Production of Interferon Alpha by Dengue Virus-infected Human Monocytes

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SUMMARY

Human monocytes appear to be very important in the pathogenesis of dengue infection. They are thought to be the most active sites of virus replication during dengue infection. We have analysed interferon (IFN) production by dengue virus from peripheral blood mononuclear cells (PBMC). IFN activity was first detected at 12 h after infection of monocytes and reached a maximum level by 48 h. Non-adherent PBMC depleted of monocytes did not produce detectable levels of IFN, and did not contain dengue antigen-positive cells after exposure to dengue virus. The IFN produced was characterized as IFN-α by neutralization tests using specific antisera to HuIFN-α, HuIFN-β and HuIFN-γ, and by radioimmunoassay. The culture fluids of dengue virus-infected monocytes, which contained IFN-α, were able to inhibit infection of human monocytes by dengue virus. These results suggest that IFN-α produced by dengue virus-infected monocytes may play an important role in controlling primary dengue virus infection.

Dengue virus infection is a major cause of morbidity especially in tropical and subtropical areas (Halstead, 1980 a). Secondary dengue virus infections with a different serotype from the virus which caused the primary infection is more commonly associated with severe complications, haemorrhagic fever and shock; however, primary dengue infections are self-limited and almost all patients recover without severe complications (Halstead, 1980 b). These observations suggest that host immune responses are effective in controlling primary dengue virus infections.

We reported earlier that dengue virus-infected cells are lysed by lymphocytes of non-immune donors to a greater degree than uninfected cells (Kurane et al., 1984). Natural killing of dengue virus-infected cells may be one of the immune responses which is responsible for controlling primary dengue virus infections. Another host defence mechanism which should be considered is interferon (IFN). It has been reported that IFN has an important role in controlling viral infections (Gresser et al., 1976); however, the role of IFN in dengue virus infection has not been analysed. We recently reported that non-immune peripheral blood lymphocytes produce high titres of IFN-α when they contact dengue virus-infected monocytes (Kurane & Ennis, 1987). In this report, we investigate IFN production by dengue virus-infected monocytes, because monocytes are the cells which predominantly support dengue virus infection.

Human peripheral blood specimens were obtained from healthy blood donors from Massachusetts who did not have antibodies to dengue virus as determined in a plaque reduction neutralization test. Peripheral blood mononuclear cells (PBMC) were separated by the Ficoll-Hypaque density gradient centrifugation method. Adherent cells were isolated from PBMC by incubating $2 \times 10^7$ PBMC in 4 ml of RPMI/10% foetal calf serum (FCS) in a plastic Petri dish with a 6 cm diameter (Falcon) at 37 °C for 20 h. The dish was rinsed several times with RPMI/10% FCS until no non-adherent cells were observed under an inverted microscope. These adherent cells were considered to be monocytes.
Table 1. Production of IFN by dengue virus-infected monocytes

<table>
<thead>
<tr>
<th>Donor</th>
<th>IFN (U/ml)</th>
<th>% Dengue antigen-positive cells</th>
<th>IFN (U/ml)</th>
<th>% Dengue antigen-positive cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>400</td>
<td>52</td>
<td>&lt;6</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>100</td>
<td>22</td>
<td>&lt;6</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>400</td>
<td>50</td>
<td>&lt;6</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>100</td>
<td>30</td>
<td>&lt;6</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>150</td>
<td>42</td>
<td>&lt;6</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>600</td>
<td>84</td>
<td>&lt;6</td>
<td>0</td>
</tr>
<tr>
<td>H</td>
<td>200</td>
<td>32</td>
<td>&lt;6</td>
<td>0</td>
</tr>
</tbody>
</table>

* Monocytes were infected with dengue virus at an m.o.i. of 10 and cultured at a concentration of $1 \times 10^6$/ml for 48 h. Culture fluids were collected and examined for IFN activity in bioassays. The percentage of dengue antigen-positive cells was determined by indirect immunofluorescence.

Dengue virus type 2 (New Guinea C strain) was used for infection of monocytes. Virus was kindly supplied by Dr Walter E. Brandt of Walter Reed Army Institute of Research. Dengue virus ($5 \times 10^7$ p.f.u./ml) was incubated with $1:10000$ diluted anti-dengue virus type 2 serum at 4°C for 1 h. Monocytes were infected with dengue virus at an m.o.i. of 5 to 10 p.f.u./cell at 37°C for 2 h. Infected cells were washed once and cultured at the concentration of $1 \times 10^6$ cells/ml in RPMI/10% FCS for 48 h unless stated. Fixation of dengue virus-infected monocytes with paraformaldehyde was performed as described previously (Kurane & Ennis, 1987). Cells were washed three times with phosphate-buffered saline (PBS), treated with 3-5% paraformaldehyde in PBS, and washed three times with PBS at 4°C.

Intracellular dengue antigens were detected by immunofluorescent staining as reported (Kurane et al., 1984). Cells were air-dried on glass slides and fixed with ethanol at $-20$°C for 10 min. The fixed cells were reacted with mouse antisera to dengue virus type 2 and fluorescein isothiocyanate-conjugated anti-mouse IgG (Cappel Laboratories).

IFN was assessed by a c.p.e. reduction assay (bioassay) (Ennis & Meager, 1981). Twofold serial dilutions of culture fluids obtained from dengue virus-infected monocytes were incubated on human fibroblast cells (trisomic for chromosome 21) for 20 h at 37°C. They were then challenged with vesicular stomatitis virus. An international IFN standard was included in each assay, and the titres were read after 24 h. The IFN content of culture fluids was also measured in radioimmunoassays (RIA) specific for IFN-α and IFN-γ as previously described (Kurane et al., 1986). RIAs were calibrated using relevant international standards for HuIFN-α and HuIFN-γ. Neutralization of antiviral activity was performed using specific antisera to IFN-α, IFN-β and IFN-γ. Antisera to IFN-α and IFN-β were purchased from Enzo Biochemicals (New York, N.Y., U.S.A.). Antiserum to IFN-γ was purchased from Interferon Sciences (New Brunswick, N.J., U.S.A.). Interferon samples were incubated with a 10- to 50-fold excess of antiserum to each type for 1 h at 37°C and residual IFN activity was titrated.

PBMC of non-immune donors were infected with dengue virus, and culture fluids were examined for IFN activity. IFN activity at titres of 40 to 150 units (U)/ml was detected in the culture fluids of dengue virus-infected PBMC, but no IFN activity was detected in samples from uninfected PBMC. Experiments were carried out to determine whether IFN-producing cells were contained in the adherent cell fraction, considered to be monocytes, or in non-adherent fractions. High titres of IFN were detected in the culture fluids of dengue virus-infected monocytes, but not in culture fluids of infected non-adherent cells. Dengue antigen-positive cells were detected only in the dengue virus-infected adherent fractions. Non-adherent cells which were exposed to dengue virus in the same way were not dengue antigen-positive (data not presented). When monocytes of seven donors were infected and cultured, IFN activity at titres of 100 to 600 U/ml was detected in the culture fluids of dengue virus-infected monocytes from all donors. No IFN activity was detected in the culture fluid of uninfected monocytes (Table 1). The time course of IFN production and the appearance of dengue antigens in the cytoplasm of infected
monocytes was studied. IFN activity was first detected at 12 h after infection and reached a maximum level by 48 h. Cytoplasmic dengue antigens were first detected at 6 h and the percentage of antigen-positive cells reached a maximum level by 24 h after infection (Fig. 1). These results demonstrate a rising titre of IFN production shortly after the appearance of cytoplasmic dengue antigens.

To examine the possibility that dengue virus-infected monocytes induced IFN from uninfected monocytes, infected monocytes were fixed with paraformaldehyde and cultured with uninfected monocytes. Paraformaldehyde-fixed, dengue virus-infected monocytes did not produce any IFN or infectious dengue virus. Although paraformaldehyde-fixed, dengue virus-infected monocytes were able to induce 150 U/ml of IFN from non-adherent cells (Kurane & Ennis, 1987), they did not induce IFN from uninfected monocytes. Uninfected monocytes treated with paraformaldehyde did not induce IFN from uninfected monocytes or non-adherent cells. These results indicate that the IFN detected in the culture fluids of dengue virus-infected monocytes was produced by infected monocytes themselves, and was not induced from uninfected monocytes by dengue virus-infected monocytes.

The IFN produced was characterized as IFN-α, because antiserum to HuIFN-α, but not to HuIFN-β or HuIFN-γ, neutralized all the IFN activity detected. This result was confirmed by RIA using monoclonal antibodies to HuIFN-α and HuIFN-γ.

To determine whether the IFN-α produced by dengue virus-infected monocytes could inhibit dengue virus infection of other human monocytes, monocytes were incubated for 20 h in culture fluid obtained from dengue virus-infected monocyte cultures which contained 400 U/ml of IFN-α, and then they were infected with dengue virus. None of the monocytes treated with culture fluids of dengue virus-infected monocytes contained dengue viral antigens 24 h after infection; however, 50% of untreated monocytes and monocytes treated with culture fluids from uninfected monocytes contained dengue viral antigens (Table 2). This result indicates that IFN-α produced by dengue virus-infected monocytes inhibits infection of monocytes by dengue virus.

Monocytes appear to play very important roles in dengue virus infection. They are the predominant cells that support dengue virus replication (Halstead et al., 1977). Monocytes containing dengue virus-like structures have been detected in the skin and kidney of dengue haemorrhagic fever patients (Boonpucknavig et al., 1976, 1979). In this paper we report that
Table 2. Inhibition of dengue virus infection of monocytes by induced IFN-α

<table>
<thead>
<tr>
<th>Treatment of monocytes*</th>
<th>IFN titre (U/ml) contained in the culture fluids</th>
<th>% Dengue antigen-positive cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (RPMI/10% FCS)</td>
<td>&lt;6</td>
<td>51</td>
</tr>
<tr>
<td>Culture fluid of dengue virus-infected monocytes</td>
<td>400</td>
<td>0</td>
</tr>
<tr>
<td>Culture fluid of uninfected monocytes</td>
<td>&lt;6</td>
<td>50</td>
</tr>
</tbody>
</table>

* Supernatant fluids from dengue virus-infected or uninfected monocyte cultures at a concentration of 1 × 10⁶/ml were removed and examined for IFN activity in bioassays. They were then added to other monocyte cultures at a concentration of 1 × 10⁶/ml for 20 h, prior to infection with dengue virus at an m.o.i. of 10. The percentage of monocytes infected with dengue virus was determined 24 h after infection by indirect immunofluorescence.

monocytes enriched by adherence, which react with a monoclonal antibody to monocytes (anti-Mo2 antibody) and have phagocytic activity, produced IFN-α after they were infected with dengue virus and contained dengue antigen. Non-adherent cell fractions did not produce IFN after exposure to dengue virus during 48 h of culture and did not contain dengue antigen-positive cells.

It has been reported that IFN-α activity is detected in the sera of volunteers inoculated with the 17D vaccine strain of yellow fever virus (Wheelock & Sibley, 1965) and in the plasma and cerebrospinal fluids of patients with Japanese encephalitis (Burke & Morrill, 1987). We have also detected IFN-α activity in the serum of a volunteer infected with an attenuated dengue 4 vaccine and from two volunteers infected with an attenuated dengue 3 virus strain. IFN-α was detected on days 6 to 9 after infection with titres up to 50 to 100 U/ml (data not presented). Therefore, IFN-α is likely to be present in the sera of patients at certain stages of natural dengue virus infections. Correlation of IFN-α titres detected in vivo with the symptoms and prognosis of patients with dengue may clarify the role of IFN in dengue virus infection.

Monocytes may regulate specific T and B cell responses to dengue virus by presenting antigens and producing interleukin 1, in addition to the production and induction of IFN. Therefore, the roles of monocytes and IFN-α in recovery from dengue virus infection and in the pathogenesis of dengue haemorrhagic fever and dengue shock syndrome are important subjects to be elucidated in order better to understand human immune responses to dengue virus.

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REFERENCES


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