Clin Exp Immunol 1997; **107**:282–287

Induction of hypoglycaemia in Japanese encephalitis virus infection: the role of T lymphocytes

N. KHANNA, A. MATHUR, M. BHARADWAJ & U. C. CHATURVEDI *Postgraduate Department of Microbiology, K. G. Medical College, Lucknow, India*

(Accepted for publication 11 October 1996)

SUMMARY

We report here development of hypoglycaemia in the convalescent phase of Japanese encephalitis virus (JEV) infection in mice by the induction of antigen-specific $Ly1^{-}2^{+}$ T cells in the spleen which mediate hypoglycaemia through the generation of soluble T cell hypoglycaemic factor (TCHF). The TCHF acted in a dose-dependent manner and was found to be trypsin-sensitive and thermolabile. It was purified on Superose-12 high performance liquid chromatography (HPLC) gel filtration column and purified protein migrated as a ~25-kD band on SDS–PAGE. The JEV-induced hypoglycaemia coincided with an increased circulating glucagon level, without any alterations in blood insulin and growth hormone concentrations. These effects were mimicked by TCHF. These results indicate that JEV-primed T lymphocytes mediate hypoglycaemia through the production of a soluble hypoglycaemic factor.

Keywords hypoglycaemia Japanese encephalitis virus T cell factor T suppressor cell glucagon

INTRODUCTION

Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus, responsible for epidemic encephalitis in South-East Asia [1]. Fatality rates are between 21% and 40%. Following entry into the host system, JEV replicates in various organs and generates an inflammatory response [2] with brain congestion, haemorrhage and neuronal degeneration [3]. Clinical manifestations of the disease are fever, headache, vomitting leading to convulsions and altered sensorium. Disturbance of protein, fluid and carbohydrate metabolism has been detected in large numbers of JE patients [4]. The histopathology of JE has been studied in great detail [5], while little is known regarding biochemical alteration during infection. Our earlier studies revealed a significant fall in serum iron levels in JEV infection, both in humans [6] and in mice [7], caused by the action of macrophage-derived factor, which blocks the release of iron from splenic reticuloendothelial cells [7]. We recently showed leakage of plasma protein and infectious JEV in urine [8].

Marked hypoglycaemia (blood glucose 54–70 mg%) is noticed during JEV infection [4], but its triggering mechanism is not known. Hypoglycaemia could be dangerous because glucose is the primary energy substrate of the brain and its absence may result in deranged functions and tissue damage, and could lead to death [9].

Hypoglycaemia has been reported in few viral infections. Samson *et al.* [10] have reported recurrent hypoglycaemia during viral hepatitis, which is associated with hyperinsulinaemia, while Oldstone *et al.* [11] have observed that lymphocytic choriomeningitis virus perturbs the endocrine functions without any change in serum cortisol, or insulin levels. Hypoglycaemia with hyperinsulinaemia have been reported in *Plasmodium falciparum* malaria [12,13]. Considering that the mechanism of production of hypoglycaemia during JEV infection is not known, the present study was undertaken to examine this phenomenon in great detail.

Correspondence: Professor Asha Mathur, Department of Microbiology, K. G. Medical College, Lucknow -226 003, India.

MATERIALS AND METHODS

Animals and virus used

The study was performed on inbred Swiss albino mice aged 5–6 weeks, obtained from the mouse colony maintained in this Department, and the inbred BALB/c mice were purchased from the Central Drug Research Institute, Lucknow. JEV strain 78668A was propagated in suckling mouse brain and was used as infected mouse brain suspension, as described earlier [14]. The virus titre was $10^{6·2}$ LD_{50}/ml. This produced uniform sickness and 100% mortality by day 6 when given intracerebrally in mice, but intraperitoneal (i.p.) inoculation produced no clinically evident disease.

Priming of mice

The mice were given a single i.p. dose of 0·3 ml of 10^2 LD_{50} of JEV, while control mice were inoculated with 0·3 ml of 10% normal mouse brain suspension. They were fed *ad libitum* on the standard commercial laboratory chow and water, and were fasted for 18 h, before bleeding.

Spleen cell culture

Spleens were collected from JEV-infected or control mice at different periods and teased out in chilled minimum essential medium (MEM) with HEPES containing 10% fetal calf serum (FCS) and antibiotics. The total spleen cells (5×10^6 cells/ml) were cultured in MEM–HEPES or normal saline for 24 h.

Preparation of enriched spleen cell subpopulations

The splenic single-cell suspension was fractionated to obtain enriched subpopulations of macrophages, T and B lymphocytes. Briefly, single cell suspension of mouse spleen prepared in chilled MEM–HEPES containing 10% FCS, 5×10^{-5} M 2-mercapto-ethanol (2-ME) and antibiotics was layered in glass Petri dishes and incubated in moist air with 5% CO_2 at 37°C for 2 h. The supernatant having non-adherent cells was collected. A macrophage-enriched subpopulation was obtained by collecting the glass-adherent cells. About 97% of these cells phagocytosed latex particles and were considered as macrophages. Enriched subpopulations of T and B lymphocytes were obtained by a panning procedure from glass-non-adherent cells [15]. Briefly, $1{\cdot}5 \times 15$ cm polystyrene Petri dishes were coated with anti-mouse IgG antibody (1:100 diluted in 0·05 M Tris) and then layered with 1×10^8 glass-non-adherent cells in MEM–HEPES for 70 min. Thereafter, the supernatant with non-adherent cells was collected. Of these cells, >95% consisted of T lymphocytes, as judged by treatment with anti-Thy1.2 antisera. The bound cells were gently scraped out using a rubber-tipped glass rod, washed with PBS, and the purity was checked by treating the cells with anti-mouse IgG antibody and complement. Of this cell population, >94% was considered B lymphocytes.

Assay of blood glucose

Blood glucose was assayed using Haemo-Glukotest strips (Boehringer, Mannheim, Germany) which were read in a glucometer for quantitative measurement and expressed as mg/dl. Before this, mice were fasted for 18 h but had free access to water. Blood samples were repeatedly taken from the same mice ($n = 8$–10). Data were statistically analysed by Student's *t*-test and mean ± s.e.m. was calculated.

Preparation of T cell hypoglycaemic factor

T cell hypoglycaemic factor (TCHF) was prepared from JEV-infected mouse spleen. Briefly, mice were given 0·3 ml of 10^2 LD_{50} of JEV intraperitoneally. On day 20 post-infection the spleens were collected and the splenic T cells were separated by a panning procedure as described above. Cells were washed three times with Hanks' balanced salt solution (HBSS), viability was checked and they were cultured in normal saline for 24 h at 37°C. The T cell supernatant was collected, centrifuged and assayed for hypoglycaemic activity by *in vivo* inoculation in mice.

Purification of hypoglycaemic factor

The crude JEV-stimulated splenic T cell culture supernatant was concentrated by freeze drying in Speed Vac (Savant Instruments Inc., New York, NY). One hundred micrograms of the concentrated preparation were applied to Superose 12 fast protein liquid chromatography (FPLC) column (Pharmacia, Uppsala, Sweden). PBS (0·1 M, pH 7·4) was used as elution buffer. Absorbance at 280 nm was monitored (Pharmacia LKB VWM 2141 monitor) and the corresponding peaks were recorded (Pharmacia LKB Rec. 2) and tested for hypoglycaemic activity. The active fractions were pooled, concentrated and subjected to molecular weight determination by SDS–PAGE [16]. The molecular weight markers used were: carbonic anhydrase (31 000), Trypsin soyabean inhibitor (21 000), cytochrome C (12 000), and acid aprotinin (6500).

Hormonal study

Insulin, glucagon and growth hormone levels in the fasting blood of JEV- or TCHF-inoculated mice were measured at different time periods post-inoculation using the commercially available ^{125}I radioimmunoassay (RIA) kits (Diagnostic Products Corp., Los Angeles, CA) based on double antibody technique. All the mice were bled in the morning at approximately the same time. The control group comprised normal mice of matched age and sex. The samples were kept for 24 h with specific antibodies followed by the addition of 100 μl ^{125}I-labelled ligand. Then 1·0 ml of precipitating solution was added and tubes were centrifuged at 1500 ***g*** for 15 min. The precipitate containing the antibody-bound fraction was then counted and the ct/min was measured in a gamma counter (LKB Wallac, Pharmacia). Simultaneously, a calibration curve was drawn in each case as per the manufacturer's instructions and hormonal concentrations were read. Data were subjected to Student's *t*-test, and results were expressed as mean ± s.e.m. of 8–10 values.

Preapration of anti-TCHF antisera

The anti-TCHF antibody was prepared in mice. Purified TCHF (100 μg) emulsified in Freund's complete adjuvant was injected intramuscularly in the inner side of the flank, and the same dose was repeated at 3 week intervals with Freund's incomplete adjuvant. Then three intradermal injections at weekly intervals were given without any adjuvant, followed by an intravenous injection 4 days before bleeding. The serum was collected, inactivated at 56°C for 30 min. The optimal dilution of antibody which abrogated the hypoglycaemic response was determined and sera were stored at −70°C.

RESULTS

Production of hypoglycaemia during JEV infection

The fasting plasma glucose levels of inbred Swiss albino mice were measured on alternate days after priming with 0·3 ml of 10^2 LD_{50} of JEV intraperitoneally. As presented in Fig. 1, the mice developed a transient decline in their blood glucose from day 18 p.i. The maximum decrease was observed between days 20 and 22 p.i. The mean fasting plasma glucose concentration of 58 ± 8 mg/dl in JEV-infected mice was significantly lower ($P < 0{\cdot}001$) than the mean observed in control (121 ± 14 mg/dl). These findings show the development of hypoglycaemia in the convalescent phase of JEV infection. There was no significant difference in fasting blood glucose in the initial stage of infection compared with controls.

In an attempt to investigate the effect of strain variation in production of JEV-induced hypoglycaemia, blood glucose levels in inbred BALB/c mice were measured on different days following i.p. inoculation of JEV. Findings presented in Fig. 1 show a fall in blood glucose level from day 18 following JEV infection, similar to that observed in the Swiss albino strain of mice.

In all further experiments Swiss albino mice were used.

Hormonal alterations during JEV infection

The findings presented above show a significant fall in blood glucose levels of mice during JEV infection. We investigated

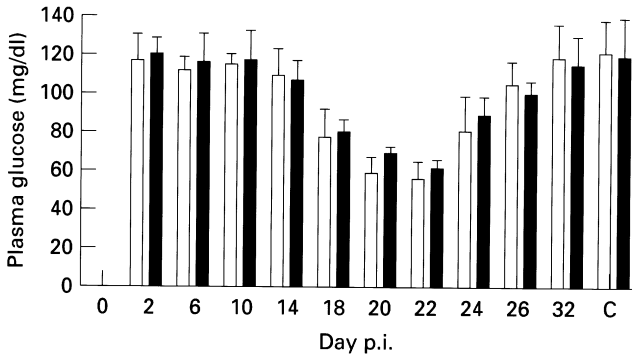

Fig. 1. Blood glucose levels following Japanese encephalitis virus (JEV) infection in Swiss albino (□) and in BALB/c (■) mice. Mice were fasted for 18 h before bleeding on different days. Data are presented as mean of 8–10 mice.

whether this could be due to alteration of synthesis of carbohydrate regulating hormones, e.g. insulin, glucagon or growth hormone. Findings summarized in Fig. 2 show no change in circulating insulin (mean value 226 ± 27·3 μU/ml on day 20 p.i. *versus* 219·87 ± 21·2 μU/ml in controls) and growth hormone levels (mean value 0·55 ± 0·03 pg/ml on day 20 p.i. *versus* 0·56 ± 0·07 pg/ml in controls) throughout the study period. The glucagon levels showed a transient rise from day 18 p.i. onwards and the increased levels were maintained up to day 20 after JEV inoculation (262 ± 19·8 pg/ml *versus* 177·28 ± 14·87 pg/ml in control) (Fig. 2).

Production of hypoglycaemic factor in spleen during JEV infection

In order to know the mechanism(s) of hypoglycaemia in JE, efforts were made to study the role of the spleen in this phenomenon. Spleens were collected 20 days after JEV infection (i.e. coinciding with the time of maximum fall in blood glucose) and the ability of the 24 h spleen cell culture supernatant to produce hypoglycaemia by *in vivo* inoculation in mice was studied. Injection of 200 μg of JEV-primed spleen culture supernatant intravenously in mice showed significant reduction in blood glucose between days 8 and 10 after inoculation compared with control mice, which received 200 μl of normal splenic cell culture supernatant (Fig. 3).

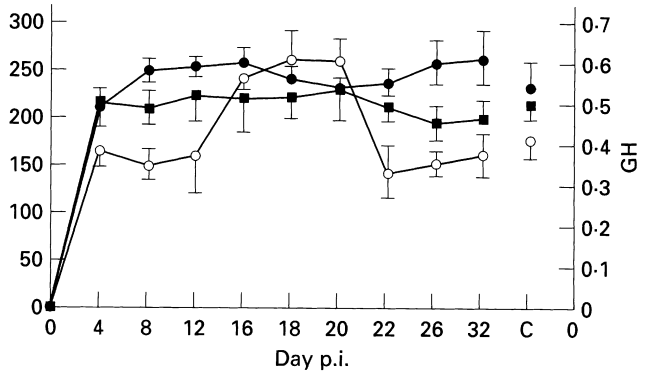

Fig. 2. Blood insulin (■; μU/ml), glucagon (○; pg/ml) and growth hormone (●; ng/ml) levels in Japanese encephalitis virus (JEV) infected and control (C) groups of mice. Each value is mean ± s.e.m. of triplicate experiments ($n = 8–10$ in each experiment).

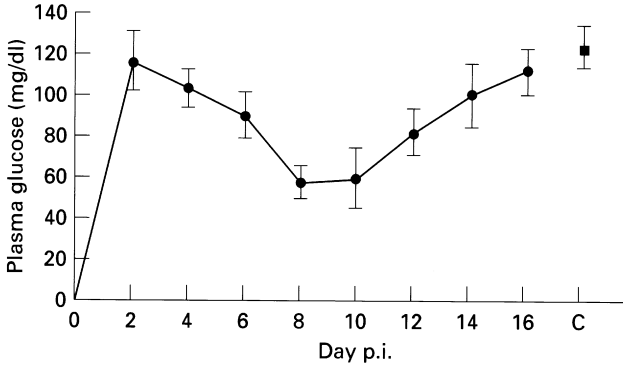

Fig. 3. Blood glucose concentration in mice given Japanese encephalitis virus (JEV)-primed splenic cell culture supernatant (●) and normal splenic cell supernatant (■). Values are mean ± s.e.m. ($n = 8–10$).

Identification of cell type producing hypoglycaemic factor

To delineate the splenic cell type responsible for the production of hypoglycaemic factor, the splenic single-cell suspension prepared on day 20 p.i. was enriched for macrophage, T and B lymphocyte subpopulations, and individual subpopulations were cultured in saline at 37°C. The 24-h culture supernatants were collected and tested for hypoglycaemic activity by i.v. inoculation into mice. The hypoglycaemia occurred 8–10 days after inoculation of JEV-primed T lymphocyte supernatant only ($P < 0·05$), while macrophage and B lymphocyte culture supernatants failed to produce any significant alteration in plasma glucose compared with controls ($P > 0·05$) (Table 1). This shows that during JEV infection, splenic T lymphocytes secrete a factor which mediates hypoglycaemia. We named this factor T cell hypoglycaemic factor (TCHF).

Surface phenotype of T cells

The splenic T cells mediating hypoglycaemia on day 20 after JEV priming were treated with anti-Ly1.2 or anti-Ly2.1 antibody and complement as described earlier [26] before they were assayed for their ability to alter blood glucose concentration in mice. It was observed that anti-Ly1 antisera treatment of JEV-primed splenic T cells did not abrogate the hypoglycaemic activity, while treatment with anti-Ly2 antisera abrogated it completely (Table 2). Hence, the culture supernatant of $Ly2^+$ splenic T cells collected on day 20 following JEV infection in mice was used as hypoglycaemic factor

Table 1. Cell type producing hypoglycaemic factor during Japanese encephalitis virus (JEV) infection

	Blood glucose (mg/dl)		
Day p.i	Macrophage supernatant	T cell supernatant	B cell supernatant
2	119·2 ± 5·8	117·3 ± 2·2	115·8 ± 2·9
4	118·4 ± 7·1	114·7 ± 2·0	112·6 ± 3·7
6	100·2 ± 2·7	82·8 ± 2·5	108·8 ± 3·2
8	111·7 ± 3·5	51·4 ± 3·2	117·3 ± 4·9
10	114·3 ± 3·4	55·2 ± 4·8	113·8 ± 4·7
12	119·6 ± 5·1	89·4 ± 5·3	119·3 ± 3·3
14	117·5 ± 6·5	117·8 ± 3·9	120·1 ± 3·0
Control	120·1 ± 8·6	130·3 ± 9·3	125·7 ± 6·3

Table 2. Identification of T cell phenotype secreting hypoglycaemic factor

	Plasma glucose (mg/dl)	
Day p.i.	Ly1-treated cell supernatant	Ly2-treated cell supernatant
4	108·6 ± 4·1	110·1 ± 3·1
6	82·7 ± 2·7	114·6 ± 5·1
8	58·4 ± 5·3	108·5 ± 3·9
10	55·7 ± 3·9	105·7 ± 5·0
12	81·0 ± 6·8	111·6 ± 4·6
14	101·5 ± 8·7	109·4 ± 5·4
Control	130·6 ± 8·3	122·0 ± 6·9

throughout the study. The supernatant was concentrated by freeze drying, 100 μg of the crude protein were applied to Superose 12 FPLC column and eluted with 0·1 M PBS pH 7·4. Figure 4 shows the elution profile of different proteins present in the supernatant. Each of the peaks obtained was concentrated and tested for hypoglycaemic activity, which was recovered in peak III. The corresponding fractions were concentrated, and repurified by reversed-phase high performance liquid chromatography (HPLC) on Pep-S column (Pharmacia) using 70% acetonitrile as elution solvent. The purified TCHF was run on SDS–PAGE in 10–15% linear gradient polyacrylamide gels for molecular weight determination. Silver staining of gels revealed that TCHF migrated as a single ~25-kD band on SDS–PAGE (Fig. 5).

Characterization of TCHF

The sensitivity of purified TCHF was screened to proteases at different time periods. The findings presented in Table 3 show that i.v. injection of TCHF in mice incubated for 4 h with trypsin resulted in no change in hypoglycaemic activity, while prolonged incubation with the serine protease caused 98·4% loss in activity, indicating that TCHF is proteinaceous in nature. TCHF was found to be heat-sensitive, as incubation at 37°C for 3 h had no effect on hypoglycaemic activity, whereas exposure to 56°C for 1 h and to 100°C for 15 min resulted in 38·5% and 53·2% reduction in hypoglycaemic response, respectively. There was partial inactivation of its activity at acid and alkaline pH.

Effect of anti-TCHF antisera treatment

The response to TCHF was inhibited by pretreatment of mice with anti-TCHF antibody (plasma glucose 111·9 ± 7·6 mg/dl *versus* TCHF induced-decrease in plasma glucose 57·2 ± 4·7 mg/dl). Normal mouse serum had no effect on lowering in glucose levels mediated by TCHF (mean value 52·8 ± 8·9 mg/dl).

Hormonal study after TCHF inoculation

Glucagon, insulin and growth hormone were measured by RIA on different days after injection of 200 μg of purified TCHF (Fig. 6). Serum glucagon levels rose gradually after injection with peak titres on day 8, and fell sharply by day 14 p.i. Serum insulin and growth hormone levels, however, remained almost the same throughout the study period. This indicates that the changes in circulating concentration of glucagon during JEV infection could be attributed to the secretion of TCHF.

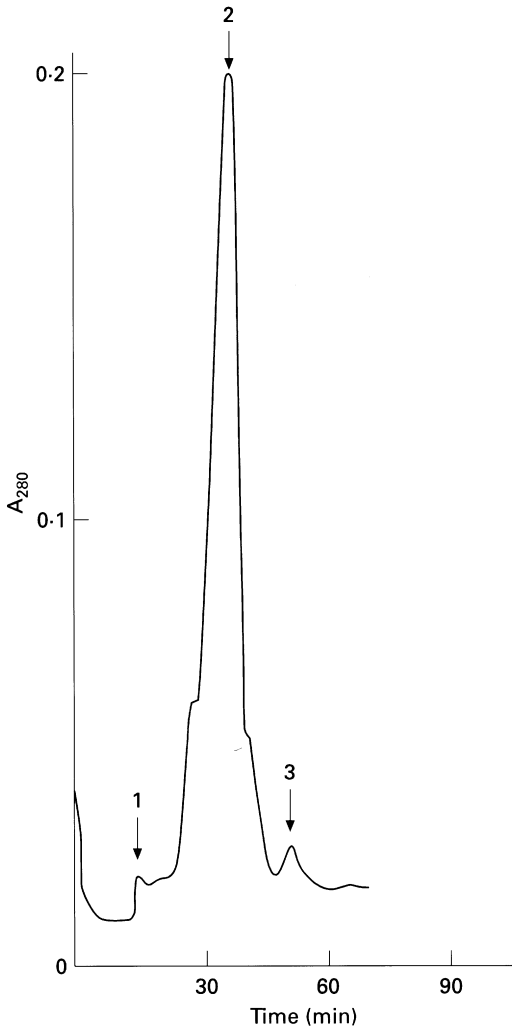

Fig. 4. Superose-12 gel filtration profile of Japanese encephalitis virus (JEV)-primed splenic T lymphocyte supernatant.

DISCUSSION

The main finding of the present study is development of severe hypoglycaemia during the convalescent phase following i.p. inoculation of JEV in mice. The lowering of blood gluocse was attributed to the secretion of a factor by $CD8^+$ JEV-primed T lymphocytes ($Ly1^-2^+$) *in vivo*. We have termed it T cell hypoglycaemic factor (TCHF). Maximum reduction in blood glucose levels in recipient mice was observed between days 8 and 10 post-inoculation, whereas the culture supernatant of normal splenic T lymphocytes did not induce alterations in blood glucose levels in mice. The hypoglycaemia-inducing activity appears to be mediated through a JEV-specific component, as pretreatment of mice with JEV-specific monoclonal antisera abrogated the fall in blood sugar level. These results are in concert with those previously observed in humans during JEV infection [4].

JEV is a mosquito-borne flavivirus. After haematogenous spread, it replicates in various organs, causing short viraemia. Host defence is mediated through a variety of specific [14] and non-specific [17] effector mechanisms. However, these responses decline after 2 weeks with the appearance of suppressor T cells (Ts), which mediate suppression through soluble suppressor factor

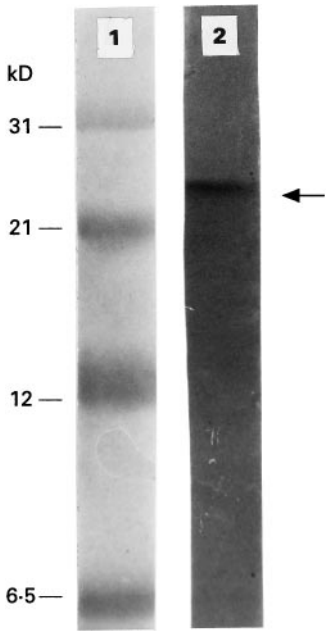

Fig. 5. SDS–PAGE of purified T cell hypoglycaemic factor (TCHF). Lane 1 shows molecular weight markers. Lane 2 shows position of TCHF (arrow).

[18,19]. Further studies have suggested that this immunosuppression leads to evasion of anti-viral immune mechanisms, resulting in persistence of virus in T cells [20]. Our present study shows that time of production of hypoglycaemia following JEV infection coincides with the appearance of suppressor cells.

The TCHF was purified by HPLC gel filtration on Superose 12 column and was found to be thermolabile and partially sensitive to acid and alkaline pH. On the basis of protease sensitivity it appears

Table 3. Biochemical characterization of T cell hypoglycaemic factor (TCHF)

Treatment	Blood glucose (mg/dl)	Reduction in activity (% of control)
Chymotrypsin		
4 h, 37°C	57·7 ± 3·3	2·6
Control	59·2 ± 4·2	—
18 h, 37°C	99·5 ± 2·8	98·4
Control	61·1 ± 5·7	—
Temperature		
37°C, 3 h	68·3 ± 6·0	Nil
56°C, 1 h	89·7 ± 2·5	37·5
100°C, 15 min	99·9 ± 4·4	53·2
−70°C	67·9 ± 5·1	Nil
Control (22°C)	65·2 ± 3·9	—
pH 2·0		
22°C, 3 h	91·6 ± 5·3	42·2
Control	64·4 ± 7·0	—
pH 9·0		
22°C, 3 h	95·7 ± 8·2	54·3
Control	62·0 ± 4·3	—

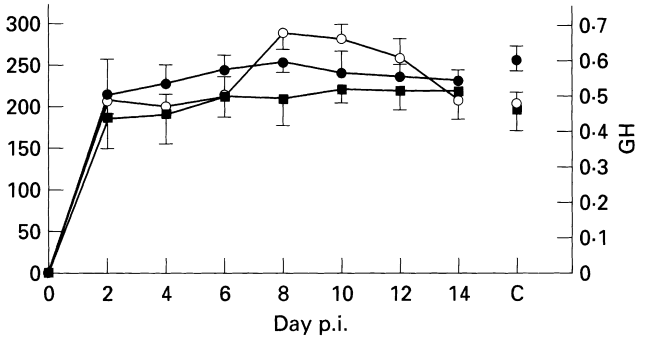

Fig. 6. Blood insulin (■; μU/ml), glucagon (○; pg/ml) and growth hormone (●; ng/ml) levels in T cell hypoglycaemic factor (TCHF)-inoculated and control (C) mice.

to be a polypeptide. The purified TCHF was shown to have an approximate mol. wt of 25 kD. We have not come across any description of release of hypoglycaemic factor during JEV infection.

A frequent feature of most of the viral infections is hyperglycaemia. Mice infected with reovirus [21], encephalomyocarditis virus [22], coxsackie B4 and mumps [23] and scrapie virus [24] show increase in blood sugar levels. However, virus-induced hypoglycaemia is rare. Despite extensive study, the factors responsible for the reduction of blood glucose are unknown. Fulminating hepatitis with recurrent hypoglycaemia has been reported [10]. In lymphocyte choriomeningitis virus (LCMV) infection in mice, perturbation of endocrine function resulting in altered synthesis of growth hormone is reported to be responsible for hypoglycaemia [11]. As a corollary to this, canine distemper virus-induced fall in blood sugar is known to occur due to a reduction in norepinephrine and dopamine concentrations [25]. Alternatively, cytokine released during the immune response could also mediate hypoglycaemia. Rey & Besedovsky [26] have reported that low doses of IL-1 can affect glucose metabolism by increasing blood insulin levels, which may in turn serve to satisfy the enhanced metabolic demands during infection.

Sufficient evidence now exists regarding the role of insulin-like growth factors (IGF)-I and II in the regulation of blood glucose. In general, they are considered to be anabolic hormones, and the changes in glucose metabolism by IGFs could be attributed to decreased hepatic glucose production, or secretion of factors that enhance glucose uptake [27].

Hypoglycaemia may be considered to be a consequence of the catabolic phase of carbohydrate metabolism coupled with secretion of counter regulatory hormones such as insulin, glucagon, epinephrine, cortisol and growth hormone [9]. In the present study we tried to find out whether JEV infection or TCHF results in alteration of circulating levels of hormones, thus contributing towards hypoglycaemia. We found that there occurred a rise in circulating glucagon levels coinciding with the production of hypoglycaemia. No change in growth hormone or insulin concentrations was observed at any time after JEV or TCHF inoculation. No infectious JE virus from the pancreas at any time following infection was isolated, suggesting thereby that a direct effect of the virus on pancreas is unlikely. Thus, this study suggests that hypoglycaemia during JEV infection may be attributed to the production of TCHF, which mimics the effects of JEV. At the present juncture, however, its mechanism is not clear. Studies are

underway to investigate whether TCHF induces a fall in blood glucose by eliciting an over-utilization of glucose in the muscle, or by increasing the hepatic glucose uptake by measuring C^{14}-glucose incorporation in organ explants.

ACKNOWLEDGMENTS

We thank Professor C. G. Agarwal for continued advice and help during this study. The work was supported in part by a grant-in-aid from Council of Scientific and Industrial Research, New Delhi.

REFERENCES

1 Umenai T, Kryzysko R, Betiminov TA, Assad FA. Japanese encephalitis: current worldwide status. Bull WHO 1985; **63:**625–31.
2 Johnson RT, Burke DS, Elwell M, Leake CJ, Nisalak A, Hoke CH, Lorsomrudee W. Japanese encephalitis: immunocytochemical studies of virus antigen and inflammatory cells in fatal cases. Ann Neurol 1985; **18**:567–73.
3 Monath TP. Flaviviruses. In: Fields BN, Knipe DM, Chanock RM *et al*., eds. Fields Virology, 2nd edn. New York: Raven Press Ltd., 1990.
4 Grascenkov NI. Japanese encephalitis in the USSR. Bull WHO 1964; **30**:161–72.
5 Shiraki H. Japanese encephalitis. In: Debre R, Celers J, eds. Clinical virology. Philadelphia: Saunders, 1970:155–75.
6 Bharadwaj M, Prakash V, Mathur A, Chaturvedi UC. Prognostic significance of serum iron levels in cases of Japanese encephalitis. Postgrad Med J 1991; **67**:247–9.
7 Bharadwaj M, Khanna N, Mathur A, Chaturvedi UC. Effect of macrophage-derived factor on hypoferraemia induced by Japanese encephalitis virus in mice. Clin Exp Immunol 1991; **83**:215–8.
8 Mathur A, Khanna N, Kulshreshtha R, Maitra SC, Chaturvedi UC. Viruria during acute Japanese encephalitis virus infection. Int J Exp Pathol 1995; **76**:103–9.
9 Foster DW, Rubenstein AH. Hypoglycemia, insulinoma and other hormone-secreting tumors of the pancreas. In: Braunwald E, Isselbacher KJ, Petersdorf RG, Wilson JD, Martin JB, Fanci AS, eds. Harrison's principles of internal medicine 2, 11th edn. Hamburg: McGraw Hill Book Co., 1987:1800–7.
10 Samson RI, Trey C, Timme AH, Med M, Saunders SJ. Fulminating hepatitis with recurrent hypoglycemia and hemorrhage. Gastroenterology 1967; **53**:293–300.
11 Oldstone MBA, Rodriguez M, Danghaday WH, Lampert PW. Viral perturbation of endocrine function: disordered cell function leads to disturbed homeostasis and disease. Nature 1984; **307**:278–81.
12 Elased K, Playfair JHL. Hypoglycemia and hyperinsulinemia in rodent models of severe malaria infection. Infect Immun 1994; **62**:5157–60.
13 Phillips RE, Looareesuwan S, Molyneux ME, Hatz C, Warrell DA. Hypoglycaemia and counterregulatory hormone responses in severe falciparum malaria: treatment with Sandostatin. Q J Med 1993; **86**:233–40.
14 Mathur A, Arora KL, Chaturvedi UC. Host defence mechanisms against Japanese encephalitis virus infection in mice. J Gen Virol 1983; **64**:805–11.
15 Wysocki LJ, Sato VL. ''Panning'' for lymphocytes: a method for cell selection. Proc Natl Acad Sci USA 1978; **75**:2844–8.
16 Laemmli UK. Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature 1970; **227**:680–5.
17 Khanna N, Srivastav S, Mathur A, Chaturvedi UC. Stimulation of neutrophil respiratory burst and degranulation by Japanese encephalitis virus-induced macrophage derived factor. Int J Exp Pathol 1993; **74**:339–45.
18 Mathur A, Rawat S, Chaturvedi UC. Suppressor T cells for delayed type hypersensitivity to Japanese encephalitis virus. Immunology 1984; **52**:395–402.
19 Mathur A, Arora KL, Rawat S, Chaturvedi UC. Persistence, latency and reactivation of Japanese encephalitis virus infection in mice. J Gen Virol 1986; **67**:381–5.
20 Mathur A, Kulshreshtha R, Chaturvedi UC. Evidence for latency of Japanese encephalitis virus in T lymphocytes. J Gen Virol 1989; **70**:461–5.
21 Onodera T, Tonido A, Ray UR, Jenson AB, Knazek RA, Notkins AL. Virus-induced diabetes mellitus XX. Polyendocrinopathy and autoimmunity. J Exp Med 1981; **153**:1457–73.
22 Kruppenbacher JP, Mertens TH, Muntefering H, Eggers HJ. Encephalomyocarditis virus and diabetes mellitus: studies on virus mutants in susceptible and non-susceptible mice. J Gen Virol 1985; **66**:727–32.
23 Lycke E. Pathogenesis of acute virus infection. In: Lycke E, Norsby E, eds. Textbook of medical virology. London: Butterworths and Co. Ltd., 1983.
24 Carp RI, Kin YS, Callahan SM. Scrapie-induced alterations in glucose tolerance in mice. J Gen Virol 1989; **70**:827–35.
25 Oldstone MBA. Viral alteration of cell function. Sci American 1989; **261**:34–40.
26 Rawat S, Mathur A, Chaturvedi UC. Characterization of Japanese encephalitis virus induced suppressor T cells and their products for delayed type hypersensitivity. Br J Exp Pathol 1986; **67**:571–9.
27 Rey A, Besedovsky H. Interleukin-1 affects glucose homeostasis. Am J Physiol 1987; **253**:794–8.
28 Sara VR, Hall K. Insulin like growth factors and their binding proteins. Physiol Rev 1990; **70**:591–614.