics is being harnessed to transform the torrents of machine-vision data into meaningful diagnostic displays."

[(Howard Sochurek in *National Geographic* 171(1):2-41, January 1987). Reproduced with permission from Press Digest. *Current Contents*®, No. 12, March 23, 1987. Published by the Institute for Scientific Information®, Philadelphia, PA, USA].

SS BHATNAGAR PRIZE FOR SCIENCE AND TECHNOLOGY FOR 1985–86

The above prize has been awarded to Dr Dilip Kumar Ganguly, Scientist, Head of the Division of Pharmacology and Experimental Therapeutics, Indian Institute of Chemical Biology, Calcutta. The prize has been awarded to Dr Ganguly's work on a chemical model of Parkinson disease. He has recently established that there is a "spinal involvement in the genesis of Parkinson tremor". Dr Ganguly is a founder-fellow of the Indian Academy of Neurosciences and has been the Vice-President of the same Academy.