BIKEN JOURNAL Vol. 29, 51–55, 1986

SHORT COMMUNICATION

ANTIBODY-MEDIATED ENHANCEMENT OF INFECTION BY DENGUE VIRUS OF THE P815 MURINE MASTOCYTOMA CELL LINE

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(Received December 10, 1985)

SUMMARY Dengue type 2 virus (DEN 2) could replicate only to a limited extent in a murine mastocytoma cell line, P815. The viral multiplication was enhanced 10- to 100-fold by mouse anti-DEN 2 antiserum or anti-DEN 2 type-specific monoclonal antibody diluted beyond their neutralizing titers. Cells incubated with virus-antibody mixtures changed morphologically, developing a mature mast cell-like appearance, 4–5 days after infection. The indirect fluorescent antibody technique showed that the enhancement of infection was caused by an increase in the number of DEN 2-infected cells. This is the first report that cells of mast cell lineage support dengue virus multiplication, and that virus production is enhanced in the presence of anti-dengue antibodies.

Dengue viruses have a predilection for lymphoid and myeloid tissues including monocytes, macrophages and histiocytes. The virus permissiveness of mononuclear phagocytes in monkeys and humans is reported to be enhanced in the presence of subneutralizing titers of anti-dengue antibodies (Brandt et al., 1982; Daughaday et al., 1981; Halstead and O'Rourke, 1977; Halstead et al., 1977; Hotta et al., 1984b). The enhanced infection was due to an increase in the number of infected cells with Fc receptors on their surface. The Fc receptors apparently acted as entrance points for the virus after forming immune complexes with antibodies. Experimental models in mice have also been used to study enhancement of infection, and have demonstrated that peritoneal macrophages from dengue type 1 virus-immune mice show greater permissiveness than those from non-immune control mice to dengue type 2 virus (Hotta and Hotta, 1982). Enhanced permissiveness of mouse macrophage cell lines to dengue virus was also observed in the pre-

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sence of anti-dengue antibodies (Halstead et al., 1983; Hotta et al., 1984a).

Clinical and laboratory studies have suggested that mast cells may be involved in antibody- and cell-mediated responese to dengue virus infection in man (George and Duraisamy, 1981; Kuberski et al., 1977; Pavri et al., 1979). However, it is still unknown whether mast cells act as a target of dengue virus replication. In the present study we investigated the multiplication of dengue virus and the infection-enhancing effect of anti-dengue antibodies in cultures of a murine mastocytoma cell line, P815.

P815 cells (Dunn and Potter, 1957), kindly donated by Dr. Yasuhiro Hosaka (Department of Preventive Medicine, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan), were cultured in Eagle's minimum essential medium supplemented with 10% fetal calf serum without antibiotics. Dengue type 2 virus (DEN 2; New Guinea C strain) was prepared as the supernatant of a 10% infected suckling mouse brain homogenate. Anti-DEN 2 antiserum was raised in eight-week-old male ICR mice by subcutaneous injection of DEN 2 mixed with 1 mg of aluminum hydroxide and subsequent booster injections on days 14 and 28. Control mouse serum was prepared in the same way except for use of uninfected suckling mouse brain homogenates. Monoclonal anti-DEN 2 antibody was a product of a hybridoma cell line (3H5), and was characterized as a type-specific neutralizing antibody and identified as murine IgG1 (Gentry et al., 1982; Henchal et al., 1982). The 50% plaque-reduction neutralizing titers ($PRNT_{50}$) of the antibody preparations were 1: 256 for the mouse antiserum and 1: 1,024 for the monoclonal IgG1. DEN 2 was inoculated into P815 cell cultures at a multiplicity of infection (M.O.I.) of 1 plaque-forming unit (PFU) per cell, in the presence or absence of antibodies, which were diluted 1: 100 and 1: 1,000 beyond their $PRNT_{50}$. After virus adsorption at 35 C for 90 min, the cells were washed with Hanks' balanced salt solution to remove unadsorbed virus, and the complete medium containing the subneutralizing antibodies was added to the cultures. Portions of the culture fluids were taken every day after infection, and the samples were titrated for virus infectivity.

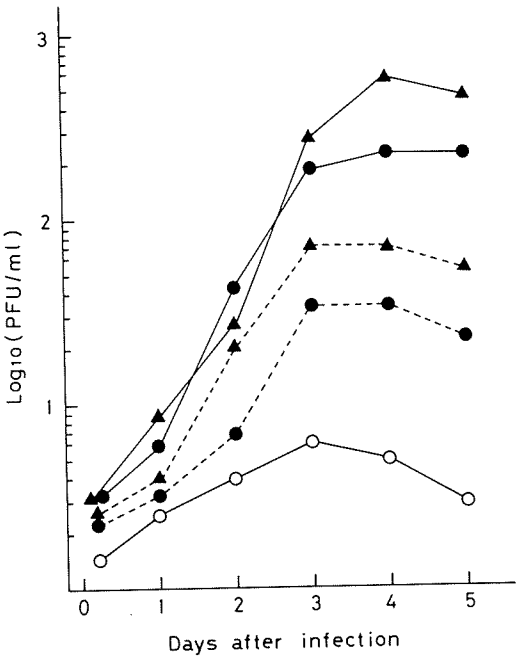

FIGURE 1. Multiplication of DEN 2 in the P815 mastocytoma cell line. DEN 2 was inoculated into cultures of P815 cells in the presence of mouse antiserum diluted 100-fold (▲----▲) and 1,000-fold (▲——▲) beyond its $PRNT_{50}$, or a monoclonal anti-DEN 2 antibody diluted 100-fold (●----●) and 1,000-fold (●——●) beyond its $PRNT_{50}$. Open circles (○——○) represent viral growth in the presence of a 1,000-fold dilution of control serum, which was obtained from mice immunized with uninfected suckling mouse brain preparations.

DEN 2 antigen in infected P815 cells was examined by the fluorescent antibody (FA) technique as described previously (Hotta and Hotta, 1982; Hotta et al., 1984a). When required, the cells were stained with Giemsa and with toluidine blue, and observed under

TABLE 1. *Morphological features of DEN 2-infected P815 cells.*

Inoculum[a]	No. of vacuoles per cell[b]	No. of granules per cell[b]	% FA-positive cells[c]
Virus alone	5.0±0.3	16.0±3.0	10.0±2.3
Virus plus antibody	17.0±2.0	193.0±5.1	36.0±3.7

[a] DEN 2 was inoculated onto P815 cells at an M.O.I. of 1 in the absence (virus alone) or presence (virus plus antibody) of mouse anti-DEN 2 antiserum diluted 1 : 1,000 from its $PRNT_{50}$.
[b] Mean value ± standard deviation for 300 cells.
[c] Mean value ± standard deviation for three different preparations.

a light microscope.

P815 cells supported DEN 2 multiplication to some extent even in the absence of the antibodies and dose-dependent virus production was observed in the range of 0.01 to 1 M.O.I. (data not shown). Viral production in the cultures was enhanced 10- or 100-fold in the presence of subneutralizing concentrations of mouse anti-DEN 2 antiserum (diluted 1: 100 or 1: 1,000 from its $PRNT_{50}$, respectively) (Fig. 1). The monoclonal neutralizing antibody caused the same extent of enhancement of infection as the polyclonal antiserum (Fig. 1).

At 5 days after infection, the number of vacuoles in the infected P815 cells had increased almost threefold and the number of metachromatic granules had increased 100-fold in the presence of subneutralizing concentrations of anti-DEN 2 antiserum (Table 1). Cell viability decreased slowly to 60% of that of the uninfected cell cultures. By phase contrast microscopy, the cells infected with virus plus antibody were seen to be large mononuclear cells with spreading cytoplasmic processes and increased cytoplasmic granulation (data not shown). These changes were not observed in cells infected with virus alone. On FA staining of DEN 2 antigen, positive fluorescence was seen in the cytoplasm but not the nucleus. The percentage of FA-positive cells was 36% in the presence of the enhancing antibody and 10% in cells infected with virus alone on day 4 after infection (Table 1).

The secretion of chemical mediators from mast cells and the role of these products in immediate and delayed type hypersensitivity have been studied extensively (Askenase, 1977; Austen et al., 1976) but there have been few studies on the role of mast cells as a host of microbial replication (Padawer, 1971). Previous studies on cell differentiation indicated that mast cells are derived from mononuclear phagocytes (Czarnetzki et al., 1979; 1981; 1982; 1983). Thus mast cells or their precursor cells may act as the target for dengue virus infection, as do mononuclear phagocytes (Brandt et al., 1979; 1982; Daughaday et al., 1981; Halstead and O'Rourke, 1977; Halstead et al., 1977; Hotta and Hotta, 1982; Hotta et al., 1984a; 1984b). The present study showed that DEN 2 replicated in the P815 mastocytoma cell line to a limited extent. A more interesting finding was that 10- to 100-fold enhancement of viral production was observed when P815 cells were infected with DEN 2 in the presence of subneutralizing concentrations of mouse anti-DEN 2 antiserum or a monoclonal anti-DEN 2 antibody (IgG1). The enhancement of infection is probably mediated by anti-DEN 2 IgG antibody through the interaction of virus-antibody complexes with IgG receptors on the surface of mast cells (Ovary, 1971). Although P815 cells show considerable dedifferentiation, as evidenced by marked reduction in the histamine content and cellular metachromasia, we consider that they are useful for studies on enhancement of infec-

tion in cells of mast cell lineage, since the immunoglobulin receptors on their surface appear to have specificity for all classes of mouse IgG molecules and show many similarities to those of macrophages and normal mast cells (Cline and Warner, 1972; Ovary, 1971).

P815 cells infected with DEN 2 in the presence of anti-DEN 2 antiserum underwent morphological alterations, i.e., they spread on plastic surfaces and developed the appearance of mature mast cells. In this connection, it is interesting that precursors of mast cells from *Schistosoma mansoni*-immune animals were found to show a tendency to differentiate when they were cultured in the presence of the same helminth antigen (Ishizaka et al., 1977), and also that a mast cell growth factor present in antiserum preparations could promote differentiation of precursor cells (Tertian et al., 1981; Yung et al., 1981). Whether the morphological alterations shown by DEN 2-infected P815 cells were due to differentiation of the cells during the course of virus infection or merely to cytopathic effects remains to be clarified.

ACKNOWLEDGMENT

One of the authors (L.F.S.L.) was recipient of a scholarship from the Japanese Government (Monbusho) from April 1979 to March 1984.

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