Immunoreactive IFN-γ in CSF in neurological disorders

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SUMMARY Interferon gamma (IFN-γ) was measured in the CSF of neurological patients using a highly specific and sensitive immunoradiometric assay. It was detected in 52% of patients presenting as suspected meningitis or encephalitis and in 83% with proven viral meningitis. In contrast IFN-γ was detected in only 26% of patients who did not have an acute infection at the time of presentation. Only 15% of patients with multiple sclerosis had detectable CSF IFN-γ. The presence of IFN-γ in CSF in response to acute viral infections of the central nervous system may be of importance in relation to the pathophysiology of immunologically mediated neurological disorders.

Interferons (IFNs) are naturally occurring proteins which were first recognised because of their antiviral properties. International nomenclature1 now subdivides IFNs into three antigenically distinct species and, whereas IFN-α and IFN-β are predominantly produced in response to viral infection,2 IFN-γ is thought to be produced by mitogenic stimulation,3 and in vivo release in response to a virus has not been demonstrated previously. IFN-γ ("immune IFN") is released from immunocompetent T cells and has powerful actions as a lymphokine modulator of immune systems.4 It is the most potent naturally occurring substance for inducing the expression of DR class II HLA antigenicity,5 and it has been suggested that this action may be involved in the subsequent development of autoimmunity.6

Bioassays have been used in most previous studies of CSF IFNs in neurological disorders,7−9 but these techniques lack the specificity of radioimmunoassays. As a result it has not been possible to characterise accurately the species of IFN being studied. We have demonstrated that newly available immunoradiometric assays (IRMA) may be used to study IFN in CSF,10,11 and now report the use of a highly sensitive and specific IRMA for IFN-γ in the study of neurological patients.

Methods

Patients studied
We studied two separate populations. Group A Consisted of 32 patients attending a regional neurological department who underwent diagnostic lumbar puncture. Fifteen patients had multiple sclerosis in an active phase, three acute Guillain Barré syndrome and three motor neuron disease. The remainder had miscellaneous disorders not thought to be immunologically or virally mediated (two epilepsy, two migraine, one dementia, one sarcoidosis, one VIth nerve palsy, one subarachnoid haemorrhage, one extradural abscess, one vertigo, one previously treated neurosyphilis).

Group B Consisted of 74 patients referred to a regional infectious disease unit with a suspected diagnosis of meningitis and/or encephalitis. It is the policy of this unit to submit all such patients to diagnostic lumbar puncture unless clinically contraindicated. These patients were subdivided into four groups according to the nature of the infection. (1) Proven viral infection (31), (2) clinically suspected viral infection (24), (3) bacterial meningitis (11), (4) other bacterial infections (8).

CSF from all patients was sent for routine cell count and biochemical analysis and, in addition, three 1 ml aliquots were stored at −70°C until used for IFN assay. CSF, throat swab, stools, urine and acute and convalescent sera were sent from Group B patients for virological study.

Assay technique
IFN-γ assays were performed using a highly sensitive IRMA assay, "Sucrosep" (Boots-Celltech Diagnostics Ltd) which is a two-site assay based upon a highly specific 125I-labelled monoclonal antibody from human IFN-γ. The assay standard is a preparation of purified natural human IFN-γ obtained from the Finnish Red Cross Blood Transfusion Service. This has been calibrated against a reference human...
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IFN-γ preparation (Gg 23-901-530-NIH, Bethesda, Maryland, USA). The concentration of analyte (IFN-γ) is quantified directly by incubation with the monoclonal antibody. The analyte 125I-labelled antibody complex is then immobilised by incubation with a sheep anti-IFN-γ antibody coupled to solid phase. Separation of bound from unbound labelled monoclonal antibody utilises a non-centrifugation system based upon gravity, "Sucrosep". This results in low non-specific binding and thereby greater assay sensitivity. There is no cross reactivity with other IFN species and the assay will detect 1 IU/ml IFN-γ. It, therefore, has many advantages over previously used biological assays.12

**Results**

**Group A**
IFN-γ was detected in low concentration in eight patients (26%) (fig 1). There was no difference in overall detection in the "immunological" groups compared with the miscellaneous disorder group (4), (two epilepsy, one subarachnoid haemorrhage, one migraine). IFN-γ was detected in only two patients with multiple sclerosis (15%).

**Group B**
IFN-γ was detected in the CSF of 39 patients overall (52%) (fig 2) and in 22 patients with proven meningitis (67%). Twelve patients in group 1 had viral meningitis (six mumps, four ECHO, one coxsackie, one herpes simplex) and IFN-γ was detected in 10 (83%) (mean IFN-γ concentration 5-9 IU/ml). In 14 patients a virus was identified as the cause of the illness (three ECHO 11, two Rota, two measles, two adenovirus, one influenza A, one influenza B, one coxsackie, one varicella, one Epstein Barr virus), but there was no CSF pleocytosis. IFN-γ was present in seven of these patients (50%) (1-9 IU/ml). The remaining five patients had viral encephalitis. IFN-γ was detected only in the two specimens taken acutely, the remaining three specimens taken 10 days after commencing antiviral treatment, did not contain detectable IFN-γ.

Group 2 consisted of 24 patients in whom a viral infection was diagnosed clinically, but no virus was identified. IFN-γ was detected in six of nine patients (66%) with aseptic meningitis (3-6 IU/ml) and in seven of 15 (47%) patients with no CSF pleocytosis (1-2 IU/ml). An organism was isolated from the CSF of eight of 11 patients with bacterial meningitis (six meningococcus, two pneumococcus), two had previously received antibiotics and one was subsequently found to have tuberculous meningitis. Five patients had detectable IFN-γ (45%) (2-2 IU/ml) (Group 3). The remaining patients (Group 4) had bacterial infections but no CSF pleocytosis (otitis media, pneumonia, septicaemia, scarlet fever, urinary tract

**Fig 1** CSF IFN-γ in neurological patients.

**Fig 2** CSF IFN-γ in infectious disease patients.
infection, tonsillitis, gastroenteritis). IFN-γ was detected in only one (12%) (0.4 IU/ml).

Discussion

IFN has generally not been detected in the circulation of normal individuals, but elevated serum levels of IFN-γ have been described in a number of conditions which are thought to be immunologically mediated.13-15 The recovery from CSF of a viral inhibitory substance with properties similar to IFN was first described by Gresser and Naficy7 who, using a bioassay, found an IFN-like substance in 23 of 58 patients with viral meningoencephalitis, and also in a smaller percentage of patients with bacterial meningitis. Using anti-IFN antibody to characterise the species of IFN Dussiaix et al16 found IFN-α in 90% of children with herpes simplex encephalitis and more than half their patients with viral meningitis. Miyazu et al17 found both IFN-α and IFN-γ in patients with a range of viral CNS disorders. Ichimura et al,18 using a radioimmunoassay, detected IFN-α in the CSF of 100% of 20 children with Echovirus 30 meningitis.

There have been fewer studies of CSF-IFN in noninfectious neurological disorders. Haahr8 found virus inhibitor in nine out of 1161 CSF specimens from neurological patients and two of these nine had acute multiple sclerosis; however, specimens had been stored for long periods under suboptimal conditions. Degre et al19 found IFN in the serum of 22 (61%) and in the CSF of 13 (36%) multiple sclerosis patients, but in none of 59 with a variety of non-inflammatory disorders. Dussiaix et al16 found no IFN-α in the CSF of children with Guillain-Barré syndrome, subacute sclerosing panencephalitis and a range of non-inflammatory disorders, although three patients with systemic lupus erythematosus had detectable IFN-α. Using a highly sensitive IRMA we found no IFN-α in 15 patients with multiple sclerosis.10 Such immunoassays possess the major advantage over bioassays that they allow definitive identification of the IFN species studied.12

In early nomenclature IFN-γ was referred to as “immune IFN” since it was thought to be released primarily in response to immunological stimulation. We recently reported the presence of IFN-γ in CSF of seven patients with viral meningitis11 and, to our knowledge, this lymphokine has not previously been shown to be released in vivo in response to a viral infection.

In this larger study IFN-γ was detected in 83% of patients with proven viral meningitis compared with 26% of controls with neurological disorders. None of these patients were suffering from an acute infectious illness at the time of lumbar puncture. Overall IFN-γ was detected in the CSF of 61% of patients with a proven viral infection (Group 1) and 54% with clinically suspected viral infection (Group 2).

CSF IFN-γ was detected in seven patients with meningitis due to an identified virus, but without CSF pleocytosis. This suggests that a generalised viral infection with meningeal irritation may lead to local IFN production in the absence of an inflammatory meningeal response. However, detection of IFN-γ was not specific for viral infection since it was also detected in five patients with acute bacterial meningitis. The presence of IFN in CSF of patients with bacterial meningitis has been noted previously.19 20

CSF IFN-γ was detected in only a minority of patients in the neurological group and was, in fact, detected less frequently in the patients with putative immunological disorders than in the miscellaneous group. There was, in particular, no evidence of increased IFN production in multiple sclerosis patients despite lumbar puncture having been performed at a time when the disease was clinically active. The two patients in whom small concentrations of IFN-γ were detected did not differ clinically from the remainder. The possible role of viral infection and subsequent IFN production in neurological diseases is still unclear. This study has demonstrated that CNS viral infections lead to production of IFN-γ in the CSF. IFN-γ may have an important role in initiating class II HLA expression on target cells involved in autoimmune disease, and further studies of IFN-γ in CSF in relation to immunologically mediated CNS diseases may be worthwhile. However, since IFN-γ is not invariably detectable in the CSF of patients with viral CNS disease, the measurement of this lymphokine is unlikely to become a useful diagnostic test.

We acknowledge the help of Dr S R Abbott (Boots Celltech Diagnostic Ltd) who supplied the IFN-γ assay kits.

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*J Neurol Neurosurg Psychiatry* 1987 50: 882-885
doi: 10.1136/jnnp.50.7.882

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