Increased transcription of interleukin-6 in the brains of mice with chronic enterovirus infection

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An animal model of chronic enteroviral infection was established by using PCR to detect viral genomes in animal tissues and to compare levels of transcription of a variety of cytokines in the brain. Chronic coxsackievirus B1 infection was found in both brain and skeletal muscle of mice infected as neonates. The viral infection cleared by 240 days post-infection. Elevated levels of tumour necrosis factor α and interleukin-6 (IL-6) would appear to be linked to acute and chronic infection respectively. Levels of IL-6 return to normal upon clearance of the virus.

Acute viral infections have traditionally attracted most interest but it has become apparent that numerous viruses, e.g. the herpes simplex, cytomegalovirus, Epstein–Barr, rubella and adenoviruses, can cause chronic viral disease (Oldstone, 1985). A variety of clinical patterns can be produced by such chronic infections and, since the pattern depends on the host response as well as the virus, the same agent may be responsible for more than one syndrome (Haywood, 1986).

Chronic viral infections can be divided into two main groups: persistent, when infectious virus can be identified, and latent, when it cannot. Examples of persistent virus infection include those due to measles, rubella, the papovavirus group and some of the herpesvirus group, but other members of the herpesvirus and retrovirus groups are associated with latency e.g. herpes simplex and varicella-zoster viruses. In both types the complete viral genome is present and is capable of the production of infectious virions.

One of the major conceptual advances in recent virology has been the realization that persistent viruses may cause disease by interfering with specialized functions in differentiated cells without killing them or interfering with their basic ‘house-keeping’ functions (Oldstone et al., 1984; de la Torre et al., 1991). It is not surprising that, if a virus succeeds in evading the immune response and establishing a persistent infection, evidence of its presence will be difficult to detect. It may not show cytopathic effect in anything other than a highly susceptible cell line, light and/or electron microscopy may not reveal viral particles and immunostaining of infected cells will be negative if viral antigen is not expressed. In addition, the patient cannot be expected to show a rising titre of specific antibody, although a constant level of antibody against the infecting agent may be maintained.

The study of viral persistence and especially of viral persistence in the brain is still in its infancy and little is known of the immune response of the brain to chronic viral infections. Persistent enterovirus infection has been implicated in three major syndromes: polymyositis, myocarditis and myalgic encephalomyelitis (Strongwater et al., 1984; Kandolf et al., 1987; Tam et al., 1991; Archard et al., 1988; Gow & Behan, 1991). The mechanism by which the virus is able to persist in patients is still not understood, nor is the long-term effect on the persistently infected cells, nor how the virus causes disease/symptoms in the absence of cytolysis.

Chronic release of cytokines has been shown to be associated with fatigue, myalgia, headache, memory impairment and sleep disturbance (Bocci, 1988), many of which are reported by patients suffering from polymyositis and myalgic encephalomyelitis, both of which are believed to have a persistent enterviral cause. The cytokine interleukin-6 (IL-6) has been demonstrated to be produced in the central nervous system during viral infection (Frei et al., 1989) and continually secreted during persistent infection of mice by lymphocytic choriomeningitis virus (Moskophidis et al., 1991). IL-6 has also recently been shown to be similar, in structure and function, to a family of neuropoietic cytokines which are known to be capable of modulating the expression of a number of neurotransmitter synthetic enzymes and neuropeptide hormones in post-mitotic neurons (Patterson, 1992).

Here, a model of chronic enterviral infection in mice...
Table 1. Observations on mice

<table>
<thead>
<tr>
<th>Time post-infection (days)</th>
<th>Number of mice studied</th>
<th>Actin gene PCR-positive</th>
<th>CVB-1* PCR-positive brain</th>
<th>CVB-1* PCR-positive muscle</th>
<th>Brain IL-1</th>
<th>IL-6</th>
<th>IL-2</th>
<th>TNFα</th>
<th>IFN-γ</th>
<th>IL-4</th>
<th>IL-5</th>
<th>Histological observations†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected (all ages)</td>
<td>30</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
</tr>
<tr>
<td>Early acute (1 to 14)</td>
<td>40</td>
<td>38</td>
<td>38 (100)†</td>
<td>38 (100)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Focal myositis in muscle</td>
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<tr>
<td>Late acute (15 to 21)</td>
<td>10</td>
<td>10</td>
<td>3</td>
<td>10 (100)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2/10 as above +</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>mitochondrial abnormalities</td>
</tr>
<tr>
<td>Interim period (21 to 34)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
</tr>
<tr>
<td>Chronic (35 to 120)</td>
<td>35</td>
<td>32</td>
<td>4</td>
<td>5 (12.5)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
</tr>
<tr>
<td>Interim period (120 to 240)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
</tr>
<tr>
<td>Recovered (240)</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
</tr>
</tbody>
</table>

* Detection of CVB-1 genome by PCR.
† No inflammatory infiltrate or tissue damage of any kind was seen in the brain at any time during infection.
‡ Percentage of actin positive animals demonstrating the presence of enteroviral genomes.
§ Percentage of CVB-1-positive animals demonstrating upregulation of TNFα in brain.
¶ Percentage of CVB-1-positive animals demonstrating upregulation of IL-6 in brain.

was examined. Newborn CD-1 mice were inoculated intraperitoneally with a sublethal dose of coxsackievirus B1 (CVB-1), originally isolated from a human source. Mice were infected with 200 p.f.u. of virus in 50 μl saline; age-matched controls received 50 μl saline only. Groups of test and control mice were perfused transcardially with isotonic saline at regular intervals from between 10 days and 8 months post-infection (p.i.) and were sacrificed by carbon dioxide asphyxiation. Rapid dissection of the animal yielded brain, heart and skeletal muscle samples for analysis, all of which were examined independently. Half of each sample was used for histological and ultrastructural examination, the other half for RNA preparation.

RNA was prepared by the acid–guanidinium thiocyanate–phenol–chloroform method of RNA extraction after the method of Chomczynski & Sacchi (1987). Positive control spleen tissue was prepared separately to avoid cross-contamination. cDNA for PCR analysis was prepared using random hexamers (Sambrook et al., 1989). PCR was employed to amplify β-actin, a basic ‘house-keeping’ gene, cytokine and enteroviral sequences from each sample of cDNA. The integrity of the cDNA preparation was assumed to be insufficient if the samples were negative for actin and these were consequently discarded.

Cytokine PCR primers were either synthesized in house according to published sequences (Hunter et al., 1991) or obtained from commercial sources (Clontech). Actin and CVB-1 primers, complementary to the 5’ nontranslated region of the viral genome, were also synthesized in house following published sequences (Hunter et al., 1991; Gow & Behan, 1991). In each case amplification was carried out for 35 cycles (94 °C for 1 min; 55 °C for 1 min; 72 °C for 1.5 min for actin and enterovirus, or 94 °C for 1 min; 60 °C for 2 min and 72 °C for 3 min for cytokines, respectively) on a programmable dry block thermocycler. Negative control PCR samples consisted of cDNA from age-matched, uninfected control mice. In addition, a water-only negative control was incorporated in the form of a PCR mixture without cDNA template.

Cytokine PCR-positive controls contained cDNA derived from mouse spleen. For enteroviral PCR cDNA from acutely infected mouse brain or muscle was used as a positive control. PCR products were electrophoresed through a 2% agarose gel, stained with ethidium bromide and photographed under u.v. illumination. In addition, all enteroviral PCR products were subjected to hybridization under standard conditions (Sambrook et al., 1989) with a radiolabelled internal probe (Gow & Behan, 1991) to confirm specificity.

Virus could not be isolated from blood specimens 4 weeks following infection. To allow for a margin of error the chronic phase of infection was defined as 6 weeks p.i. onwards.

The results, summarized in Table 1, reveal that tumour necrosis factor-alpha (TNFα) mRNA is increased in the brains of CD-1 mice acutely infected with CVB-1. Transcription of this cytokine tapers off towards the end of the acute phase of infection at 18 days p.i. Interleukin-1-alpha (IL-1α) was found to be constitutively expressed in the brains of both test and control animals.

Viral genomes were still detectable, by PCR, in the
brain or muscle of a small number of these animals at 120 days p.i. (Table 1). No animal, however, displayed a chronic infection in brain tissue and, simultaneously, in muscle tissue. During this chronic stage of infection TNF-α mRNA levels were normal but elevated levels of IL-6 transcription were detectable.

Elevated levels of interleukin-2 (IL-2), interferon gamma (IFN-γ), interleukin-4 (IL-4) and interleukin-5 (IL-5) mRNA were not detected at any time during infection. Furthermore, both acute and chronic cerebral infection occurred in the absence of any obvious inflammatory infiltrate.

Increased production of IL-6 from lymphocytes has been shown to be accompanied by increased levels of IL-4 and IL-5 (Moskophidis et al., 1991); the increased levels of IL-6 mRNA in brain tissue of animals described in this report, in the absence of IL-4 mRNA and IL-5 mRNA, indicate a neural origin for the upregulated IL-6 mRNA. The results would thus indicate that the increase in level of IL-6 mRNA is brain-associated, as evinced by the lack of inflammatory infiltrate in the brain combined with the lack of increased levels of IL-4 and IL-5 mRNA. No CVB-1 was detected in heart tissue at any time.

No animals were analysed between 120 and 240 days p.i. By 240 days p.i., however, all animals examined had recovered from the viral infection. Levels of IL-6 mRNA had returned to normal and no viral genome was detectable by PCR in either brain or muscle. Examination of animals in the intervening period, 120 to 240 days p.i., and the effect of chronic infection upon neurotransmitter function is in progress.

Although use of the purely qualitative PCR method utilized in this study is limited, in that it does not allow precise quantification of increased transcription, determination of changes in mRNA stability or post-translational modifications, it allows examination of a large number of transcripts in a relatively short period of time and gives rise to significant findings which can be more intensively investigated with a variety of quantitative methods. In addition, qualitative PCR does not allow us to determine whether the chronic enterovirus infection described in this study is present in a latent or persistent state. Enteroviruses, however, have not been reported to be capable of establishing latency.

Low-level chronic CVB-1 infection in the brain would thus appear to be accompanied by increased transcription of brain-derived IL-6 mRNA. This work thus adds support to the hypothesis that a chronic enterovirus infection could be responsible for neurological symptoms, associated with several different syndromes, by triggering an abnormal cytokine response.

We would like to thank Dr C. Hunter and Dr J. Gow for PCR primer sequences and Mrs J. Rodgers for preparation of histology material. We would also like to acknowledge, with gratitude, the generous support of the Barclay Trust of Glasgow University.

References


