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The relationship between AIDS dementia complex and the presence of macrophage tropic and non syncytium inducing isolates of human immunodeficiency virus type 1 in the cerebrospinal fluid

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We sought to determine the clinical significance of macrophage tropic and nonsyncytium inducing isolates of human immunodeficiency virus type 1 (HIV-1) in the cerebrospinal fluid (CSF) of patients with and without AIDS dementia complex (ADC). HIV-1 was isolated from the CSF of 31 patients with and without ADC. The isolates were then characterised as to the degree of macrophage tropism by quantitation of p24 production and the presence of syncytium inducing (SI) or non-syncytium inducing (NSI) isolates by MT2 assay and SupT1 coculture. The degree of macrophage tropism varied according to the donor macrophage that was used except in strongly macrophage tropic isolates. Moderate and severe ADC (stage $\geqslant 2$) was associated with the presence of highly macrophage tropic isolates in the CSF ($P=0.01$). The sensitivity and specificity values of a highly macrophage tropic isolate in the CSF for ADC stage $\geqslant 2$ were 82% and 66% respectively while the predictive value was 64%. Three of four asymptomatic patients with such highly macrophage tropic isolates in the CSF subsequently developed ADC after an average of 4 months. Twenty-eight isolates from the CSF and 23 from the blood were NSI regardless of the presence or absence of ADC. The predictive value of an SI isolate in the blood reflecting an SI isolate in the CSF was 37.5% while the predictive value of an NSI isolate in the blood reflecting an NSI in the CSF was 100%. These data suggest that host factors are essential in determining the degree of macrophage tropism in HIV-1 and that such tropism is important for the presence and possibly subsequent development of ADC. The CSF usually has NSI isolates regardless of the presence of ADC and irrespective of the presence of such isolates in the blood thereby suggesting that the CSF is behaving virologically as a separate compartment to the blood.

Keywords: AIDS dementia; CSF; marcophages; syncytium inducing

Introduction

AIDS dementia complex (ADC) is a subacute dementing illness that occurs relatively frequently in advanced HIV-1 infection (McArthur *et al*, 1993). The cause of the disorder remains unclear as its severity is not directly related to productive HIV-1 infection of the brain (Glass *et al*, 1993; Tyor *et al*, 1992) athough HIV-1 infection appears to be the driving force behind its expression (Price, 1994). The only neural cells that have consistently been found to support productive HIV-1 infection are cells of the mononuclear phagocytic type, including both macrophages and microglia (Budka, 1990; Michaels *et al*, 1988; Watkins *et al*, 1990). Moreover, it is known that certain macrophage products are toxic to the central nervous system (Epstein and Gendelman, 1993) and from individual case studies that brain or cerebrospinal fluid (CSF) derived isolates of HIV-1 are characterised by their ability to infect and replicate in macrophages (O'Brien, 1994). It would therefore appear that such macrophage tropic isolates of HIV-1 are important in the causation of ADC but how often such isolates occur and their relationship to the presence and severity of ADC has not been explored. Additionally,

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because most macrophage tropic isolates of HIV-1 are non-syncytium inducing (NSI) we hypothesised that HIV-1 isolated from the CSF of patients with ADC would be more likely to be macrophage tropic and NSI than isolates from patients without ADC.

Results

Patient characteristics

HIV-1 could be cultured from the CSF of 31 of the 176 patients; these 31 patients are the basis of this report. Varying degrees of ADC were seen: the numbers of patients for each of these stages are as follows: stage 0, seven patients; stage 0.5, six patients; stage 1, seven patients; stage 2, eight patients; stage 3, three patients. All patients were significantly immunodeficient with a mean CD4 cell count of 72 ± 18 cells/μl. Ten of the patients were taking zidovuine (ZDV) within 4 weeks of the time of assessment, one was taking didanosine (ddI) and 20 were on no treatment. Lumbar puncture was performed to further investigate ADC (21 patients), to assess the presence of headache for which no cause apart from HIV was subsequently found (four patients), to diagnose cryptococcal meningitis (four patients) and to assess the extent of systemic lymphoma (two patients each of whom had no evidence of metastatic spread).

Virus isolation

All of the 31 isolates gave the same tropism results when run in duplicate on the same donor macrophages. However, 26 isolates were studied on two or more different donor cells to determine how often a particular isolate gave consistent tropism results. There was exact concordance of tropism in nine isolates and each of these had 4^+ macrophage tropism. In five isolates there were discordant results with one donor showing 4^+ and the other showing no evidence of tropism. In three instances one set of donor cells showed 4^+ and the other showed 1^+ tropism.

Conversely, the frequency of a particular host giving consistent tropism results with different isolates was determined by using 15 different donor macrophages. The mean number of isolates that were used to inoculate any one macrophage culture from a single donor was six. Exact concordance of tropism was found in only one donor macrophage culture.

In order to analyse the data for the degree of macrophage tropism of a particular isolate, the mean value of the tropism scores for the different hosts was calculated for each isolate. These were then used to determine if there was a relationship between the mean macrophage tropism score and ADC using previously defined criteria. As shown in the table, of the isolates that were $\geqslant 3^+$ macrophage tropic, nine were from patients with stage $\geqslant 2$ and five were from patients with stage <2, while the isolates that were $<3^+$ macrophage tropic, there were two from patients with stage $\geqslant 2$ and 10 with stage <2. As shown in the figure, there was a significant relationship between isolates that were highly macrophage tropic ($\geqslant 3^+$) and ADC stage $\geqslant 2$ ($\chi^2=6$, $P=0.01$, relative risk=3.86 and 95% confidence limits are 1–14.5). When the same analysis was used with ADC stage $\geqslant 1$ and stage <1 there was no relationship to the degree of macrophage tropism. The sensitivity and specificity of a CSF macrophage tropic ($\geqslant 3^+$) isolate for the presence of ADC stage $\geqslant 1$ was 66% and 75% respectively while the predictive value was 86%. The same parameters for ADC stage $\geqslant 2$ were 82%, 66% and 64% respectively. In the 10 patients who were taking ZDV five of the CSF isolates had tropism scores $\geqslant 3^+$.

There were four patients with an ADC stage of <2 and CSF isolates with macrophage tropism scores of $\geqslant 3^+$ and four patients with an ADC stage of <2 and

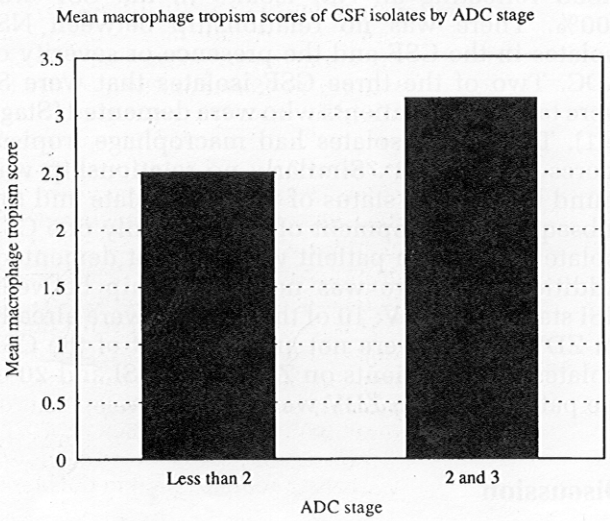

Figure 1 The relationship between the mean macrophage tropism score of CSF derived isolates and the presence of AIDS dementia complex of varying severity.

Table 1 Mean macrophage tropism (MMT) scores of CSF isolates and ADC stage

	ADC Stage 0	*ADC Stage 0.5*	*ADC Stage 1*	*ADC Stage 2*	*ADC Stage 3*
MMT: 0>×<1	1			1	
MMT: 1>×<2		2	1		
MMT: 2>×<3	1	2	3	1	
MMT: 3>×<4		2	3	6	3

CSF isolates with macrophage tropism scores of $<3^+$ for whom there was adequate follow-up. Of the patients in the first group, three developed ADC $\geqslant 2$ at 3, 4 and 6 months after the CSF had been taken; the other patient died 2 months after the CSF study without developing ADC. None of the second group developed definite signs of ADC although the length of follow-up to death was variable (one to 10 months) and the development of progressive multifocal leukoencephalopathy in one patient 10 months after the CSF study could have clouded the presence of ADC.

In regard to the presence of NSI and SI isolates in the CSF, 28 isolates were NSI and three were SI, while 23 of the 31 blood isolates were NSI with each of these having an NSI isolate in the CSF. There were three patients with an SI isolate in both the blood and CSF while the remaining five patients with SI isolates in the blood had NSI isolates in the CSF. The predictive value of an SI isolate in the blood reflecting an SI isolate in the CSF was 37.5% while the predictive value of an NSI isolate in the blood reflecting an NSI isolate in the CSF was 100%. There was no relationship between NSI isolates in the CSF and the presence or severity of ADC. Two of the three CSF isolates that were SI were taken from patients who were demented (Stage $\geqslant 1$). These two isolates had macrophage tropism scores of 4^+ and 2^+. Similarly no relationship was found between SI status of the CSF isolate and the subsequent development of ADC, as only one CSF isolate was from a patient who was not demented. Additionally, there was no relationship between NSI status and ZDV: 10 of the patients were already on ZDV and 21 were not and yet eight of the CSF isolates of the patients on ZDV were NSI and 20 of the patients not on ZDV were NSI.

Discussion

This study highlights the importance of several virological aspects of HIV-1 isolates obtained from the CSF and their relationship to ADC. Firstly, the presence and degree of macrophage tropism of HIV-1 may vary with some isolates according to the donor macrophages that are used in the assessment. Secondly, despite the variability of macrophage tropism there is a relationship between marked macrophage tropism of HIV-1 in the CSF and the presence of moderately severe ADC. Thirdly, the presence of a markedly macrophage tropic isolate in the CSF is possibly associated with the subsequent development of ADC at least in the small number of samples studied here. Lastly, in contradistinction to macrophage tropism, the NSI/SI status of the CSF isolates is not related to the presence or the severity of ADC or the subsequent development of ADC and has a variable relationship to the NSI/SI status of the blood.

The variability of macrophage tropism according to the donor macrophages has been described previously for blood derived isolates (Fouchier *et al*, 1994; Sharpless *et al*, 1992). The data presented here show that it occurs frequently in CSF derived isolates and that both host and viral factors determine macrophage tropism. Currently, macrophage tropism is considered to be predominantly although not exclusively related to the V3 loop of the envelope region of HIV-1 (Hwang *et al*, 1991; Kim *et al*, 1995; York-Higgins *et al*, 1990). Our data emphasise that as yet undefined host factors are also important as suggested by Fouchier *et al*. In light of the current view of the pathogenetic importance of macrophage tropism in ADC (Epstein and Gendelman, 1993), our observations may explain in part why only approximately 15–20% of patients with AIDS have ADC (McArthur *et al*, 1993; Portegies *et al*, 1993).

The association between marked macrophage tropism of CSF derived HIV-1 isolates and moderate to severe ADC is in keeping with current *in vitro* and *in vivo* data. Only macrophage tropic isolates of HIV-1 have been able to infect primary neural cell cultures (Jordan *et al*, 1991) and cells of the monocyte macrophage lineage are the only type that can support productive infection in the brain (Budka, 1990; Glass *et al*, 1993; Kure *et al*, 1990). Moreover, productive infection of the brain is found only in patients with more severe ADC (Brew *et al*, 1995; Glass *et al*, 1993).

However, it should be recognised that our results pertain to bulk isolates of HIV-1 and as such minor subpopulations of HIV-1 with potentially pathogenetically important mutations may have been missed. As has been shown by Monken *et al*, there was a considerable number of such populations of HIV-1 in the single patient that they studied. Nonetheless, it is exceptionally difficult from a practical aspect to analyse every genotypically different subpopulation of HIV-1 in a large number of patients with ADC.

Having emphasised the importance of macrophage tropic isolates in the causation of ADC it is somewhat surprising that such isolates were not found even more frequently in patients with ADC and less frequently in those without ADC. There are at least four possible reasons. Firstly, the nature of the isolate obtained from the CSF may be different from that obtained from the brain. Koyanagi *et al* have shown that HIV-$1_{JR\text{-}CSF}$ from the CSF of a patient with severe ADC had modest macrophage tropism while the isolate derived from the same patient's brain, HIV-$1_{JR\text{-}FL}$, had very marked macrophage tropism. Secondly, isolation of HIV-1 from patients using HIV-1 seronegative peripheral blood mononuclear cells may skew the virological properties of the isolated virus. Thirdly, a particular isolate may be tropic for that host's macrophages but when tested *in vitro* on other hosts it may not be

macrophage tropic. Lastly, macrophage tropic isolates may be found in patients without ADC because the presence of such isolates may substantially increase the risk of developing ADC in the near future. Our data from these current experiments would tend to support both the last two possibilities as three of the four non-demented patients with a highly macrophage tropic isolate developed ADC while none of the four without such a highly macrophage tropic isolate developed ADC.

Our observation that isolates from the CSF of HIV-1 infected patients are almost always NSI whereas isolates from the blood of the same individuals are less frequently NSI has potential practical significance. The patients in this study all had advanced HIV infection as evidenced by the mean CD4 cell count of 72 cells/μl. Previous data have shown that SI isolates in the blood become much more common when the CD4 cell count falls below 500 μl and that at the time of an AIDS diagnosis approximately half the patients will have SI isolates (Koot *et al*, 1993). In the CSF, however, we have found that the isolates remain almost exclusively NSI despite the marked advanced state of immunodeficiency in the systemic circulation. Whether the patient was taking ZDV also seemed not to be important. This is to some extent already borne out by data from Boucher *et al*, who have demonstrated that ZDV does not prevent the conversion from NSI to SI. Our observation that CSF and blood compartments sometimes behave differently in regard to the presence of biological phenotypes of HIV-1 has potential therapeutic implications. Because there is some evidence that patients with SI isolates have a poor response to ZDV compared to those with NSI isolates (Boucher *et al*, 1992) it is conceivable that there will be situations where ZDV has failed in the systemic circulation but is still effective in the CSF. Current therapeutic strategies would suggest that ZDV should be switched to another antiretroviral agent but our data would support the addition of another antiretroviral agent to ZDV.

In conclusion, we have shown that the degree of macrophage tropism of a particular CSF derived isolate of HIV-1 is determined in part by host factors and that significant macrophage tropism is associated with the presence and possibly subsequent development of severe ADC. Finally, HIV-1 isolates from the CSF are almost always NSI whereas this phenotype is less common in the blood thereby emphasising that the CSF and blood compartments behave differently and possibly respond differently to antiretroviral therapies.

Methods

Patient selection and ADC staging

Over a 36 month period, paired CSF and blood specimens were collected from 176 HIV-1 infected patients. None of the CSF samples had detectable red cells thereby excluding for practical purposes viral contamination from the blood compartment. Inclusion in this study was based on performance of a clinically indicated diagnostic lumbar puncture. All patients were seen by a neurologist and additional diagnostic investigations were performed according to the clinical setting. Patients were diagnosed as having ADC if abnormal neurologic symptoms and signs and characteristic neuroimaging findings were present. Severity was rated on a functionally based scale ranging from 0 to 4 (Price and Brew, 1988): stage 0 (normal), stage 0.5 (equivocally abnormal symptoms or signs), stage 1 (mild), stage 2 (moderate), stage 3 (severe), stage 4 (very severe).

Cells

Human peripheral blood mononuclear cells (PBMC) from the buffy coats of HIV-1-seronegative individuals were prepared on Ficoll/Hypaque gradients and propagated in RPMI-1640 medium containing 10% heat-inactivated fetal calf serum (FCS) 56°C for 30 min), 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 10 mM HEPES buffer and 3 μg/ml pHA. The cells were incubated (5% CO_2 at 37°C) for 72 h before use. Primary monocytes were obtained from PBMC by plastic-adherence in 24 well plates: of 5×10^6 cells 1×10^6 became adherent and confluent. The cells were counted on day 10 in a standardised section of the plate and then multiplied by the area of the plate. The cells were cultured for 10–12 days in RPMI-1640 medium supplemented with 10% heat-inactivated human serum to allow differentiation into macrophages. The CD4+ T-lymphocyte cell lines, MT2 and Sup-T1, were maintained at 1×10^6 cells per ml, in RPMI-1640 medium with 10% heat-inactivated FCS, 2 mM glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin.

Virus isolation from patient samples

Cerebrospinal fluid (CSF) was filtered (0.4 μm filter) and inoculated onto 3×10^6 PHA-stimulated PBMC which had been pretreated with DEAE-dextran (25 μg/ml) at 3×10^6 cells/ml for 45 min. Supernatants were sampled and assayed for the appearance of p24 antigen (ELISA; Genetic Systems and Coulter Corporation Florida, USA) every 3 days. The cultures were maintained for 30 days in RPMI-1640 supplemented with 10% fetal calf serum and 10% rIL-2 and received 1×10^6 fresh PHA-stimulated PBMC every 6 days. Virus isolates were also obtained by co-cultivation of PBMC from each subject with normal PBMC. Virus stocks were prepared by inoculating PHA-stimulated and DEAE-dextran pre-treated PBMC (40×10^6) with 1 ml of virus containing culture supernatant for 2 h at 37°C. The cells were washed and resuspended at 2.5×10^5 cells/ml. Culture supernatants

were assayed on day 7 for p24, the supernatants were harvested and aliquots were frozen at −70°C. The range of p24 values was 7000 to 29 700 pg/ml. Ten-fold dilutions of the supernatants were titrated for infectivity using PHA-stimulated PBMC as the target cell. The $TCID_{50}$ titre was defined as the reciprocal of the dilution which resulted in 50% of the cultures being positive for p24 antigen using the Kärber formula (Karber, 1931).

MT2 assays and Sup-T1 coculture for syncytia induction

The MT2 T cell line (1×10^6 cells/well) was inoculated with 1 ml of virus-containing culture supernatant (1000 $TCID_{50}$) for 1 h at 37°C. Cells were then washed three times and maintained in RPMI-1640 containing 10% heat-inactivated FCS. Culture supernatants were harvested at 3 day intervals and assayed for p24 antigen. Cytopathic effects (CPE) were assessed by observing for the presence of syncytium formation each day for up to 30 days. Duplicate PBMC (5×10^4 cells) that were productively infected with different HIV-1 clinical isolates were cocultured with either 5×10^4 or 5×10^5 Sup-T1 cells in 96-well plates (flat bottom). The cells were incubated at 37°C and evaluated for presence of multinucleated giant cells after 2, 4, 24 and 48 h. Each assay was run in duplicate.

Macrophage tropism assay

Macrophages were inoculated with 1 ml of virus-containing supernatant (1000 $TCID_{50}$) for 2 h at 37°C. Cells were washed six times and maintained in RPMI-1640 containing 10% heat-inactivated human serum. Culture supernatants were assayed for p24 antigen levels at 3 day intervals for 30 days. The isolates were then characterised according to the degree of macrophage tropism by the number of consecutive times at 3 day intervals the p24 supernatant concentration exceeded 500 pg/ml: + = one point > 500 pg/ml, ++ = two time points > 500 pg/ml, +++=three time points > 500 pg/ml and ++++ = four time points > 500 pg/ml. Each CSF isolate was inoculated into at least two sets of macrophages each derived from a different HIV-1 seronegative donor. Each macrophage tropism assay was run in duplicate.

Statistics

The CSF isolates were grouped according to ADC stage and according to macrophage tropism. Because there is controversy as to whether to use ADC stage $\geqslant 1$ or $\geqslant 2$ to define the presence of HIV related dementia (Brew and Perdices, 1992), two thresholds for dementia were used and so there were two groups for ADC stage: ADC stage $\geqslant 2$, ADC stage < 2 and ADC stage $\geqslant 1$, ADC stage < 1. The degree of macrophage tropism was dichotomised into those isolates whose mean score was $\geqslant 3^+$ and those whose mean score was $< 3^+$. These were then analysed by χ^2 test with a P value of < 0.05 taken as significant. Sensitivity and specificity values (Bourke *et al*, 1985) of a highly macrophage tropic isolate in the CSF to detect ADC stage $\geqslant 2$ were calculated.

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