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Variations in Enzyme Activity of Macrophages During Dengue Virus Infection

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Effect of dengue type 2 virus (DV) infection on the activity of various enzymes of peritoneal macrophages (Mϕ) of mice was investigated. Following intracerebral inoculation of DV the activity of alkaline phosphatase, acid and alkaline proteases, aldolase and 5′ nucleotidase was maximum on days 5-6 postinfection, while acid phosphatase was highest on the 3rd day. Majority of the enzymes showed least activity during early or later periods. In intraperitoneally inoculated mice, the maximum enzyme activity was noted during the early phase of infection. Activity of lysozyme was reduced during DV infection, while production of acid and alkaline proteases was induced. The findings of the present study, in conjuction with our earlier reports, may indicate a role of the virus-induced cytokine in the production of these changes.

Key Words: Dengue virus, Macrophages, Enzymes

Introduction

Dengue type 2 virus (DV) replicates mainly in macrophages (Mϕ) and adversely affects its functions. As the infection progresses, total number of Mϕ in the peritoneal cavity of mice reduces; and their phagocytic, migratory and the Fc-receptor-binding functions are markedly depressed. Also depressed is the function of presentation of heterologous antigen to B cells (Gulati et al. 1982, Chaturvedi et al. 1983, Morahan et al. 1985, Rizvi et al. 1987). Mϕ display a remarkable responsiveness to various stimuli and respond by producing secreting a large variety of enzymes and biologically active substances that modulate a wide range of functions. In the present study, an effort is made to investigate the alterations in different enzymes of Mϕ during DV infection of mice.

Materials and Methods

Animals: Inbred Swiss albino mice aged 2-4 months were used in the study.

Virus: Dengue type 2 virus (DV), strain P23085 was used in the form of infected mouse brain suspension (Chaturvedi et al.1977). Mice inoculated with 10^3 LD_{50} of DV intracerebrally (ic) were sacrificed in batches of 6 to 10 mice from day 1 to 10. Similarly mice inoculated intraperitoneally (ip) were sacrificed at 1, 3 and 5 hr and day 1 to 10 after inoculation. The control mice were inoculated with normal mouse brain suspension.

Chemicals: The special chemicals used were bovine serum albumin (BSA), lysozyme, adenosine monophosphatase fructose 1, 6 diphosphate and dihydroxy acetone (Sigma Chemical Co., USA), 4-nitrophenyl phosphate, disodium salt (Sisco Research Laboratories,

India), heparin (Biological E, Limited, India), haemoglobin (Loba-Chemie Indo-austronal Co.), cysteine hydrochloride, tyrosine (E Merck AG, Dasmstadt, West Germany).

Preparation of macrophage monolayers: Mϕ monolayers were prepared from the cells collected by lavage of the mouse peritoneal cavity with 5 ml heparinized Eagle's minimum essential medium containing 5% calf serum (MEM). The aspirated cells were layered on glass Petri dishes and incubated for 2 hr at 37°C in presence of 5% CO_2. Non-adherent cells were removed by washing three times with Hank's balanced salt solution (HBSS). The glass-adherent cell sheet contained more than 95% phagocytic cells, as shown by the ingestion of latex particles (Chaturvedi et al. 1982) and were considered as Mϕ in the present study.

Preparation of macrophage extract: Mϕ cell sheets were scrapped off from the Petri dishes by rubber-tipped policeman rod in unit volume distilled water. After sonication (Ultrasonic Processor, W-380, Heat Systems-Ultrasonics, INC., USA), for 3-5 min the extract was centrifuged in cold at 3000 g for 10 min. The clear supernatant obtained was stored at −20°C for protein and enzyme estimation.

Protein and enzyme assay: The protein content of the Mϕ extract was estimated by the method of Lowry et al. (1951) after precipitation with trichloroacetic acid (TCA). The acid phosphatase (EC 3.1, 3.2) and alkaline phosphatase (EC 3.1, 3.1) were estimated by the method of Leinhardt and Walter (1963) using *p*-nitrophenyl phosphate as substrate. Estimation of acid and alkaline protease was done by the method of Anson (1938). Aldolase (ALD, EC 4.1, 2.7) activity was estimated by the method of Pinto et al. (1969) using fructose, 1, 6 diphosphate as substrate. Assay of lysozyme (EC 3.2, 1.17) activity was carried out according to the procedure of Smolelis and Hortsell (1949) using Micrococcus lysodeikticus as a substrate. 5′ Nucleotidase (EC 3.1, 3.5) activity was estimated by the method of Campbel (1962) using adenosine monophosphate as a substrate.

All the chemicals used were of AR grade and demineralized water was used throughout the study. All the enzyme activities have been expressed in terms of unit in 100 mg of protein. Mean values of the enzyme activities obtained in repeated experiments with ± standard deviation have been presented. The data were analysed using student's *t* test for *p* value. A *p* value of more than 0.05 was considered insignificant.

Results

Effect on acid phosphatase: The findings of acid phosphatase activity in Mϕ collected from the DV ic inoculated mice at different periods are summarized in table 1. The enzyme activity in the Mϕ obtained from control mice was 883 ± 35 units/100 mg protein. After a brief decline on the day 2, the activity showed a sharp rise on the 3rd day, the values being 4650 ± 655 units ($p = 0.001$). The enzyme activity then gradually declined, being 1523 ± 387 units on the day 4 and 557 ± 57 to 621 ± 110 units from the 7th day onwards (table 1). Acid phosphatase activity in Mϕ following DV ip inoculation showed (table 2) a decline from 1 hr to 2 days. The enzyme activity increased on the 3rd and 4th day, followed by an irregular pattern of decline and increase.

Effect on alkaline phosphatase: The alkaline phosphatase activity in control Mϕ was 156 ± 49 units/100 mg protein. It was observed that from the day 2 to 5 after ic inoculation of DV the enzyme activity was not different significantly from that of control (table 1). The increase in the enzyme activity on days 8 to 10 was about three times that of controls, while on days 6 and 7 the increase was 7 to 10 folds (figure 1). The activity of alkaline phosphatase in Mϕ from DV ip inoculated mice was increased at 1 hr, the increase being two folds. This was followed by a sharp decline in the activity at the 5th hr and again on the 4th day when the activity was 40 ± 8 and 48 ± 15 units. A lower activity was observed on the 5th, 9th and 10th day but the difference from controls was insignificant (table 2). At other periods the increase in activity was 2-4 fold (figure 1).

Effect on acid proteases: Control Mϕ had no acid protease activity. In Mϕ obtained from the DV ic inoculated mice the acid protease activity

Table 1 *Enzyme activity in peritoneal macrophages of mice inoculated intracerebrally with DV*

Days after virus inoculation	Enzyme activity/100 mg protein of the macrophages						
	Acid phosphatase	Alkaline phosphatase	Acid protease	Alkaline protease	Lysozyme	Aldolase	5′ Nucleotidase
1	953±127	670±67	12±3	0.6±0.1	840±36	500±75	4500±540
2	490±23	164±26	12±2	0.4±0.2	1090±220	750±77	600±87
3	4650±655	225±31	8±2	0.9±0.3	1330±400	550±71	330±45
4	1523±387	246±39	23±17	0.4±0.1	790±221	463±47	600±62
5	1480±108	263±48	24±16	1.6±0.9	1050±260	1772±138	1500±154
6	918±243	1618±231	28±10	0.7±0.4	316±47	2833±109	5600±680
7	631±178	1139±134	24±6	0.7±0.2	440±29	1750±207	1750±238
8	621±110	462±128	3±1	1.6±0.5	597±86	760±260	1420±184
9	651±219	496±19	2±1	1.3±0.2	167±50	1330±386	1250±109
10	557±57	461±45	2±1	0.9±0.1	730±160	1180±238	2200±348
Controls*	883±35	156±49	0	0	1310±170	1800±198	2660±400

*The value of enzymes was similar at different days in control mice, therefore, for ease of presentation they have been pooled and mean value expressed

Table 2 *Enzyme activity in peritoneal macrophages of mice inoculated intraperitoneally with DV*

Period after virus inoculation	Enzyme activity/100 mg protein of the macrophages				
	Acid phosphatase	Alkaline phosphatase	Acid protease	Alkaline protease	Lysozyme
1 hr	656±172	340±70	6±4	24±9	1000±200
3 "	335±115	184±77	9±4	11±6	1800±100
5 "	304±41	40±8	4±1	3±1	1200±100
1 day	860±110	592±53	0	5±1	1315±166
2 "	477±77	244±49	0	1±1	922±142
3 "	2680±950	195±52	0	3±1	471±170
4 "	3200±640	48±15	0	2±1	816±222
5 "	504±55	143±17	0	0.8±0.1	870±170
6 "	128±28	486±67	0	2±0.5	517±230
7 "	2120±312	313±80	0	14±3	725±95
8 "	3995±255	330±58	0	1±0.2	700±81
9 "	365±73	119±35	0	1±0.6	840±250
10 "	1795±405	150±23	0	1.5±0.7	800±238
Controls*	883±35	156±49	0	0	1310±170

*The value of enzymes was similar at different days in control mice, therefore, for ease of presentation they have been pooled and mean value expressed

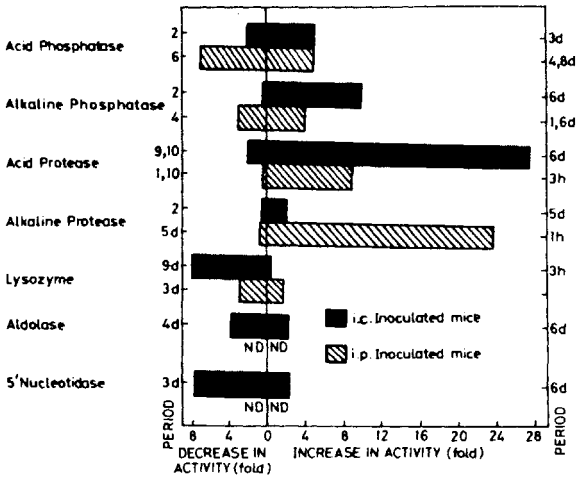

Figure 1 The period of highest and lowest activity of an enzyme as compared to controls. *ND*, not done; *d*, days post infection; hr, hours post-infection

gradually increased from the 1st day and was maximum on the 6th day, the activity being 28 ± 10 units/100 mg protein. The enzyme activity was negligible on 8th to 10th day (table 1). In Mϕ from DV ip inoculated mice, the enzyme activity was 4 ± 1 units/100 mg protein to 9 ± 4 units at 1 to 5 hr period while at later periods, from the day 1 to 10, the enzyme activity was absent (table 2).

Effect on alkaline proteases: Alkaline proteases were absent in Mϕ of control mice. In Mϕ of DV ic inoculated mice, a minimal enzyme activity started appearing from the day 1 being 0.6 ± 0.1 unit/100 mg protein. A maximum value of 1.6 ± 0.9 unit was observed on the 5th day of infection. At other periods the activity was at lower levels (table 1). In contrast, the enzyme activity in Mϕ from DV ip inoculated mice was maximum at the Ist hr (figure 1), being 24 ± 9 units/100 mg protein. Then the enzyme activity gradually decreased. Following a sudden increase in the enzyme activity on the 7th day (14.2 ± 3 units), the values were reduced to 1 to 1.5 units on the 8th to 10th days (table 2).

Effect on lysozyme: The activity of lysozyme in control Mϕ was 1310 ± 170 units/100 mg protein (table 1). Following i c inoculation of DV the enzyme activity was reduced, the decline being more marked from the 6th post-inoculation day onwards. The lowest activity of 167 ± 50 was noted on the 9th day (table 1). A similar pattern was seen in ip inoculated mice, but the decline started earlier, the lowest value being 471 ± 170 on the 3rd day (table 2).

Effect on aldolase: In control Mϕ the aldolase activity was 1800 ± 198 units/100 mg protein. Following ic inoculation of DV the enzyme activity was significantly decreased except between the 5th and 7th day. The lowest value of 463 ± 47 was recorded on the 4th day (table 1 and figure 1).

Effect on 5' nucleotidase: The activity of this enzyme in control Mϕ was 2660 ± 400 units/100 mg protein. In DV ic inoculated mice, the enzyme activity was significantly reduced except on the 1st and 6th post-inoculation day. The lowest activity of 330 ± 45 units was recorded on the 3rd day (figure 1) while the highest activity of 5600 ± 680 units was seen on the 6th day (table 1).

Discussion

The findings show a pattern of changes in the activity of various enzymes of Mϕ during dengue virus infection of mice. The pattern was more clearly demarcated in ic inoculated mice which showed that peak activity of most of the enzymes was present on the 6th day while lower activity was found early (2 to 4 days) or late (9-10 day) during the infection. On the other hand, peak activity of most of the enzymes, studied in ip inoculated mice, was found with in 24 hr of infection; the activities were lower at the later periods (figure 1). In an early study on the enzymes of skeletal muscles mice infected ic with DV, we observed two biochemical stages; the initial phase lasting up to 5 days, is of an increased glycolysis and glycogenolysis, probably caused by metabolic stress due to virus replication; and the later phase could be a metabolic degeneration due to cell injury (Agrawal et al. 1978).

Following ic inoculation in mice DV is present in muscles from the 2nd post-inoculation day (Agrawal et al. 1978) and in Mϕ from the 4th-5th day (Chaturvedi et al. 1983). On ip inoculation DV could be detected in Mϕ from 24 hr (Rizvi et al.

1987). The main cell to replicate DV is Mϕ (reviewed by Chaturvedi 1989). It has been demonstrated that Mϕ uptake DV, process it with the help of serine-like proteases and then present the antigen to B lymphocytes leading to their clonal expansion (Rizvi et al. 1989, 1990). Therefore, outburst of biochemical activity occurs in Mϕ as soon as DV enters it. In ic inoculated mice DV first replicates in the brain tissue and then spreads to the peripheral organs while on ip inoculation the Mϕ are immediately exposed to a large dose of the virus. Since the time period of exposure of Mϕ to DV differs in the two routes of inoculation, therefore, difference in the pattern of enzyme changes is not unexpected. Changes in enzyme pattern have been reported in tissue culture cells exposed to viruses, bacterial toxin and ultraviolet irradiation (Bahuguna & Chaturvedi 1974, Chaturvedi & Bahuguna 1976, Chaturvedi et al. 1975).

Mϕ produce and secrete a large variety of enzymes and biologically active substances which play very important role in immunoregulation and killing of invading microbes etc. Most of the enzymes produced are secreted out, therefore, intracellular levels of activity represent less than 20 per cent of total activity produced. In the present study, therefore, the amount of enzyme activity was lower. Some of these, like lysozyme, are constitutively secreted while production of others are induced (Gordon et al. 1974, Werb et al. 1980, Werb & Chin 1983). It was observed that DV down regulated the lysozyme and 5′ nucleotidase activity but induced production of acid and alkaline proteases. Various enzymes have been shown to be the markers of different subcellular organelles (Shnitka & Seligman 1971) and alterations in them may indicate effect on the injurious agent on them. The findings of the present study may thus, indicate involvement during DV infection of mitochondria (5′ nucleotidase), lysosomes (acid phosphatase) and plasma membrane (alkaline phosphatase) of the Mϕ.

The point which needs comment is the mechanism of the alterations in enzyme contents of Mϕ. This could either be mediated by replication of the virus in Mϕ, or via action of some cytokines on Mϕ. We have observed that 0.2 to 0.8 per cent peritoneal Mϕ are infected with DV when the virus is given by ic route (Chaturvedi et al. 1983) and 10 to 25 per cent by ip route (Rizvi et al. 1987). Thus, greater extent of changes seen in ic inoculated mice in the present study, does not correlate with the virus-infected cells. We have shown production of a number of cytokines in DV infected mice which produce different immunopathological effects (reviewed by Chaturvedi 1989). It is, therefore, likely that the alterations observed in the Mϕ, in the present study, are mediated by a DV-induced cytokine.

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