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SENDAI VIRUS STIMULATES CHEMILUMINESCENCE IN MOUSE SPLEEN CELLS

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SUMMARY

Sendai virus stimulates chemiluminescence within a few seconds after it is added to a suspension of mouse spleen cells. Virus rendered non infectious by irradiation with ultraviolet light induces a similar burst of chemiluminescence. Heating or pronase treatment of the virus abrogate this reaction, as does sonication of the cells before the addition of the virus. The ability of the virus to stimulate chemiluminescence is correlated with its hemagglutination, neuraminidase, cell fusion and hemolytic properties. It is suggested that Sendai virus-induced chemiluminescence is initiated by the interaction of the virus envelope spike glycoproteins with the cell membrane.

INTRODUCTION

Chemiluminescence has been observed in phagocytic cells stimulated with particles or various soluble agents [reviewed in 1]. More recently, rat thymocytes have been shown to emit light when activated with the mitogenic lectin Concanavalin A or the calcium ionophore A23187[2]. The biochemical reactions leading to CL are not well understood. In phagocytes, a membrane bound NAD(P)H linked dehydrogenase is believed to be activated by the binding of particles to

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Abbreviations: HAU: hemagglutinating unit, EID_{50}: egg infectious $dose_{50}$, CL: chemiluminescence, HANA: hemagglutinin-neuraminidase, HEPES: N-2-hydroxiethylpiperazine-N-2-ethane-sulfonic acid, PBS: phosphate buffered saline.

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the cell membrane. This enzyme forms superoxide anion and hydrogen peroxide, which, together with other highly unstable oxygen species, then react with polyunsaturated fatty acids, carbohydrates, or artificial easily oxidized substrates such as luminol (5-amino-2, 3-dihydro-1, 4-phthalazinedione), inducing electronically-excited states in these molecules. Finally, light is emitted upon relaxation to the ground state[1]. There seems to be a close association between the binding of the stimulating agent to specific receptors on the cell surface and the induction of CL, as shown by experiments with opsonized and histamine-coated particles[3,4]. Most viruses are believed to bind to specific receptors on the cell surface[5,6], but very little is known about biochemical changes in the host cell during this stage of viral replication. The adsorption of viruses to cells has been reported to be associated with alterations of membrane fluidity and potential, monovalent cation flux, and patching/capping of the virus-receptor complex on the cell surface[7-11].

The early interactions of Sendai virus with cells are of particular interest. This virus binds to its cell-surface receptor through a virus envelope spike glycoprotein with hemagglutinating and neuraminidase activities (HANA spike). The viral envelope then fuses with the cell membrane and fusion between neighbouring cells follows. Fusion is known to be dependent on the second envelope spike, formed by the F-glycoprotein [reviewed in 12]. Moreover, the binding of viral hemagglutinin to its receptor on mouse spleen cells[3] has been shown to be sufficient for the induction of interferon in these cells[14]. Both this

observation and the cell-cell fusion demonstrate that the virus profoundly influences the cell long before the events associated with the replication of the viral genome are initiated.

We report here a new observation which further supports this idea: Sendai virus stimulates CL in mouse spleen cells within a few seconds post-infection.

MATERIALS AND METHODS

Virus: Egg-grown Sendai virus was purified from allantoic fluid by pelleting it in a 21 Spinco rotor at 18,000 RPM for 110 minutes after previous clearing of the allantoic fluid at 4000xg for 10 minutes. Resuspended pellets were layered onto a 20-60% sucrose gradient, 10mM Hepes pH 7.6, and spun at 24,000 RPM for 60 minutes in a SW27 rotor. The resulting virus band was collected, diluted with PBS, pelleted, resuspended in PBS and stored at -70°C.

Cells: Spleens from 5-12 week old C57Bl/6 mice were teased on a steel mesh immersed in chilled F15 tissue culture medium containing 10% fetal calf serum. After removal of adherent cells using the carbonyl iron method [15] and separation of dead and red cells from viable cells on a Ficoll-Hypaque cushion [16], $2x10^7$ cells in 2.5 ml F15 medium containing 1% FCS were suspended in sterile glass scintillation vials. No sodium azide was used in the separation of dead from viable cells because it interfered with CL.

RESULTS

Figures 1a and b show CL induced by various concentrations of Sendai virus. Light emission increased within seconds after addition of virus, reaching a peak after 4-8 minutes and declining thereafter. CL depended on the dose of virus and seemed to be very responsive to low doses of virus, since 3HAU/10^6 cells induced a two-fold increase in CL. Virus in the absence of cells did not increase background CL (not shown) and sonication of the cells before addition of virus completely abrogated the increase in CL (Fig. 2), thus establishing that CL was

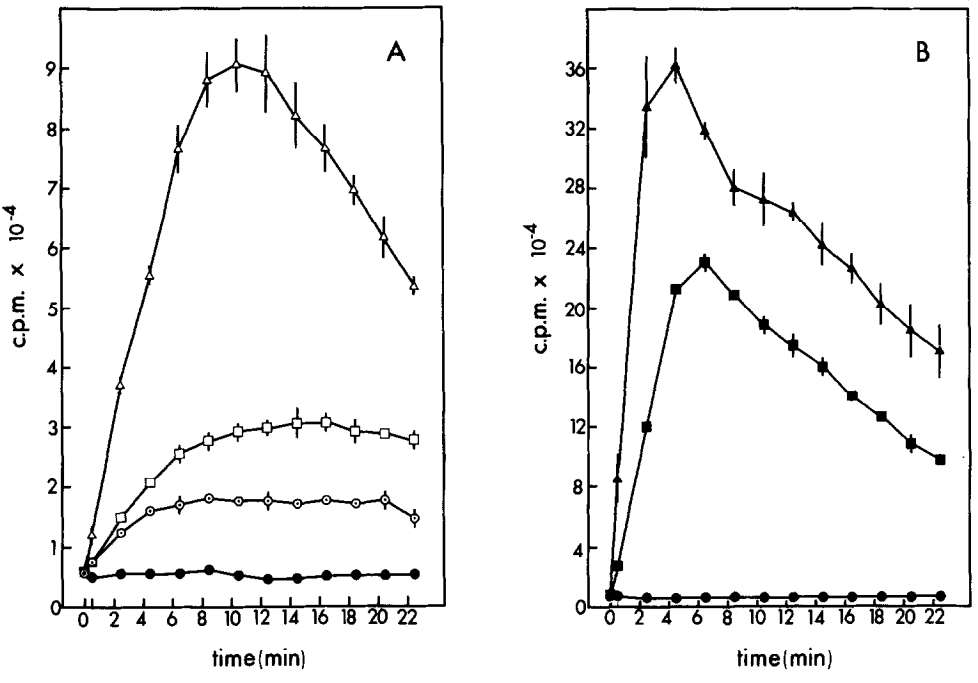

Fig.1 Effect of virus dose on chemiluminescence

Non-adherent spleen cells suspended in scintillation vials were adapted to the dark at 37°C for 45 minutes. 50µl of 4% bovine serum albumin saturated with luminol was then added to each vial, followed by Sendai virus 8 minutes later. Chemiluminescence was determined for 0.1 min periods at two-minute intervals starting 30 seconds after the addition of virus. The Packard model 3320 liquid scintillation spectrometer was operated at room temperature in the out-of-coincidence mode [1]. Discriminator setting was 50-1000 and gain 50%. Between countings, cells were incubated at 37°C in a water bath. Each point represents the mean of 3 cultures. Bars indicate S.D.

Control (PBS added)
3 HAU/10^6 cells
9 HAU/10^6 cells
27 HAU/10^6 cells
81 HAU/10^6 cells
243 HAU/10^6 cells

generated by a cell-virus interaction and depended on the intact structure of the cells. Peak levels of CL stimulated by a given dose of virus showed considerable variation between different cell batches; e.g. 85 HAU/10^6 cells used in experiments 2 and 3 (Figures 2 and 3) were as effective as 243 HAU in experiment 1 (Figure 1b).

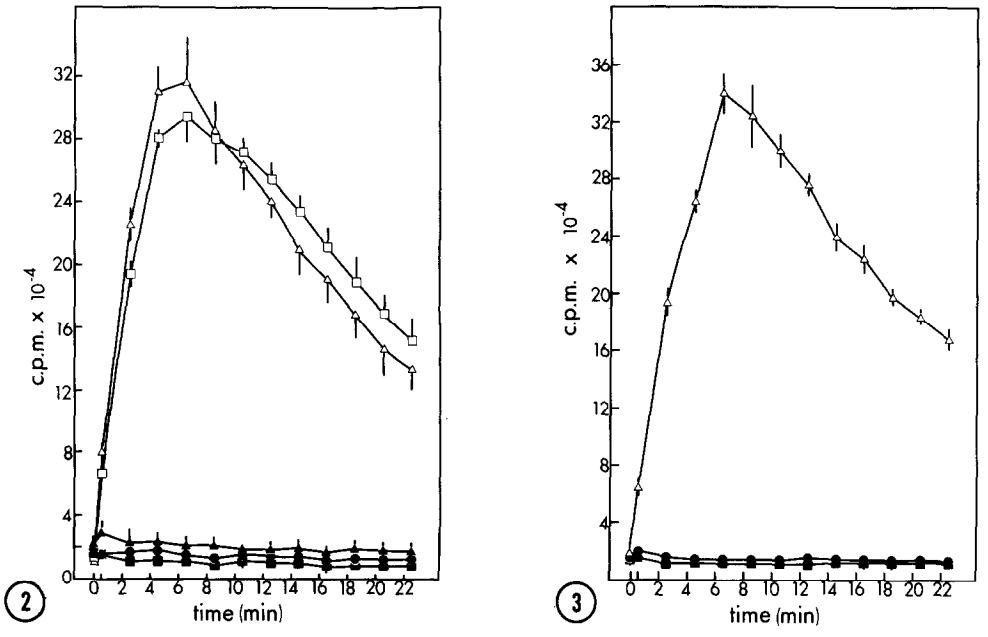

Fig.2 Effect of infectivity of the virus and viability of the cells on CL

Sendai virus was inactivated by irradiation with a UV lamp at a distance of 18cm for 7 minutes (27erg mm^{-2} sec^{-1}) or by heating, at 56°C, for 30 minutes. Equal amounts (85 HAU/10^6 cells) of untreated and treated virus, respectively, were added to the cells and CL measured as described in the legend to Figure 1.

For sonication of the cells, a Branson sonifier B-12 fitted with a microtip was used (40W/60 sec).

Samples were run in triplicate and results given as mean ± S.D.

- untreated Sendai virus
- untreated Sendai virus in sonicated cells
- UV-irradiated Sendai virus
- Heat inactivated Sendai virus
- uninfected control (PBS)

Fig.3 Effect of pronase treatment of virus on CL

Sendai virus ($2x10^5$ HAU ml^{-1}) was incubated with 1mg ml^{-1} pronase (B Grade, Calbiochem) at 37°C for 30 minutes. Control virus was incubated in the absence of enzyme. Virus was then diluted with ice-cold PBS and pelleted at 80,000xg for 30 minutes. The pellets were resuspended to the initial volume of the virus sample and cells infected with equal amounts of treated/untreated virus (85 HAU/10^6 cells). Samples were run in triplicate and results given as mean ± S.D.

- untreated virus
- Pronase-treated virus
- uninfected control

Table 1

Biological activity of Sendai virus

Virus	Infectivity EID_{50}/0.1ml	Hemagglutination HAU/0.1ml	neuraminidase activity OD_{549}/0.1ml	Hemolysis OD_{575}/0.1ml	Cell fusion %
Control	1.59×10^9	5120	21.70	13.70	31
UV-irradiated	<60	5120	20.90	13.20	29
Heat inactivated	<60	<20	0.28	0.00	ND
Pronase treated	<60	<20	0.00	0.00	ND

This table summarizes the biological activities of the various virus preparations used in the chemiluminescence experiments. Infectivity of the virus was determined by injection of 0.1 ml of virus suspension into 11 day old eggs and detection of hemagglutinin in the allantoic fluid 48 hours later.

Hemagglutinin was titrated by doubling dilutions in 200μl in plastic trays and was detected by the addition of 20μl of 5% chicken red cells. The highest dilution showing agglutination was taken as 1 hemagglutinating unit (HAU).

Neuraminidase activity was assayed by incubation of 512 HAU of untreated and UV-irradiated or equal volume of heat and pronase-treated virus, respectively, with Fetuin (4 mg/ml,pH 5.9) for 30 minutes 37° C. N-Acetyl neuraminic acid released was estimated according to Aminoff [22]. Cell fusing activity was screenedby cell counting after incubation of HeLa monolayer cell cultures with 512 HAU/ml for 3 hours at 37° C and is expressed as percent reduction in total cell number compared to untreated controls. Hemolysis was estimated using chicken red cells and 512 HAU of virus [23]. Note that all activities are expressed per 0.1 ml of virus suspension, except for cell fusion which refers to 512 HAU or equal volume of non-hemagglutinating virus suspension.

Abbreviations used: % :percent reduction in cell number
ND :not detected

We next investigated whether the infectivity of the virus was required for CL induction. Virus was inactivated using two different methods: UV-irradiation, which damages the RNA of the virus, and heating, which destroys infectivity by denaturing viral proteins.

Figure 2 shows that UV-irradiated virus still increased CL whilst heat-inactivated virus was ineffective. This suggested that structural components of the virus rather than the genome itself were responsible for the stimulation of light emission. While UV-irradiation of the virus did not significantly alter its haemagglutination, neuraminidase, cell fusion and hemolytic properties, heat inactivation did cause a loss of all these properties that are mediated by the envelope spikes (Table 1).

To obtain further evidence for a role of the spike proteins in CL induction, a virus suspension was incubated with pronase which strips off the spikes, leaving the envelope nucleocapsid intact [17]. Pronase-treated virus no longer stimulated light emission by spleen cells (Fig. 3) and also had lost all other spike activities (Table 1).

DISCUSSION

The observations reported in this paper show, for the first time, that a virus is able to stimulate CL in animal cells.

With respect to the virus, the most obvious feature connected with CL is the presence of biologically active spike proteins on the virus envelope. The experiments reported here do not establish whether the two envelope proteins must both be present for the stimulation of CL. However, the virus must bind to the cell before the fusion

of the envelope with the cell membrane can proceed. This suggests that the initial reaction leading to the generation of CL is the binding of the HANA protein to its receptor on the cell surface. Using purified envelope spikes, we are currently investigating the individual contributions of each protein to CL induction.

Of numerous cell types tested, we found virus-induced CL only in cells of the immune system: mouse peritoneal macrophages, spleen cells, lymph node cells, and thymocytes. Carbonyl iron treatment reduced the proportion of phagocytic cells in the spleen cell population from 6-8% to less than 1%, as estimated by staining for Esterase, [18], while reducing CL by only 5-15%. This suggests that the lymphocytes, rather than the 'classical' chemiluminescent cells (Macrophages, neutrophilic Granulocytes [1]) are the main source of CL in the non-adherent spleen cell populations used in our experiments. In this respect, it is of interest that a number of mitogenic lectins (lentil lectin, Concanavalin A, Phytohemagglutinin, Lipopolysaccharide) also stimulate CL in mouse spleen cells (E.P., unpublished). Concanavalin A-induced CL has been shown to be an early event in the activation of rat thymocytes [2]. Some Myxo- and Paramyxoviruses cause alterations in immune response, in particular, they inhibit the mitogenic activation of lymphocytes by lectins [19,20], or are mitogens themselves [21]. This makes it necessary to investigate whether virus-induced CL plays a role in these phenomena.

As a first step, we are defining the subpopulations of cells which chemiluminesce in our non-adherent spleen cell

preparation. Together with the elucidation of the biochemical reactions responsible for it, this is essential for understanding what CL means, functionally, both for the virus and the immune system.

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