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IN VIVO HYPORESPONSIVENESS INDUCED BY SENDAI VIRUS IN CFLP MICE

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Summary. — Sendai and Semliki Forest viruses (SFV) raised the interferon (IFN) level in blood and suppressed the acute inflammatory response induced by carrageenan in CFLP mice. After Sendai virus had been inculuted, unresponsiveness developed to repeated challenge either with the same virus or with SFV. The hyporeactive state culminated 48 hr after first virus inoculation. It was characterized (1) by absence of IFN induction and (2) by disappearance of the virus-induced anti-inflammatory effect. In contrast, the anti-inflammatory effect of indomethacin and dexamethasone remained unchanged. In addition, peripheral white blood cells were counted upon Sendai virus inoculation either in normal or in hyporesponsive mice. Six hr after inoculation, Sendai virus induced a marked granulocytosis with lymphopenia. In hyporesponsive mice leukocytosis was observed. Repeated Sendai virus injection was followed by a less pronounced granulocytosis, while the decreased number of mononuclear cells remained unchanged. These alterations in mice inoculated with Sendai virus offers a model of hyporesponsiveness established in vivo.

Key words: Sendai virus; Semliki Forest virus; hyporesponsiveness; interferon production; anti-inflammatory effect; CFLP mice

Introduction

It has been long known that exposure of cultured cells or living organism to an appropriate virus leads to interferon (IFN) induction. IFN may not be produced if the first virus challenge is followed by a second inoculation in vitro (Ho *et al.*, 1965) or in vivo (Younger and Stinebring, 1965). This phenomenon, termed as hyporesponsiveness or hyporeactive state, makes impossible to use repeatedly various IFN inducers in experimental animals or clinical patients.

Presented experiments were undertaken to study the development of an in vivo hyporesponsive state and its characterization by the absence of IFN production, anti-inflammatory effect and appearance of granulocytosis in-

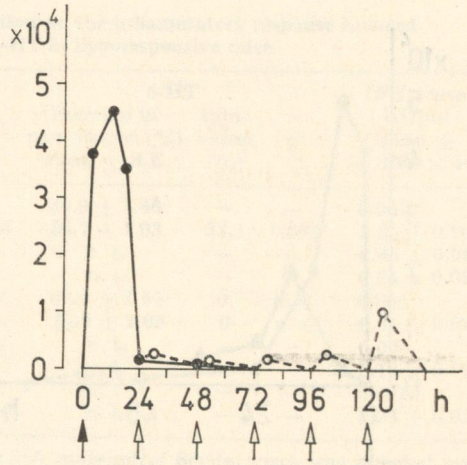

Fig. 1.
IFN induction and hyporesponsiveness after Sendai virus inoculation to CFLP mice
Filled arrow — first intravenous inoculation of 1600 HA units of Sendai virus
Open arrows — second inoculation of Sendai virus.
IFN levels in blood after the first inoculation of virus (●———●); IFN levels after the second inoculation of virus (○·····○).
Control chorioallantoic fluid did not change IFN level in blood. A representative out of 5 experiments.

duced by repeated virus inoculation. Since Sendai virus is a prominent IFN inducer and it is not lethal to mice, its inoculation was considered to appropriate for inducing hyporesponsiveness.

Materials and Methods

Animals. Eight weeks old randomly bred female CFLP mice weighing 27—29 g were fed with commercial food pellet and allowed to drink tap water ad libitum.

Production and standardization of Sendai virus and SFV. Sendai virus was submitted from Dr. K. Cantell (State Serum Institute, Helsinki, Finland); it was inoculated into the chorioallantoic cavity of 10 days old embryonated hen eggs, which were incubated at 38 °C for 72 hr. Haemagglutination (HA) and HA inhibition (HAI) tests were carried out in Linbro or Takátsy microplates using 0.5% suspension of fresh chicken red blood cells in phosphate buffered saline, pH 7.2 (Dulbecco, 1954). Standard virus suspensions containing 4000 or 8000 HA units per ml respectively were injected intravenously in 0.4 ml volume. The chorioallantoic fluid of uninfected eggs served as control.

SFV (Kumba serotype) was obtained from the brain of intraperitoneally infected CFLP mice; the standard suspension contained 5×10^9 PFU in 2 mg homogenized brain tissue prepared in 0.2 ml isotonic NaCl. Normal brain tissue homogenate was used as control. Virus preparations were stored at —70 °C until use.

IFN induction and titration. Mice were inoculated with standard Sendai virus and SFV preparations or their controls and bled at different intervals; pooled sera from 6—10 animals were assayed for IFN in L-929 cell cultures. Vesicular stomatitis virus (Indiana serotype) was used as challenging virus. Titration was performed in Linbro plates using a semi-micromethod (Armstrong, 1971). Readings were made 48 hr after the challenge. Titres were expressed in reference units (RU per ml) as compared to a standard mouse IFN (No. G-002-904-511) kindly supplied by N.I.H., Bethesda, Maryland, U.S.A.

The inflammatory response. Acute inflammatory response was induced in the hind paw with 300 μg carrageenan (Viscarin 402, Marine Colloids, Inc.) or with 0.85 μg serotonin (5-HT) creatinine sulphate (Fluka, F.R.G.; the dose refers to the base). Swelling was measured according to Levy (1969) 3 hrs after carrageenan or 30 min after 5-HT and the results were evaluated as described earlier (Koltai *et al.*, 1981). At the same time blood samples were collected for IFN titration and in some cases for HA test.

Sendai virus, SFV as well as indimethacin (Chinoin, Hungary) and dexamethasone sodium phosphate (N. V. Organon Oss Holland) were used as anti-inflammatory agents in normal and hyporeactive mice. Statistical analysis was made by the unpaired Student's t-test.

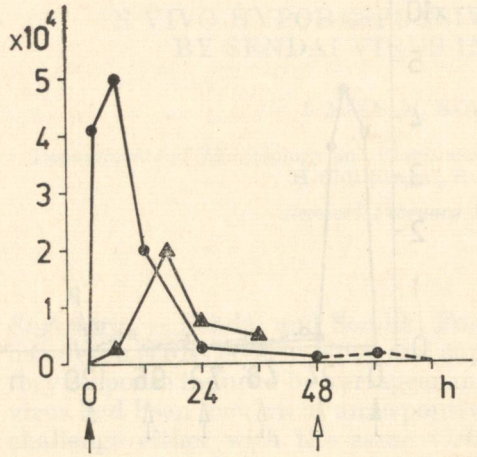

Fig. 2.

Sendai virus induced hyporesponsiveness to SFV

Filled arrow — inoculation of 1600 HA units Sendai virus or of 5×10^9 PFU SFV, respectively

Open arrow — administration of SFV after Sendai virus

IFN levels induced by Sendai virus (●———●); IFN induction by SFV inoculated at 0 hr (▲———▲); IFN production when SFV was given 48 hrs after Sendai virus inoculation (● — — ●). Neither the control chorioallantoic fluid nor the normal brain suspension enhanced the IFN level in blood. A representative out of 3 experiments.

Peripheral white blood cell counts. Leukocytes in blood withdrawn from the tail vessels were determined in a haemocytometer. Differential count of granulocytes and mononuclear cells were performed in smears stained according to Giemsa; in each group 8 mice were examined.

Results

IFN production by Sendai virus and SFV in untreated mice

When 1600 or 3200 HA units of Sendai virus were injected intravenously, the sharp elevation of IFN concentration in blood (Fig. 1 and 2) reached maximum at 6 hr; then a steep drop ensued by time. Very low level was usually found 24 hr after inoculation and practically no IFN was detected 48 hr after administration of the virus. The rise in IFN concentration following intraperitoneal inoculation of SFV was much slower; peak levels were found at 14 hr. On the other hand, IFN induction was more delayed as observed with Sendai virus, thus the two time-response curves crossed each other (Fig. 2).

Hyporesponsiveness produced by Sendai virus as measured by IFN induction

Repeated injections of Sendai virus into CFLP mice markedly reduced their ability to react to IFN induction (Fig. 1). As early as 24 hr after the first inoculation, the second virus injection produced only a negligible enhancement in the blood IFN concentration. Using different groups of mice, the virus was given in 24 hr intervals up to 120 hr. The lowest IFN production was seen 48 hr after the first Sendai virus inoculation. Later on a very slight increase of IFN producing capacity was constantly observed. At the end of the observation period, the reinjection of the virus again caused a marked marked elevation of serum IFN which reached about one-fifth of the initial response. HA tests performed at daily intervals showed no antibodies against Sendai virus.

Table 1. Effect of Sendai virus inoculation on the inflammatory response induced by carrageenan and 5-HT in hyporesponsive mice

Time of Sendai virus inoculation (hr)		Carrageenan			5-HT			IFN titres RU/ml
First[a]	Second[b]	Increase in paw weight (%) mean ± S.E.[c]	Inhibition (%)	p	Increase in paw weight (%) mean ± S.E.	Inhibition (%)	p	mean ± S.E. × 10^4
Control		42.7 ± 1.90	—	—	39.9 ± 1.46	—	—	0.001
1	0	23.8 ± 1.92	44.3	0.005	26.7 ± 1.93	33.1	0.001	3.85 ± 0.20
24	0	35.5 ± 1.78	16.8	n. s.	n. t.	—	—	0.48 ± 0.01
24	1	29.6 ± 1.65	16.7*	n. s.	n. t.	—	—	0.64 ± 0.03
48	0	41.0 ± 1.88	4.0	n. s.	41.4 ± 1.67	0	n. s.	0.005
48	1	42.4 ± 1.98	0	n. s.	41.9 ± 2.05	0	n. s.	0.08 ± 0.02
72	0	39.9 ± 1.66	6.6	n. s.	n. t.	—	—	0.005
72	1	33.3 ± 1.59	16.6	n. s.	n. t.	—	—	0.12 ± 0.02
96	0	41.0 ± 1.68	4.0	n. s.	n. t.	—	—	0.001
96	1	31.7 ± 1.84	22.7	n. s.	n. t.	—	—	0.24 ± 0.01

[a] 0.4 ml chorioallantoic fluid containing 4000 HA units/ml of Sendai virus was injected intravenously at the time indicated. Controls received 0.4 ml of virus-free chorioallantoic fluid.
[b] The same inoculum was given 1 hr before the irritants.
[c] Data obtained from 3 experiments each performed on groups of 15 control and 10 Sendai virus-treated mice.
* These values were compared to those obtained in mice not receiving a second virus inoculation.
n. s. = not significant, n. t. = not tested.

Since the peak appearance of hyporesponsiveness was found at 48 hr after the first Sendai virus inoculation, experiments were also performed with SFV at this time. As indicated in Fig. 2, SFV hardly induced IFN when injected 48 hr after the Sendai virus inoculation.

Anti-inflammatory effect in hyporesponsive mice

The inflammatory response induced by carrageenan or 5-HT was not changed by administration of virus-free chorioallantoic fluid or mouse brain

Table 2. Effect of SFV on the carrageenan-induced inflammatory response in mice with Sendai virus-established hyporesponsivity

Time of inoculation (hr)		Increase in paw weight (%)	Inhibition (%)	p	IFN titres RU/ml
Sendai[a]	SFV[b]	mean ± S. E.[c]			mean ± S. E. × 10^4
Control[a]		41.8 ± 1.84	—	—	0.001
1	0	20.6 ± 1.68	50.8	0.0005	5.00 ± 0.28
0	14	23.8 ± 1.57	43.1	0.005	2.40 ± 0.19
48	0	38.7 ± 1.68	7.4	n. s.	0.08 ± 0.01
48	14	37.2 ± 1.82	3.9*	n. s.	0.08 ± 0.01

[a] 3200 HA units of Sendai virus in 0.4 ml chorioallantoic fluid were inoculated before carrageenen at the time indicated. Controls received 0.4 ml virus-free chorioallantoic fluid.
[b] 5×10^9 plaque forming units of SFV were injected before the phlogogen at the time indicated. Controls received 0.2 ml virus-free mouse brain suspension.
[c] Data obtained from 3 experiments each performed on groups of 12 control and 9—10 virus-treated mice.
* This value was compared to that obtained from mice not given SFV.
n. s. = not significant.

Table 3. Peripheral white blood cell counts in mice injected with Sendai virus

Time of Sendai virus inoculation (hr) First	Second	Number of leukocytes (per μl) mean ± S.E.	Change (%)	Granulocytes per μl mean ± S.E.	Change (%)	Mononuclear cells per μl mean ± S.E.	Change (%)	Mo/Gr** ratio
Control		10 400 ± 404	—	1 703 ± 106	—	8 697 ± 303	—	5.11
6	0	15 668 ± 867	+50.7	9 983 ± 529	+486.2	5 685 ± 328	−34.6	0.57
48	0	26 330 ± 966	—	5 288 ± 404	—	21,045 ± 832	—	3.98
48	54	21,800 ± 757	−17.2*	9,883 ± 695	+86.9	11,917 ± 625	−43.4	1.21

Groups of 8 mice were injected intravenously with 1600 HA units of Sendai virus at the time indicated. The volume of the inoculum was 0.4 ml. Controls received 0.4 ml virus-free chorioallantoic fluid.

* This value was compared with the group in which cells were determined 48 hr after the first virus challenge (hyporeactive mice). Data obtained in hyporeactive animals were not compared to controls.

** Mo = mononuclear cells; Gr = granulocytes.

suspension. The values obtained under these conditions are referred to as controls.

In hyporesponsive mice, carrageenan inflammation was studied during a 96 hr period, whereas the effect of 5-HT was checked only at 48 hr. As shown in Table 1 the inflammatory responses were considerably inhibited by the first Sendai virus application. As far as with carrageenan, 25 hr after the first inoculation there was still a slight but statistically not significant inhibition. When the virus was then reinjected, a further suppression of the paw edema was observed. The inflammatory reactions returned to normal 48 hr after the first inoculation, however, the reinjection of the virus failed to reduce paw swelling, i.e. the anti-inflammatory effect completely disappeared. On the consecutive days, a gradual increase in the inhibition of the carrageenan-induced inflammatory reaction was detected again following the second virus inoculum. The anti-inflammatory effect became statistically significant by the 4th day.

Paw edema induced by carrageenan but not by 5-HT was also remarkably suppressed when tested 14 hr after intraperitoneal SFV injection. At that time, no sighs of virus-induced lesions were seen in the CNS of mice. The inhibition of the carrageenan response by SFV was no longer detectable 48 hr after Sendai virus inoculation (Table 2).

Indomethacin administered orally 1 hr before the irritant in doses 1, 3 and 9 mg/kg, as well as dexamethasone given subcutaneously in doses of 0.125, 0.25 and 0.5 mg/kg immediately before the intrapedal injection of carrageenan, produced a dose-dependent inhibition of the paw edema evoked by carrageenan, and proved to be equipotent in normal and hyporesponsive mice.

Effect of Sendai virus inoculation on the peripheral white blood cells

Six hr after the first Sendai virus injection, the total white blood cell count was enhanced (Table 3). This was due to an extensive granulocytosis. At that

time, a moderate lymphopenia was also observed. When 48 hr elapsed after the intravenous Sendai virus application, a marked leukocytosis was detected. The reinjection of the virus did not cause a further elevation in the total white blood cell count. The number of circulating granulocytes was much less increased as compared to that after the first virus challenge. The decreased mononuclear cell count was comparable with the initial response.

Discussion

The present results indicate that under in vivo conditions, Sendai virus inoculation is followed by a hyporesponsive state during which the reinjection of either the same virus or SFV is found to be incapable of (1) inducing IFN, (2) suppresing acute non-immune inflammation, and (3) producing excessive granulocytosis. The inability of cultured cells to produce IFN upon repeated administration of some inducers has been known from the fundamental studies of Ho *et al.*, (1965). In vivo hyporesponsiveness has been described by Youngner and Stinebring (1965). The anti-inflammatory effect of SFV against carrageenan-induced paw edema has previously been reported (Koltai and Mécs, 1973), while Sendai virus has proved to be able to suppress the inflammatory response produced by carrageenan and 5-HT (Koltai *et al.*, 1981). Degré (1973) has estimated the peripheral white blood cells in mice treated with poly I : poly C. Ne found correlation between IFN induction and granulocytosis. Our results are in accordance with his findings, since Sendai virus also caused an elevation in number of circulating granulocytes with a concominant lymphopenia when the inducer produced peak level of IFN in the blood. Lymphopenia, presumably due to the direct effect of the virus, was still found upon the second challenge 48 hr after the first inoculation, but only a moderate increase in polymorphonuclear cell count was detected.

The hyporeactive state was in part verified by HAI test; no HA antibodies were found in the blood after Sendai virus inoculation during the examination period. The possibility of early appearance of IgM should be also excluded, since Fiebig *et al.* (1979) detected IgM in the blood as early as 3 days after special immunization. In their experiments the IgM level was found to be gradually increased, then remained unchanged for a long time. In our studies with Sendai virus, the peak appearance of hyporesponsiveness was seen at 48 hr after inoculation, and later a modest but continuous recovery in IFN producing capacity was detected. It seems therefore unlikely that a very early IgM response would be responsible for the hyporesponsiveness. The most convincing evidence against this is that 48 hr after Sendai virus injection, the mice failed to respond to SFV having completely different antigenic structure.

It is pertinent to note that hyporesponsiveness was always accompanied by an absence of virus-induced anti-inflammatory effect, while indomethacin and dexamethasone, having different sites of action but both interfering prostaglandin (PG) production (Vane, 1971; Smith and Willis, 1971; Kantorowitz *et al.*, 1975) were still capable of inhibiting carrageenan-induced

inflammatory response. This is in favour of the assumption that these drugs can suppress PG production even in hyporesponsive animals. Stringfellow (1978) has recently reported that PGs administered together with some IFN inducers restore to normal the IFN production of cultured peritoneal cells obtained from hyporesponsive mice. This finding suggests that PGs might have a therapeutic value in hyporesponsive state. It is not clear, however, whether his interesting observation might be related to our findings. The relationship between PG synthesis and hyporesponsiveness remains to be determined by forthcoming studies. In vivo experiments in the field may be facilitated by using our model.

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