UNIVERSITY OF CALIFORNIA, SAN DIEGO

Novel Role of Mannose Binding Lectin in Neuroinflammation and Neurocognitive Consequences in HIV-1 Infected Brain

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in

Biology

by

Dong Mai Tran

Committee in charge:

Kumud Singh, Chair
Michael David, Co-Chair
Elvira Tour

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The Thesis of Dong Mai Tran is approved and it is accepted in quality and form for publication on microfilm and electronically:

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Co-Chair

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Chair

University of California, San Diego

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ABSTRACT OF THE THESIS

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by

Dong Mai Tran

Master of Science in Biology

University of California, San Diego, 2014

Kumud Singh, Chair

Michael David, Co-Chair

Mannose binding lectin (MBL) is known to interact directly with mannose N-linked glycans on the HIV-1 gp120 envelope and with beta amyloid (bA). We hypothesized that MBL unique interactions with both gp120 and bA, in HIV encephalitis (HIVE), and with bA in Alzheimer's disease (AD), facilitate immune complex (IC) deposition and neuroinflammation. Post-mortem brain frontal cortex tissues obtained from California NeuroAIDS Tissue Network and Alzheimer's Disease Research Center were evaluated for the expression and colocalization of MBL, bA, gp120 and monocyte chemoattractant protein -1 (MCP-1) in HIV- controls (n=5), in those with and without HIVE (n=15 each) and AD cases (n=10) using double immunofluorescence and confocal

microscopy. Cellular fractionated tissue from frontal cortex of those with and without HIVE and with and without AD was evaluated for MBL and bA expression via western blot. Expression of MBL and bA was enhanced twofold each ($p<0.01$) and colocalization of the two proteins was found to be increased 1.5 fold ($p<0.05$) respectively in HIVE vs. nonHIVE brain. Also, a 1.7 fold increased colocalization of MBL, bA and gp120 ICs, ($p<0.01$) was observed in HIVE vs. nonHIVE cases. Almost tenfold increase of MBL-bA and eightfold increase in MBL-bA-MCP-1 ICs were observed in AD vs. AD- cases ($p<0.01$ each). Older individuals with HIVE showed higher MBL-bA deposits ($p<0.05$ each). HIV- and healthy AD- control showed no cytosolic MBL compared to those with and without HIVE and those with AD. bA localized in membrane fraction was found to be increased 1.3 fold when comparing those with and without HIVE to HIV- control. Increased expression and novel interactions of MBL with gp120 and bA proteins potentially elicit complement mediated IC deposition and inflammation in HIV neuropathogenesis and AD.

INTRODUCTION

HIV Infection and Neurocognitive Impairment

Human immunodeficiency virus (HIV) infection has caused immense morbidity and mortality over last several decades. The development of highly active anti-retroviral therapy (HAART) that increased survival and higher infection in older adults has increased the proportion of older adults living with HIV/AIDS from 17% to nearly 31% by 2008 (Centers for Disease Control and Prevention 2005, 2009). Additionally, by 2015, it is projected that more than 50% HIV infected individuals will be greater than 50 years old and more than half of the infected individuals will develop some kind of neurocognitive impairment (NCI) or neuropsychiatric disorder (CDC Fact Sheet). Older HIV infected adults are at a higher risk for developing neurological disorders. Prolonged HIV infection and HAART exposure seem to increase their risk of central nervous system (CNS) impairment among other physiological and metabolism complications that are observed during normal aging. HIV infection can lead to cognitive decline, progressing with age such as HIV-associated dementia (HAD) resulting in impairment in both motor function and cognition. Therefore, improved management of the health problems of older individuals with HIV/AIDS will require a better understanding of the interface between aging, HIV, and HIV associated neurocognitive and neuropsychiatric disorders, and concomitant HAART treatment.

The importance of enhanced research on HIV in aging populations is recognized by the NIH Office of AIDS Research that established the Working Group on HIV and

aging, identifying current and high priority research opportunities in HIV and aging (Effros 2008, High 2012). Despite antiretroviral therapy (ART) availability, HIV infected patients have an overall shorter average lifespan compared to HIV negative counterparts (Heaton 2009). HIV infection has a synergistic effect on abnormal physiological conditions associated with aging: cardiovascular disease, cancer, and immunosenescence (Valcour 2001). Age is a significant risk modifier for HAND, and there is a growing body of evidence indicating overlapping neuropathology between aging associated neurodegenerative diseases and HAND (Goodkin 2001). Although brain pathology differs between HAND and Alzheimer's disease, brains from patients with HAND often show accumulation of abnormal protein such as amyloid-beta (Andras 2013).

HIV-1 is unable to cross blood brain barrier (BBB), but HIV infected macrophage can cross BBB and viral replication in brain begins early in disease progression (Williams 2002). The majority of antiretroviral drugs fail to penetrate the BBB to combat viral replication in the brain. HIV infection in brain can lead to HIV encephalitis (HIVE), inflammation of the brain caused by a response to HIV infection in CNS. A diagnosis of HIVE is based on presence of mononuclear giant cells (MNGC), HIV-infected microglial cells and viral load (Williams 2002). While HIVE is a pathological finding, there is some evidence that HIVE status predicts neurocognitive impairment, including HAD (Gelman (2013).

MBL and Lectin Complement Activation in HIV-1 Infection

Complement activation has long been implicated in neuroinflammation and neurocognitive impairment in several diseases including neuroinflammatory and neurodegenerative diseases like Alzheimer's disease and CNS infection of various viruses

(Kolev 2009). However, actual interactions of complement proteins with other host proteins involved in neurodegenerative diseases and infectious agents are unclear. We have recently shown that lectin complement activation pathway (Sanders 2002, Saifuddin 2000, Ying 2004) activated by the binding of mannose binding lectin (MBL) with HIV-1 gp120 is involved in enhanced MBL expression in HIV-1 infected brain (Singh 2011) and is characterized by MBL-gp120 immune complex deposition and expression of neuroinflammatory monocyte chemoattractant protein-1 (MCP-1) in frontal cortex.

MBL's carbohydrate recognition domain binds to the high-mannose N-linked glycans on the HIV-1 gp120 envelope protein and activates the complement pathway that leads to virus opsonization and phagocytosis. Also, MBL has been shown recently to interact directly with beta amyloid (bA) through its cysteine-rich domains and to modulate inflammation (Larvie 2012). Higher expression and aggregation of beta amyloid in HIV-1 infected brain is a characteristic feature of HIV encephalitis and also observed in cerebrospinal fluid (Steinbrink 2012, Gisslen 2009, Clifford 2009, Brew 2005, Vehmas 2004).

The Singh group observed increased expression of MBL in HIVE vs. nonHIVE frontal cortex of post-mortem brain tissue. Additionally, increased expression of neuroinflammatory cytokine monocyte chemotactic protein-1 (MCP-1) suggests that an activation of lectin pathway can contribute to neuroinflammation (Singh 2011). It is generally believed that MBL associated serine protease -2 (MASP-2) initiates the lectin complement pathway.

Beta Amyloid Accumulation in HIV-1 Infected Brain

Beta amyloid (bA) is an insoluble protein, the main component of amyloid plaques

and associated with Alzheimer's disease and is derived from cleavage of amyloid-beta precursor protein (APP) via secretases (Barger 2008). HIV-1 infection results in increased bA deposition (Pulliam 2009). Immunohistochemical staining showed an increased accumulation of intraneuronal bA in brain of individuals with HIV compared to healthy control subjects. Additionally, cases of HIVE had higher levels of bA as compared to nonHIVE cases suggesting that long term survival with HIV might interfere with clearance of bA and worsen neuronal damage and cognitive impairment (Achim 2009). There are several hypotheses that attempt to explain bA accumulation in HIV-1 infected brain. One hypothesis proposes that HIV-1 Tat transcriptional protein inhibits neprilysin, an endopeptidase that degrades beta amyloid (Rempel 2005). Intracellular HIV-1 Tat can also interact with APP and influence its trafficking and processing into bA (Kim 2013). Another hypothesis suggests that MCP-1 expression via infected astrocytes can cause an increase in beta amyloid deposition (Williams 2002). While it is not definitively known whether ARTs can significantly affect bA accumulation in the brain, there is some evidence that ART drugs can increase bA production in neurons and inhibit macrophage bA degradation (Lan 2012, Giunta 2011). Also of interest is the finding that bA is known to be localized in different cellular compartments with ART use. It is found that there is increased intraneuronal bA in ART treated HIVE brain vs. untreated HIVE brain and extracellular bA being present in ART treated HAD brain but not untreated HAD brain (Xu 2010).

MCP-1 by itself can also induce bA oligomer formation and deposition (Kiyota 2009). Increased MCP-1 expression in the CNS, commonly seen in HIV, accelerates beta amyloidosis, a process that correlates with damage to synapses and neurocognitive

impairment (Kiyota 2009). MCP-1 deficiency influences behavioral abnormalities and disease progression in bA precursor protein/presenilin-1 double-transgenic mice (Kiyota 2012). Here, increased cortical and hippocampal bA deposition is coincident with the formulation of bA oligomers. Presence of genetic variants in *MBL2* (Larsenet 2009, Jernigan 2011, Fennema-Notestine 2011) and *APP* (Fennema-Notestine 2013, Provencher 2001, Naumova 2011, Singh 2008, Naumova 2011) has been shown to alter innate immune response in elderly and those with AD disease. *MBL2* variants that express moderate levels of MBL have been implicated in successful aging as well (Hooli 2012).

MBL is also known to have modulatory effects on cytokine responses, including TNF-alpha, with a possible role in HIV-1 disease progression via induction of a persistent inflammatory response (Heggelund 2005), enhancement of bA deposition and suppression of bA clearance (Yamamoto 2007), and neurotoxicity (Pickering 2005), and IL-1beta, which can lead to cognitive deficits and neuronal death (Xu 2010).

Because of MBL's ability to bind to gp120 and bA, possibly leading to immune complex deposition in the brain, it is a possible candidate for involvement in bA accumulation in HIV-1 infected brain. That is, we hypothesized that MBL unique interactions with both gp120 and bA, in HIV encephalitis (HIVE), and with bA in Alzheimer's disease (AD) will facilitate immune complex deposition, induce the inflammation and neuronal damage in HIVE and AD brain respectively.

MATERIALS AND METHODS

Post-mortem brain tissues from HIV disease subjects

Human brain frontal cortex tissues were obtained from the California NeuroAIDS Tissue Network and Alzheimer's Disease Research Center. Double immunofluorescence, confocal microscopy and western blot analyses were used to evaluate the expression and colocalization of MBL, bA, viral gp120 and monocyte chemoattractant protein -1 (MCP-1) in the frontal cortex of the post-mortem brain tissues from HIV negative healthy controls (n=5), those with and without HIV encephalitis (HIVE) (n=15 each). Half of the subjects in the HIV groups were reported to have been on ARTs at their last assessment with three-fourths having taken at least one ART in their lifetime. Average age at time of death was 44 years old. Three-fourths of the subjects were male and one-third was Hispanic.

Immunofluorescence and Confocal microscopy

Immunofluorescence protocol is as follows. Paraffinized tissue sections were incubated at 65°C for 1 hour, washed with citrisolve clearing agent and rehydrated in decreasing concentrations of ethanol (50:50 citrisolve and ethanol, 100%, 70%, 50%, 20% ethanol, and 1X phosphate buffer saline, PBS). Sections were washed with 1X PBS 3 times at 5 minutes each in between each of the following steps. Sections were treated with permeabilization buffer containing saponin for 10 minutes at room temperature followed by heat induced epitope retrieval in 10mM sodium citrate buffer, pH 6.0. For epitope retrieval, tissue sections were placed in a 650W microwave oven and heated

intermittently for 15 minutes and allowed to cool to room temperature. Sections and nonspecific sites were blocked in 10% bovine serum albumin (BSA) for 1 hour at room temperature.

Primary antibodies were diluted to 1:200 with 1% BSA. 200ql of diluted primary antibody was added to sections and incubation was done overnight at 4°C. Slides were incubated in the dark for one hour at room temperature, with secondary antibodies diluted to a 1:400 dilution ratio in 1% BSA. Prolong Gold anti-fade reagent with nuclear staining reagent (4', 6-diamidino-2-pheylindole, DAPI) was added and sections were covered with a cover slip. Mounted sections were air dried overnight and analyzed by confocal microscope. Confocal microscope (Olympus FV1000) was used for analysis and imaging.

Confocal microscopy images from 5 sections of the frontal cortex slides (1 in center of the section and 4 at the corners) were evaluated. Cells positive for specific antibodies were counted and added together. A ratio of the average number of positive cells between HIVE vs. nonHIVE groups was determined to calculate the fold change in expression. Unpaired two-tail t test was used to compare differential expression of markers in different cases (Tables 1). Primary antibodies included rabbit anti-MBL, mouse anti-bA, goat anti-gp120, goat anti-MCP-1 and goat anti-MASP-2. Secondary antibodies included donkey anti-rabbit AF488, anti-mouse AF568, and anti-goat AF647.

Post-mortem brain tissues from Alzheimer's disease subjects

Age matched samples of frontal brain tissue from 10 Alzheimer's disease patients with no additional pathological condition and 3 healthy control cases with no AD or other pathology were studied. For the 10 AD cases, 6 were male, mean age was 83.7 years and

mean braak stage was 5.8. For healthy controls mean age was 85.3 years and braak stage was between 0.1 and 1. Braak stage assessment indicated full AD pathology for AD cases and no symptoms of AD in healthy controls. Postmortem was done within 20 hours of death for all subjects. For immunofluorescence studies, rabbit anti-MBL, mouse anti-bA, goat anti-MCP-1 and goat anti-MASP-2 primary antibodies were used. Donkey anti-rabbit AF488, donkey anti-mouse AF568, and donkey anti-goat AF647 secondary antibodies were used. An average of cells positive for proteins were taken between AD and healthy samples, and a ratio of the average number of positive cells between the two groups was determined (Table 2). Methods were same as described above.

Western blot of cellular fractions of HIV and Alzheimer's disease tissue

Cellular fractionation to separate membrane and cytosolic fractions of tissue was done via ultracentrifugation in buffer A (PBS based, 7.4 pH, 0.32M sucrose, 50mM Hepes, 25mM MgCl2, 0.5mM DTT, 200ug/mL PMSF, 2ug/mL pepstatin A, 4ug/mL leupeptin, 30ug/mL benzamidine HCl). Tissue was homogenized in buffer A followed by 5 minute spin at 4 degrees Celsius and 5000 RCF. The supernatant was then ultracentrifuged at 100,000K for 1 hour at 4 degrees Celsius. The supernatant was extracted as the cytosolic fraction and the membrane fraction was obtained by sonicating the pellet fraction in buffer A until it was resuspended. Western blot was done with 30ug of cytosolic and membrane fractions using 4-12% Bis Tris gel and transferred to PVDF membrane via a wet transfer system. Antibody included rabbit anti-MBL, mouse 82e1 anti-bA, Mouse beta Actin, and alkaline phosphatase conjugated rabbit and mouse secondary antibody. Expression of proteins of interest was determined in each fraction and the protein amount was normalized to beta Actin bands. Ratio of MBL and bA

between HIVE vs. nonHIVE tissue, with HIV- healthy control, and between AD vs. AD-healthy control was determined (Table 3).

RESULTS

Expression and immunoreactivity of lectin complement pathway protein (MBL, MASP-2) with beta amyloid in HIVE vs. nonHIVE in the brain of HIV-1 infected individuals

Higher MBL and MASP-2 expression was observed in HIVE (n=12) vs. nonHIVE (n=14) cases ($p<.01$ for both, Figure 1)). A higher expression of bA (Figure 1) was also observed in HIVE vs. nonHIVE cases ($p<.01$) (Table 1). We expected to find higher MBL, bA, and MASP-2 expression as higher expression of those single biomarkers in HIVE vs. nonHIVE is consistent with previous work (Singh 2011, Achim 2009). Building on previous work by the Achim and Singh groups, we asked whether increased MBL-bA and MBL-bA-MASP2 colocalization would be seen in HIVE vs. nonHIVE. As MBL is able to bind to bA (Larvie 2012), MBL is a candidate for being associated with increased bA deposition in HIVE vs. nonHIVE. Increased colocalization of MBL, bA, and MASP-2 would possibly show MBL mediated lectin complement activation in bA deposition in HIVE brain. An overall increase in MBL-bA immunoreactivity and colocalization was seen in HIVE vs. nonHIVE cases ($p<.01$, Figure 1). Additionally, an increase in expression and colocalization of MBL, bA, and MASP-2 was seen in HIVE vs. nonHIVE cases ($p<.01$, Figure 1) (Table 1).

Immunoreactivity and colocalization of MBL, gp120 and bA in HIVE vs. nonHIVE brain

Next, we examined if MBL and viral gp120 immunoreact and colocalize in HIVE brain and if that this immune complex interacts with bA as MBL has been reported to

interact with both gp120 and bA. Increased MBL-bA-gp120 colocalization suggests that immune complex deposition via MBL, bA, and gp120 interactions can possibly lead to lectin complement activation and increased neuroinflammation in HIV-1 infected individuals. Results showed that there was increased colocalization of MBL, bA, and gp120 in HIVE compared to nonHIVE ($p<.01$, Figure 2), as is expected with previous results showing higher expression of single biomarkers MBL and gp120 (Singh 2011) and bA (Achim 2009) in HIVE vs. nonHIVE.

Higher expression of lectin pathway proteins, HIV-1 gp120, bA and immune complex deposition in HIVE brain by age

Since we observed MBL-bA deposition in HIVE cases, we determined if deposition of MBL-bA immune complexes increased in the HIV-1 infected brain in older age group. We found that indeed older age group of 55+ years had higher fold change compared to those in 35-44 or 45-54 years old groups (Table 1). Specifically, 55+ years old group showed almost two fold increase in MBL-bA, MBL-bA-MASP-2 and MBL-bA-gp120 immune complexes in HIVE vs. nonHIVE cases ($p<0.05$ for each) (Table 1). These results suggest that MBL potentially plays a role in HIV-1 induced bA accumulation in HIV-1 infected older individuals.

Expression and immunoreactivity of lectin complement pathway protein (MBL, MASP-2) and bA in Alzheimer's disease brain

We hypothesized that MBL interactions with bA facilitates immune complex deposition, inflammation and neuronal damage in Alzheimer's disease (AD). As bA is known to be increased in AD brain and we found evidence that MBL-bA-MASP2 interactions

possibly lead to neuroinflammation and be responsible for deposition of immune complexes in HIVE vs. nonHIVE, we asked whether we may find a similar role of MBL in AD bA deposition. Complement activation in AD frontal cortex brain was detected with antibodies against MBL and MASP-2, in conjunction with bA. Almost tenfold increase of MBL-bA complexes was observed in age matched frontal cortex brain tissues from advanced AD patients (n=10) with braak stage 5 or higher vs. AD- or lower braak stage (1 or lower) cases (n=3) ($p<0.01$) (Figure 3, Table 2). Independently, MBL and bA had 3 and 1.8-fold increase in AD vs. AD- healthy brain ($p<0.05$ each). Furthermore, a 15-fold increased immunoreactivity of MBL, bA, and MASP-2 immune complexes was observed in AD vs. age matched AD- healthy control ($p<.01$) (Figure 3, Table 2). Likewise, about eightfold increase in MBL-bA-MCP-1 immune complexes was observed in AD vs. AD- cases ($p<0.01$) (Figure 3, Table 2). Moreover, almost 8-fold increase in MBL-bA-MCP1 immune complex deposition was observed in AD vs. control brain ($p<0.01$) (Table 2), suggesting that MBL-bA interaction, and consequent activation of lectin complement pathway, may lead to neuroinflammation.

Expression of MBL and bA oligomers in HIVE and Alzheimer's disease brain frontal cortex

We evaluated the expression of MBL and bA oligomers in HIVE vs. nonHIVE with HIV- healthy control and in AD vs. AD- healthy control cases (Table 3). In summary, HIV- cases showed almost no cytosolic MBL compared to nonHIVE and HIVE cases; and AD cases showed higher MBL expression than AD- control. While we expected to find increased expression of MBL and bA in nonHIVE vs. HIV-, we did not

find increased expression of MBL or bA in HIVE vs. nonHIVE cases, as was expected from results in table 3. HIVE and nonHIVE cases showed higher membrane localized bA trimer than HIV- control, and AD cases showed more membrane fraction bA trimer and monomer than AD- control (Figure 4). No MBL was detected in membrane fraction and no bA was found in cytosolic fraction.

DISCUSSION AND CONCLUSION

In this study, we determined if innate immune response related lectin complement pathway is activated and involved in eliciting neuroinflammation and immune complex deposition in HIV-1 infected brain. Furthermore, we determined if MBL interacted with both HIV-1 gp120 and beta amyloid (bA) resulting in unique immune complex formation and neuroinflammation in HIVE and Alzheimer disease brain compared to aged matched controls. Finally, we examined the expression of MBL and bA in cytosolic and membrane fractions of frontal cortex brain tissues from HIVE/nonHIVE vs. HIV- control and in AD vs. AD- control cases.

First, we determined the expression and immunoreactivity of lectin complement activation pathway proteins, MBL and MASP-2 in HIV encephalitis (HIVE) brain frontal cortex. Since bA expression in HIVE brain and MBL interactions with both HIV-1 gp120 and bA, individually have been reported (Singh 2011, Larvie 2012), we hypothesized that MBL interactions with both HIV-1 gp120 and bA will result in formation and deposition of unique immune complexes. We found that indeed MBL-gp120-bA not only colocalized but were also deposited across HIVE brain frontal cortex. We also observed similar findings with MBL-MASP-2-bA immune complexes. These findings suggest that lectin complement pathway proteins MBL and MASP-2 could potentially induce neuroinflammatiuon in HIVE brain via deposition of MBL-gp120-bA immune complexes.

Second, since bA deposition is a characteristic of older individuals with AD, we hypothesized that there will be higher deposition of MBL-bA in HIV-1 infected older individuals. Indeed we found higher deposition of MBL-bA and MBL-gp120-bA immune

complexes in HIVE brain of 55+ years old individuals vs. age matched nonHIVE brain. An age dependent increase in colocalization of MBL, MASP-2, gp120, and bA in HIVE vs. nonHIVE suggests that MBL potentially plays a role in HIV-1 induced bA accumulation in older individuals.

Third, we hypothesized that MBL-bA interactions could be a common feature in neurodegenerative disease like AD and their novel immunoreactivity could also be involved in bA deposition and related neuroinflammation in AD brain. We found that there was indeed a significant increase in expression of MBL, MASP-2 and their colocalization with bA in AD brain tissues compared to age matched healthy AD- cases. Also, MBL-bA deposits showed immunoreactivity with neuroinflammatory cytokine MCP-1 in AD brain suggesting that their interactions potentially play an essential role in complement mediated immune complex deposition and neuroinflammation in Alzheimer's disease.

Finally, we looked at the expression of MBL and bA oligomers via cellular fractionation and western blot. We found increased cytosolic MBL and membrane bA in nonHIVE vs. HIV- and in AD vs. AD- cases. However, we did not find increased MBL or bA in HIVE vs. nonHIVE cases, as was expected from results in immunofluorescence experiments. This discrepancy can possibly be explained by the small sample size in our study. While we made much effort to characterize the use of ART and HAD status of our samples, the limited frozen tissue samples that we had for western blot experiments meant that we had to use samples in which we had limited information of the HAD status and ART use. Since bA is known to be localized in different cellular compartments

depending on HAD status and ART use, as increased intraneuronal bA is associated with ART use in HIVE vs. nonHIVE brain and extracellular bA being present in ART treated HAD brain but not in ART untreated HAD brain (Xu 2010). Expansion of the current pool of samples and further work needs to be done to confirm these preliminary findings and to clarify the host factors in the differential localization of bA and MBL, and explain the discrepancy between the results of table 1 and 3.

While this study does help to understand the phenomenon of increased beta amyloidosis and neuroinflammation in HIV-1 infection, there are some limitations to the current survey. The age of infection of subjects was not known, as subjects were enrolled at different times. It is assumed that older age at the time of death corresponds to longer duration of infection. Actual duration of the infection and the time of HIVE development are not known because HIVE is a neuropathological finding. However, despite antiretroviral therapy (ART) HIVE was observed at the time of death in all HIVE cases,.

In summary, our studies showed that MBL expression was increased and colocalized with bA in HIVE vs. nonHIVE brain. Additionally, the immune complex deposition via MBL-bA-gp120 interaction can lead to activation of the lectin complement pathway, and possibly to increased inflammation in HIV-1 infected brain. While the mechanism of bA accumulation in HIV-1 infected individuals is not well documented in the literature, there is a body of evidence that suggests that it may be because of ART use, viral protein mediated, or as a result of inflammation (Rempel 2005, Kim 2013, Lan 2012, Giunta 2011). Here, we present evidence that suggests that increased expression, immunoreactivity and colocalization of MBL with HIV-1 gp120 and beta amyloid proteins and their interactions potentially play an essential role in complement mediated

immune complex deposition, neuroinflammation and neuronal damage in HIV neuropathogenesis (Figure 5). Our studies also showed MBL-bA immunoreactivity and colocalization in AD vs. AD- brain suggesting that novel MBL-bA interactions may play a critical role in initiation and progression of AD.

FIGURES AND TABLES

Table 1. *Increased expression and immune complex deposition of MBL, beta amyloid (bA) and gp120 in HIVE vs. nonHIVE brain frontal cortex.* Table shows fold change of MBL, bA, gp120, and MASP-2, and associated immune complexes (MBL-bA, MBL-bA-gp120, and MBL-bA-MASP-2), in HIVE vs. nonHIVE. Analysis of fold change of single biomarkers and immune complexes in HIVE vs. nonHIVE grouped by age is also shown. * = p < .01, ** = p < .05

Fold change by single biomarker			
	MBL	bA	MASP-2
HIVE (n=12) vs. nonHIVE (n=14)	1.54*	1.62*	1.52*
Fold change by immune complex deposition			
	MBL-bA	MBL-bA-gp120	MBL-bA-MASP-2
HIVE (n=12) vs. nonHIVE (n=14)	1.65*	1.52*	1.47*
MBL-bA-gp120 immune complex deposition by age groups			
Age (yrs) (5 each)	35-44 yrs HIVE/nonHIVE	45-54 yrs HIVE/nonHIVE	55+ yrs HIVE/nonHIVE
MBL	1.45*	1.51**	1.53*
bA	1.42*	1.44**	1.89*
MBL-bA	1.35*	1.38**	1.50*
MBL-bA-gp120	1.37*	1.37**	1.69*
MASP-2	1.54*	1.56**	1.56*
MBL-bA-MASP-2	1.36*	1.46**	1.58*

Table 2. *Increased expression and immune complex deposition of MBL, beta amyloid (bA), MASP-2, and MCP-1, in Alzheimer's disease (AD) vs. AD- brain*. Table shows fold change of single biomarker expression and immune complex deposition (MBL-bA, MBL-MASP2, MBL-bA-MASP2, MBL-MCP1, and MBL-bA-MCP-1) in AD vs. AD- age matched control.

Higher MBL-bA deposition in AD vs. AD- brain						
	MBL	bA	MBL-bA	MASP2	MBL-MASP2	MBL-bA-MASP2
Fold Change	3.13	1.77	10.50	1.44	10.58	14.50
p-value	<.05	NS	<.01	<.05	<.01	<.01

MBL-bA deposition predicts higher MCP-1 in AD vs. non-AD brain			
	MCP1	MBL-MCP1	MBL-bA-MCP1
Fold Change	2.41	7.06	8.29
p-value	<.05	<.01	<.01

Table 3. *Increased cytosolic MBL and membrane bound bA in HIV-, HIV+, and HIVE cases, and AD- and AD cases.* Table shows fold changes for cytosolic MBL expression in nonHIVE vs. HIV-, HIVE vs. nonHIVE, and AD vs. AD- for 90kD trimer, 51kD dimer, and 32kD monomer and total MBL. Fold change for total cytosolic MBL was calculated by adding trimer, dimer, and monomer MBL in one group and divided by the comparative group (nonHIVE vs. HIV-, and HIVE vs. nonHIVE). Fold changes of membrane bound bA 12kD trimer and 4kD monomer for nonHIVE vs. HIV-, HIVE vs. nonHIVE, and AD vs. AD- are also shown. Protein amount was normalized to beta actin bands. Of note, MBL was not detected in membrane fraction and bA was not detected in cytosol fraction.

Fold Change of Cytosolic MBL			
	nonHIVE vs. HIV-	HIVE vs. nonHIVE	AD vs. AD-
90kD Trimer	5.04	0.94	1.59
51kD Dimer	13.27	0.83	3.16
32kD Monomer	18.03	0.62	1.79
Total MBL	7.63	0.79	-
Fold Change of Membrane Bound bA			
	nonHIVE vs. HIV-	HIVE vs. nonHIVE	AD vs. AD-
12kD Trimer	1.36	0.91	1.29
4kD Monomer	-	-	3.22

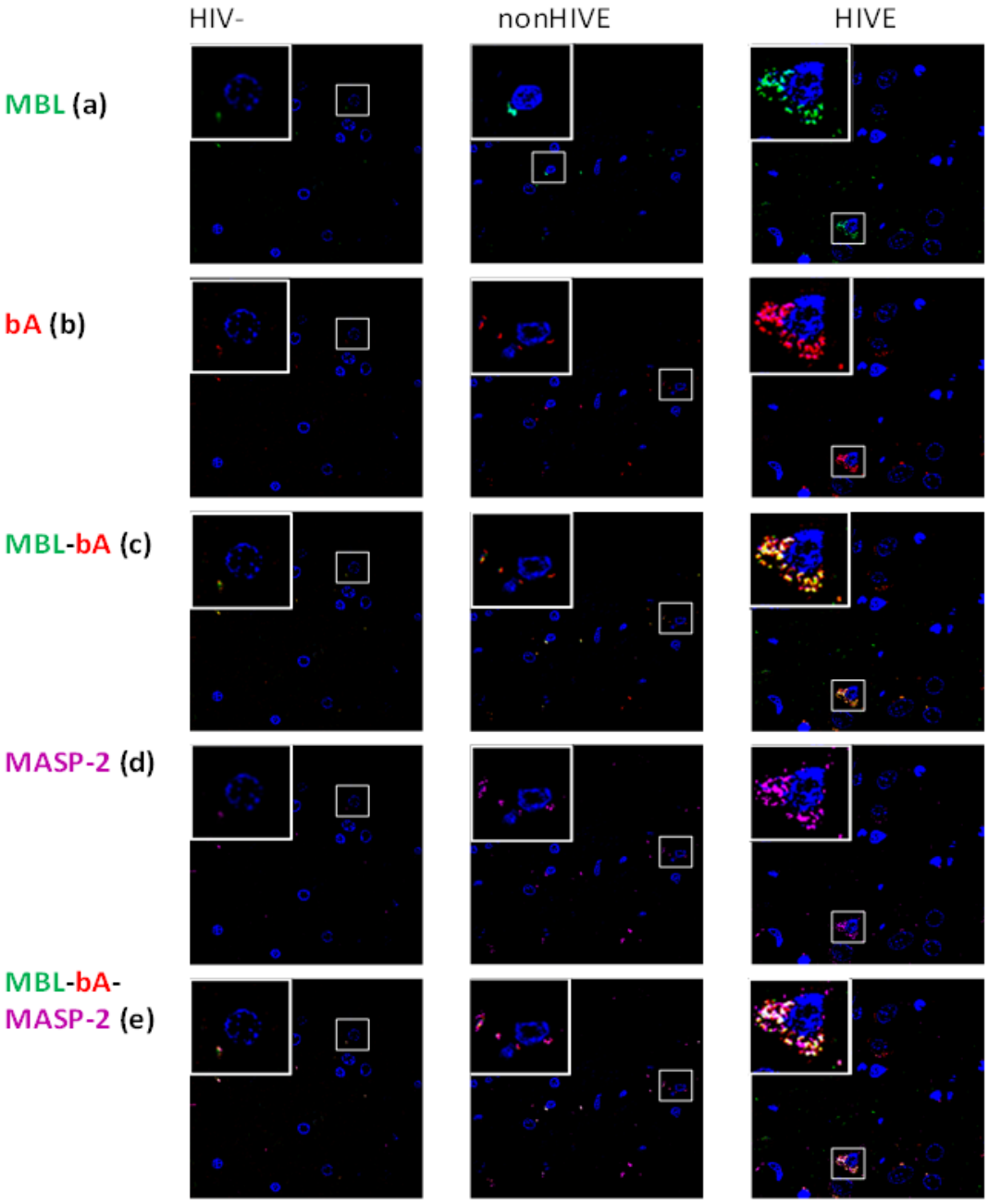

Figure 1. *Increased expression and immune complex deposition of MBL and MASP-2 with bA in HIV-, nonHIVE and HIVE brain frontal cortex*. Panels a, b, c, d, e show MBL, bA, MBL-bA, MASP-2 and MBL-bA-MASP-2 respectively, at 120um scale and 100X magnification. Green fluorescence represents MBL, red represents bA and the yellow fluorescence represents the co-localization of MBL and bA. Magenta fluorescence represents MASP-2 and co-localization of green (MBL), red (bA), and magenta (MASP-2) merged as white. Blue fluorescence represents the presence of DNA in an intact nucleus stained by DAPI.

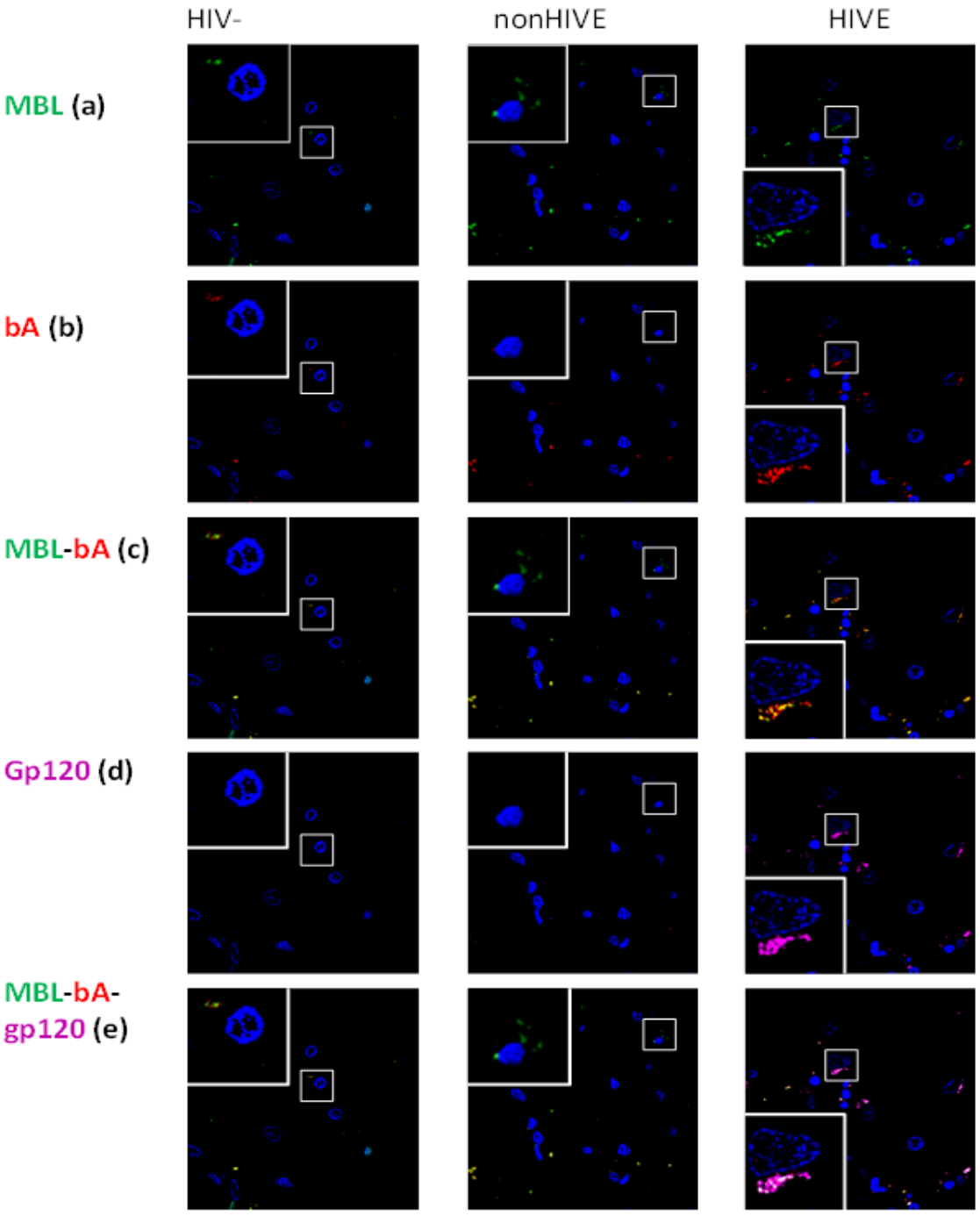

Figure 2. *Increased expression and colocalization of MBL and gp120 with bA in HIV-, nonHIVE and HIVE brain frontal cortex*. Methods, scale, magnification and fluorescence colors are same as in Figure 1.

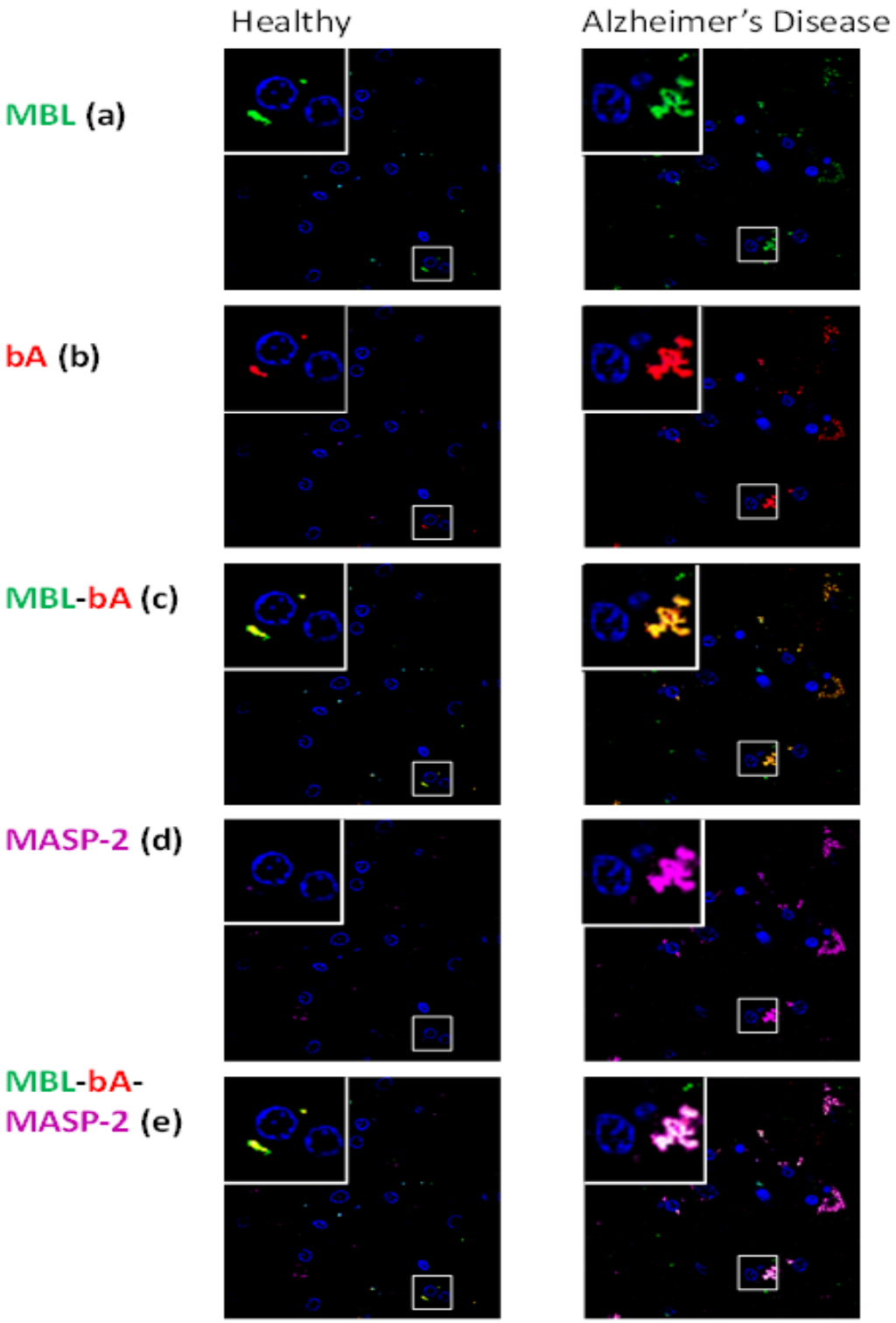

Figure 3. *Increased expression and colocalization of MBL and MASP-2 with bA in AD- and AD brain frontal cortex*. Methods, scale, magnification and fluorescence colors are same as in Figure 1.

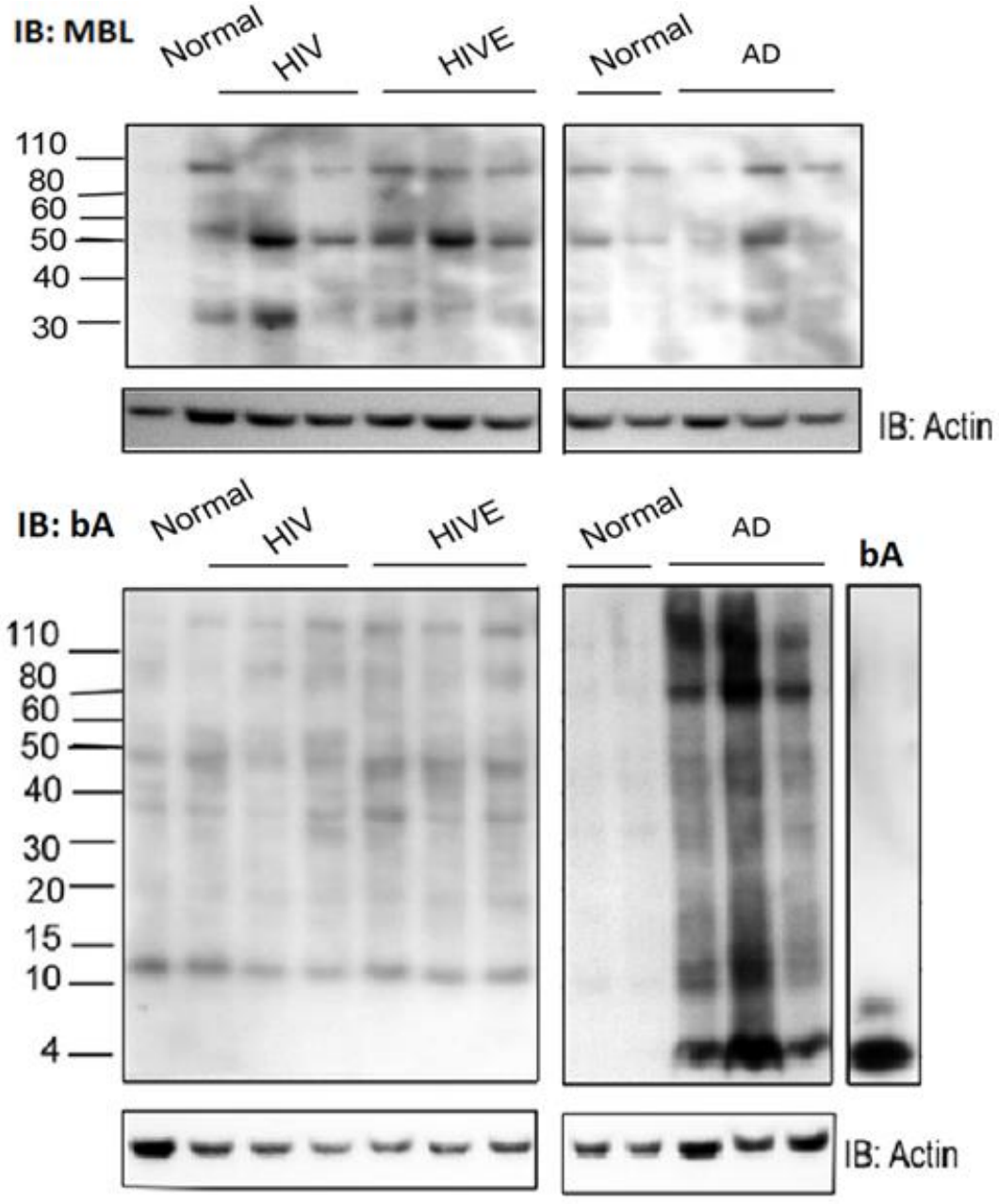

Figure 4. *Immunoblot of cytosolic MBL and membrane bA in HIV-, HIV+, and HIVE cases, and AD- and AD cases*. Immunoblot (top) shows cytosolic MBL bands in HIV- normal, HIV+ (nonHIVE), HIVE, AD- normal, and AD cases. Immunoblot (bottom) shows membrane bA bands in HIV-, HIV+ (nonHIVE), HIVE, AD- normal, and AD cases. Recombinant bA is shown in bA lane.

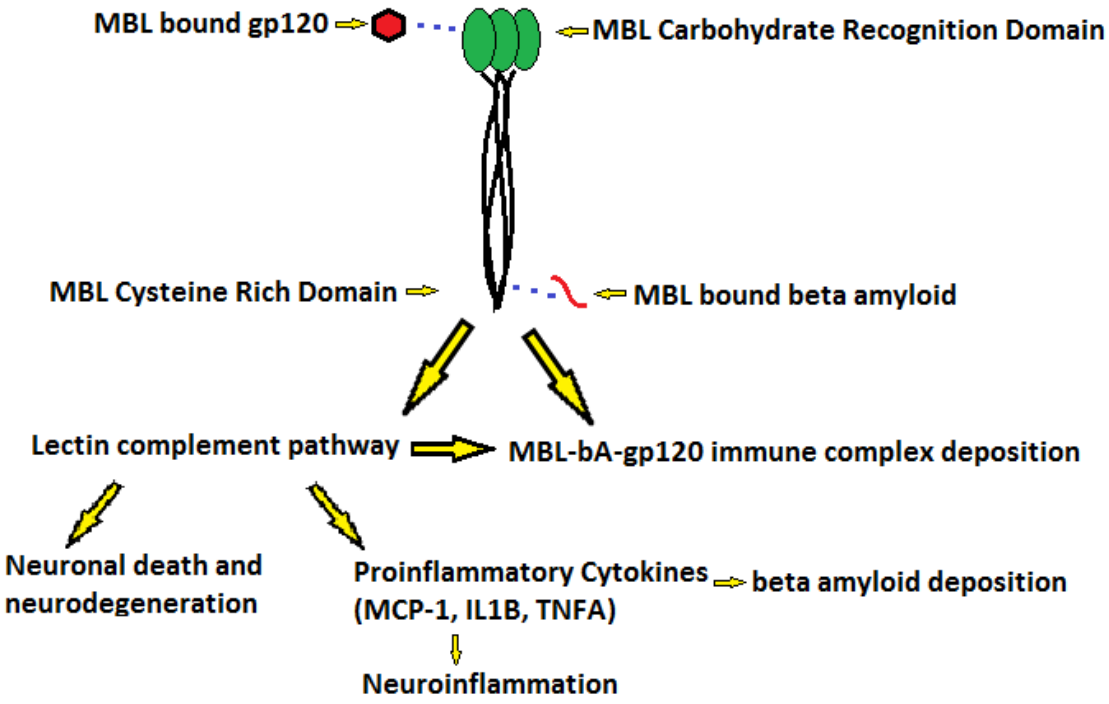

Figure 5. *Diagram of MBL binding of beta amyloid and gp120.* Diagram shows possible effects of MBL binding to bA and to gp120. Lectin complement pathway activation leads to MBL-bA-gp120 immune complex deposition, neuronal death and neurodegeneration, and the release of proinflammatory cytokines. MCP-1, IL-1 beta, and TNF alpha are capable of inducing neuroinflammation and the increased deposition of bA in HIV-1 infection.

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