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Determinants of HIV-induced brain changes in three different periods of the early clinical course: A data mining analysis

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ABSTRACT

To inform an understanding of brain status in HIV infection, quantitative imaging measurements were derived at structural, microstructural and macromolecular levels in three different periods of early infection and then analyzed simultaneously at each stage using data mining. Support vector machine recursive feature elimination was then used for simultaneous analysis of subject characteristics, clinical and behavioral variables, and immunologic measures in plasma and CSF to rank features associated with the most discriminating brain alterations in each period. The results indicate alterations beginning in initial infection and in all periods studied. The severity of immunosuppression in the initial virus host interaction was the most highly ranked determinant of earliest brain alterations. These results shed light on the initial brain changes induced by a neurotropic virus and their subsequent evolution. The pattern of ongoing alterations occurring during and beyond the period in which virus is suppressed in the systemic circulation supports the brain as a viral reservoir that may preclude eradication in the host. Data mining capabilities that can address high dimensionality and simultaneous analysis of disparate information sources have considerable utility for identifying mechanisms underlying onset of neurological injury and for informing new therapeutic targets.

1. Introduction

Injury to the brain is a serious complication of Human Immunodeficiency Virus (HIV). Early viral invasion of the brain is evidenced by virus in cerebrospinal fluid within only 7–10 days of transmission (Valcour, 2012a). Subsequent changes, however, are not well characterized because the infection often remains undiagnosed in the earliest stages. Additionally, to evaluate the brain in subclinical periods requires quantitative imaging which is not routinely available. This investigation used Magnetic Resonance (MR) imaging to examine the brain at structural, microstructural and macromolecular levels in three different periods of the early clinical course: Primary Infection, 4–12 months post-infection (pi) and >12–24 months pi. Duration of infection was determined by antibody non-reactivity (for Primary Infection) and assay values from a recent infection testing algorithm (Keating, 2012). Volumetric measurements were derived from high resolution neuroanatomic images for the major tissue classes with SIENAX (Smith, 2002) and for approximately fifty individual brain regions with Freesurfer (Fischl, 2002). Diffusion Tensor Imaging (DTI) parameters, fractional anisotropy (FA) and mean diffusivity (MD), were used to quantify microstructural alterations at levels approximating cellular dimensions (Basser and Pierpaoli, 1996a). Magnetization Transfer Ratio (MTR) was used to quantify macromolecular changes (e.g. in lipids, membranes, myelin) (Wolff and Balaban, 1994). DTI and MT parameters (FA, MD and MTR) were calculated for 3D volumes of interest, including cerebral cortex, cerebral white matter, corpus callosum, caudate, putamen, thalamus and hippocampus. Data mining methods were used to analyze measurements from the multiple MRI sequences simultaneously in order to determine the most discriminating brain measures (features) in each period of HIV infection compared to normative values from age matched seronegative controls. Recursive feature selection was also used to determine factors most highly associated with a composite measure of the brain alterations identified in each period. This analysis considered subject characteristics, clinical laboratory measures (e.g. HIV RNA and complete blood count), medical history information, behavioral measures (e.g. alcohol/substance use), and immunologic measures in plasma and CSF.

2. Materials and methods

Northwestern University Institutional Review Board approved this investigation, which was conducted in compliance with U.S. federal

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guidelines. Informed consent was obtained from all subjects. The study included 56 HIV (50 males, 6 females; mean age: 33.3 ± 10.1 years) and 21 seronegative control (16 males, 5 females; mean age: 31.4 ± 8.9 years) subjects. Study exclusion criteria included chronic neurological disorder, head injury, uncontrolled seizure disorder, experimental drugs or vaccination within the past 15 days, radiation or chemotherapy within prior month, mental condition involving inability to understand, chronic alcohol or drug abuse, pregnancy, opportunistic infection, cancer, medical condition (heart, liver or kidney) or MR contraindication. None of the participants had history of Kaposi's sarcoma, primary CNS lymphoma, non-CNS lymphoma or prior radiation therapy. Demographic and clinical information is presented in Table 1. HIV and seronegative groups did not differ in age, gender, racial composition or education level. Marijuana use was reported by more participants in the HIV than control group.

Blood samples were collected from all subjects; CSF was acquired from consenting HIV subjects (n = 11). HIV serostatus was determined by enzyme-linked immunosorbent assay and Western blot. An early infection assay (EIA) was used to assess duration of infection (Blood Systems Research Institute, San Francisco, CA). Antibody non-reactivity was used to define Primary Infection (estimated as less than 4 months pi; n = 15); 4–12 months pi (n = 15; EIA: 7.65–35.5), infected >12–24 months (n = 26; EIA >35.5). For the HIV group, absolute CD4 T cell counts ranged from 139 to 1282 cells/μL (mean: 546 ± 254.0 cells/μL); plasma viral load (log_{10}) ranged from undetectable to 5.54 copies/mL (mean: 3.34 ± 1.5 copies/mL). Plasma viral load was undetectable (<50 copies/mL) in 11 of the 56 HIV subjects, including 10 suppressed on antiretroviral therapy (ART) (0–4 months pi: n = 2; 4–12 months pi: n = 2, >12–24 months pi: n = 6). Thirty of the HIV subjects were ART naïve and 26 had initiated treatment with subgroup distribution as follows: (0–4 months pi: 7 naïve, 8 ART), (4–12 months pi: 11 naïve, 4 ART) and (>12–24 months pi: 12 naïve; 14 ART).

2.1. Cytokine/chemokine quantification

Multiplex analyses were conducted at Blood Systems Research Institute (San Francisco). Plasma/CSF was aliquoted locally, frozen and shipped for long-term storage and for batched analysis. The high-sensitivity MilliPlex kit (Millipore) was used for IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 p70, IL-13, IFN-γ, GM-CSF and TNF-α. The standard-sensitivity Milliplex Map kit (Millipore) was used for epidermal growth factor (EGF), Eotaxin, fibroblast growth factor (FGF)-2, Fractalkine, IL-1α, IL-1Rα, IL-9, IL-12(p40), IL-15, IL-17, IP-10, monocyte chemotactic protein (MCP)-1, MCP-3, macrophage-derived chemokine (MDC), macrophage inflammatory protein (MIP)-1α, MIP-1β, sIL-2Rα, TNFβ, and vascular endothelial growth factor (VEGF). The MMP panel 2 was used for MMP-1, MMP-2, MMP-7, MMP-9, MMP-10 and a singleplex was used for SDF-1. Manufacturer's protocols were followed. Serum was incubated overnight with antibody-coupled beads followed by incubation with biotinylated detection antibody, and finally, incubation with streptavidin-PE. Each sample was assayed in duplicate and cytokine standards, and controls, supplied by the manufacturer, were run on each plate. In addition, manufacturer controls and in-house controls consisting of supernatants of PBMCs stimulated with mitogen in culture are also run. Multi-analyte profiling was performed using a Luminex-100 system and data were analyzed using BioPlex 6.1 software (BioPlex). Luminex Standard Curve: A 5-PL curve fit is used to graph the 7-point standard curve. The curve for every analyte was checked for the fit of the standard data points. If there were errors or more than a 30% CV for any standard, those points were dropped. The curve fit parameter was altered if necessary depending on the number of standard points.

Table 1
Demographic characteristics.

	HIV (n = 56)	Control (n = 21)	p
Age (mean years ± SD)	33.3 ± 10.1	31.4 ± 8.9	0.45
Gender (% male)	89%	76%	0.22
Race (% White)	62%	76%	0.22
Education (% college)	81%	90%	0.29
Substance use (past month)			
Alcohol (5 or more drinks)	7	4	0.51
Marijuana	20	3	0.04
Cocaine	3	0	0.08
Amphetamines	2	0	0.39
Glue or solvent sniffing	0	0	–
Heroin	0	0	–
Other	3	0	0.08

2.2. Flow cytometry

Whole blood was collected into ethylenediaminetetraacetic acid (EDTA) anticoagulant and assayed within 24 h. Samples were processed using a simple lyse-wash-stain-wash technique. For a given sample, sixteen 5-mL polystyrene tubes were each filled with 100 μL of whole blood (seven tubes for seven marker panels, one unstained tube, and eight for compensation control). To lyse red blood cells, 2 mL of freshly-prepared 1× NH_4Cl solution was added to each tube and incubated for 10 min at room temperature. Cells were then spun down by centrifuge at 1500 rpm for 6 min at 4 °C, washed twice with 2 mL PBS + 2% BSA, and resuspended in 50 μL of PBS + 2% BSA solution for blocking. Antibodies were added to corresponding tubes and incubated in the dark at 4 °C for 45 min. Cells were removed from incubation, washed again twice with PBS + 2% BSA, and resuspended in PBS containing 0.5% formaldehyde. Cell populations were gated on a minimum of 250,000 cells and sorted on basis of 99% purity. Samples were acquired initially on Cyan ADP (Beckman Coulter, Inc.) and later on BD LSRFortessa flow cytometers (Beckton, Dickinson and Company). Logarithmic amplifier linearity and dynamic range were tested with Rainbow beads (Spherotech, Libertyville, IL). Compensation matrix was created using single-color compensation controls to correct for any spectral overlap/spillover. Compensation settings were adjusted using singly-stained peripheral blood samples. Sample analysis was done using FlowJo (Treestar, Inc., Ashland, OR).

2.3. MR imaging

Imaging data were acquired on a single MR scanner, a 3 T MAGNETOM Tim Trio (Siemens, Erlangen, Germany) with maximum gradient slew rate, 200 mT/m/s, maximum gradient strength, 40 mT/m, using a 12 channel receive-only head coil. Sagittal whole brain Magnetization Prepared Rapid Acquisition Gradient Echo images were acquired [parameters: TR/TI/TE: 2300/900/2.91 ms; flip angle: 9°; field of view: 256 × 256 mm; slice thickness: 1 mm; resolution: 1 mm × 1 mm; slices: 176]. For DTI, a 2D double refocused spin echo sequence with echo planar readout was used for acquisition [parameters: Axial, TR/TE: 9700/90 ms, flip-angle: 90°, field of view: 256 × 256 mm, in-plane resolution: 2 × 2 mm, slice thickness: 2 mm, slices: 72, bandwidth: 1326 Hz/Px, averages: 1, acceleration factor: 2, directions: 64, b = [0; 1000] s/mm^2]. High-resolution MT was acquired using a 3D MT-weighted fast spoiled gradient echo (GRE) pulse sequence with the following parameters: TR = 43 ms, TE = 5 ms, flip angle 10°, and 144 1.0-mm slices scanned for axial plane. The scanning matrix was 256 × 256 with a field of view of 256 mm, resulting in a voxel size of 1.0 × 1.0 × 1.0 mm^3. To decrease scan time while maintaining resolution, receiver bandwidth was 200 Hz/pixel and an 81.3% rectangular field of view was used.