Short Communication

Increased quinolinate immunoreactivity in the peripheral blood monocytes/macrophages from SIV-infected monkeys

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Quinolinate (QUIN), a metabolite in the kynurenine pathway of tryptophan degradation and a neurotoxin that is thought to act through the NMDA receptor system, was localized in cultured peripheral blood monocytes/macrophages from SIV-infected monkeys using a recently developed immunohistochemical method. Significant increases in QUIN immunoreactive (IR) cells were detected in all five SIV-infected monkeys examined. Multinucleated giant cells, a hallmark of lentiviral infection, were visible in selected samples. Treatment with the QUIN precursors, tryptophan and kynurenine, increased the number of QUIN-IR cells in both the control and SIV-infected preparations, perhaps by a mass action mechanism. We hypothesize that in SIV-infected monkeys, infiltrating monocytes/macrophages contribute to the high level of brain QUIN and associated neuropathology.

Keywords: quinolinic acid; tryptophan; kynurenine; excitotoxicity; lentivirus

Quinolinate (QUIN), a metabolite in the kynurenine pathway of tryptophan degradation, is an endogenous neurotoxin, the metabolism of which has been implicated in Huntington's disease (Schwarcz et al., 1984). Other studies have shown that the kynurenine pathway of tryptophan degradation is activated by a variety of infections (Yoshida et al., 1979; Werner-Felmayer et al., 1989). This activation is caused by a large increase in the activity of indoleamine dioxygenase (IDO), the first and rate-limiting enzyme in the kynurenine pathway (Yoshida and Hayashi, 1978; Yoshida et al., 1981; Yasui et al., 1986). As a result, concentration of QUIN is increased in specific tissues, blood and cerebrospinal fluid (CSF) (Heyes et al., 1992a). Increases in QUIN were recently demonstrated in HIV and SIV infections; increasing concentrations of QUIN in CSF were positively correlated with the cognitive impairment and motor dysfunction resulting from these viral infections (Heyes et al., 1991, 1992b; Rausch et al., 1994). These studies imply that at least some of the multiple neurotoxic abnormalities resulting from these infections may be causally linked to QUIN.

Recently, we produced highly specific rabbit polyclonal antibodies against QUIN and demonstrated further that QUIN is present in dendritic cells and select macrophages but not in neurons or astrocytes (Moffett et al., 1993a, 1994). We have also shown that QUIN immunoreactivity (QUIN-IR) is increased in dendritic cells and specific macrophage populations during immune stimulation in a temporally and spatially defined manner (Espey et al., 1995). Infection of human macrophages (MO) by HTLV-1 in vitro showed an increased number of QUIN-IR cells (Venkateshan et al., 1996). These results raise the possibility that QUIN may act as an immune regulator, similar to cytokines, or have some other specific role in select immune cells.

In the present study, we have examined localization of QUIN in peripheral blood monocytes/macrophages (PMO) from SIV-infected rhesus monkeys. The results show that QUIN is found...
mainly in monocyte/macrophages (MOs), and that SIV infection increases QUIN-IR in these cells. SIV is known to infect MOs/microglial cells both in vitro and in vivo (Unger et al., 1992; Baskin et al., 1992; Brinkmann et al., 1993; Jurrias et al., 1994; Zhu et al., 1995). The significance of these findings to the neuropathology observed during SIV infection is discussed.

Five SIV-infected and two noninfected rhesus monkeys (Macaca mulatta) were studied. Animals were housed in individual primate cages at the NIH Animals Center at Poolesville, MD. Their diet consisted of monkey chow, fresh fruits and vegetables, fed twice daily. Water was provided ad libitum. Each of the five juvenile rhesus monkeys (male/female, 2–4 kg) were inoculated intravenously with 1 ml of SIVsm (Strain F236) infected tissue culture supernatant fluid containing approximately 100,000 TCID<sub>50</sub>/ml. Control animals received normal physiological saline. Animals were anesthetized with ketamine and acepromazine. Blood samples were collected from the femoral vein into heparinized tubes. Mononuclear cell fractions of heparinized peripheral blood from seven rhesus monkeys were collected at monthly intervals by centrifugation on lymphocyte separation medium (LSM, Organ Teknika Corp., Durham, NC). To determine SIV infection status, peripheral blood mononuclear cells (PBMC) from each of the rhesus monkeys were co-cultured in vitro each month with uninfected foamy virus-free PBMC from normal rhesus monkeys. PBMC were activated by phytohemagglutinin treatment and grown at 37°C in an atmosphere of 5% CO<sub>2</sub> utilizing RPMI-1640 medium (Mediatech Inc., Herndon, VA) containing 50 µg/ml gentamycin, 20% heat-inactivated (56°C/30 min) fetal bovine serum and 10% interleukin-2 (ABI, Columbia, MD) (Blackburn et al., 1994). Supernatant medium from the co-cultures was monitored at weekly intervals over a 5-week period for the presence of SIV by either a standard reverse transcriptase assay employing tritiated thymidine as the radiolabel and poly rA:oligo dT<sub>12-16</sub> as the template-primer or by SIV p<sub>27</sub> antigen capture enzyme immunoassay (Coulter Corp., Hialeah, FL) (Golos et al., 1994).

Peripheral blood was collected as described above and the mononuclear cells were separated on an LSM gradient. The cells were washed three times in PBS, and then suspended in monocyte/macrophage medium (GIBCO-BRL) at a concentration of 2 × 10<sup>6</sup> cells/25 cm<sup>2</sup> culture flasks (Costar, Cambridge, MA) or 0.1 × 10<sup>6</sup> cells/well into chamber slides (NUNC, Gaithersburg, MD). MO media contained 2 mM glutamine, penicillin (100 U/ml) and streptomycin (10 µg/ml). IL-2 was not used, and all long-term culture medium contained 1% FBS (Intergen, Purchase, NY). Cultures were undisturbed for 3–5 days. The medium was then removed along with the unattached cells. Flasks and chamber slides were then gently washed twice using serum-free RPMI to remove all unattached cells. Fresh serum-free MO medium was added and the incubation continued. The medium was changed every 3 days. Throughout the study (3–6 weeks), adherent cells were predominantly MOs. Cells were fixed at day 0 and subsequently at 1, 2, 3, 4, 5 and 6 weeks for QUIN-IR determination. For the day 0 fixation of cells, the cells were cultured for 1–3 h and unattached cells were removed by washing 3–4 times in serum-free RPMI medium. Day 0 cells were also predominantly MO. Stimulation of QUIN production by precursors was performed as follows.

Cells were cultured in serum-free MO medium for 33 h, after which the medium was removed and replaced with medium containing either tryptophan (5 mM) or kynurenine (5 mM). At 36 h, media were removed, and the cells were fixed for QUIN immunocytochemistry using carbodiimide as described below.

Characterization of QUIN antibodies and their use in immunohistochemistry have been described (Moffett et al., 1994a). For immunocytochemistry, the immunohistochemical procedure was modified as detailed below. The cells were washed three times with saline for 15 s, and then a fixative containing 1% 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) (Sigma Chemical Co, St Louis, MO), 0.5% DMSO and 1 mM N-hydroxysuccinimide in water was added and the cells were incubated at room temperature for 45 min. Following the carbodiimide fixation, cells were left overnight in 4% formaldehyde in PBS. Cells were then treated with 0.3% H<sub>2</sub>O<sub>2</sub> in methanol for 30 min to irreversibly inhibit the endogenous peroxidase activity, and washed three times with PBS; non-specific binding was blocked using 2% normal goat serum (NGS) for 30 min. Cells were then treated overnight with the QUIN antibody preparation at room temperature, and subsequently with the biotin-avidin-peroxidase amplification system as described (Moffett et al., 1993b). The specificity of QUIN-IR was determined by removing QUIN-specific antibodies from the antibody preparations using a nitrocellulose strip containing protein-conjugated QUIN. The details of this procedure were described earlier (Moffett et al., 1994a). Briefly, the QUIN antibody preparation was diluted 1:8000 in 2% NGS and incubated overnight with constant rotary agitation with nitrocellulose strips containing protein-conjugated QUIN (10 µg/ml QUIN-BSA). The nitrocellulose strips were removed and the antibody preparation was subsequently applied to the carbodiimide-fixed cell preparation.

After inoculation with SIVsm, all five of the rhesus monkeys studied became infected as verified by the presence of reverse transcriptase activity or SIV p<sub>27</sub> antigen in supernatant fluid of co-cultures of their lymphocytes in vitro. All animals also developed typical symptoms of simian AIDS (Maul et al., 1986;
Lackner et al., 1989). Infected animals were shown to be immunosuppressed by mixed lymphocyte reactivity and were shown to have low lymphocyte counts by cytofluorography (data not given).

In the cultured MOs from the normal monkeys, about 10% of the cells were positive for QUIN-IR on day 0, remained at this level up to the end of the second week in culture, and then decreased gradually to about 3% by the end of the fourth week (Figure 1). Cell numbers also decreased to 40% of the initial number by the end of the 4th week, with selected cells showing clear morphologic alterations indicative of their transformation into macrophages and dendritic cells (Figures 2 and 3). The percentage of QUIN-IR cells in the cultures from the SIV-infected monkeys was initially higher (19%) than those from noninfected animals and increased to about 35% by the end of the second week (Figure 1). In an earlier study, the SIV-infected animals used in the present study were found to have increased levels of QUIN in the blood (Heyes et al., 1992b). As found in cultures from the normal animals, the total number of cells and the number of QUIN-IR cells decreased significantly in cultures from SIV-infected animals by the end of the fourth week. The IR disappeared in both the control cultures and the cultures from SIV-infected monkeys when the QUIN-adsorbed antibody preparation was used.

Treatment with precursors, tryptophan and kynurenine, increased QUIN-IR in the cultured MOs (Table 1). These results were expected based on earlier reports showing increased QUIN-IR and QUIN production after tryptophan administration (During et al., 1989; Espey et al., 1995; Venkatesh et al., 1996). Our results also served as confirmation for the specificity of QUIN-IR.

Only a small percentage of macrophages in vitro were shown to be QUIN-IR. Thus, QUIN synthetic capacity is not uniformly distributed in all MOs, suggesting that there are different macrophage subpopulations in cultures, an observation that is consistent with earlier reports that only a selected subpopulation of MOs can synthesize QUIN even under conditions of immune stimulation and viral

![Figure 1](image1.png) **Figure 1** Time course of the changes in QUIN-IR in SIV-infected monkey MOs cultured in vitro. Monocytes were obtained from five SIV-infected (○) and two normal monkeys (■), cultured in vitro for different durations, and analyzed for QUIN-IR as described in the text. Number of QUIN-IR cells and total cells were determined from an average of 8–10 fields, and are expressed as percentages of QUIN-IR cells. With prolonged incubation, the total number of cultured cells from both normal and SIV-infected monkeys decreased, falling to about 50% of the original level by the end of the 6th week. On visual inspection, the cells were considered positive for QUIN-IR when their staining intensity was significantly above the background levels. A wide range of QUIN-IR intensity was detectable in all samples.

![Figure 2](image2.png) **Figure 2** Increase in QUIN-IR in cultured peripheral blood MOs from SIV-infected monkeys. Monocytes from five SIV-infected (#3–7) and two normal (#1–2) monkeys were cultured for two weeks as described in the text, and QUIN-IR was analyzed at the end of the second week. Duplicate experiments were performed and after examining 8–10 fields typical results were reported. The monocytes appeared round and undifferentiated for up to 3 days in culture, and differentiation into macrophages was clearly evident by the second week. A higher level of QUIN-IR was seen in all five SIV-infected animals.
infection (Espey et al., 1995; Venkateshan et al., 1996). The functional basis of this macrophage heterogeneity remains unclear.

The results of the present study may contribute to an understanding of the pathophysiology of dementia in inflammatory diseases, including AIDS, in which there is infiltration of MOs into the brain. Increased QUIN in the blood and CSF has been reported in a number of these conditions and has been correlated with the development of neurologic deficits (Heyes et al., 1991, 1992b; Rausch et al., 1994). However, the cell source from which QUIN emanates in the brain is controversial. Recent reports suggest that the source may be peripheral rather than local (Saito et al., 1993). We have shown that increased QUIN-IR in the brain in response to acute systemic immune stimulation is restricted to putative monocyte/macrophages in the choroid plexus, meninges, and vasculature (Moffett et al., 1994b). However, significant numbers of QUIN-IR macrophages/microglia were detectable in the brain of rats containing implanted brain tumors or injected intracerebrally with lipopolysaccharide (Moffett et al., 1994c; Espey et al., 1994).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Unstained (%)</th>
<th>Light staining (%)</th>
<th>Intense staining (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>88.38 ± 2.60</td>
<td>7.375 ± 1.73</td>
<td>3.88 ± 0.93</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>79.38 ± 2.45</td>
<td>12.88 ± 2.57</td>
<td>8.25 ± 1.09</td>
</tr>
<tr>
<td>Kynurenine</td>
<td>80.13 ± 3.70</td>
<td>14.38 ± 7.25</td>
<td>8.00 ± 2.06</td>
</tr>
</tbody>
</table>

The QUIN-IR cells from 8–10 fields were counted, averaged and expressed as percentage of QUIN-IR cells. After visual inspection, the QUIN-IR cells were categorized as moderately or intensely stained using an arbitrary scale.

It should be stressed that only a limited number of circulating MOs is likely to leave the blood and enter the brain during the disease, and of these only a small proportion (10–20%) is likely to contain QUIN. Therefore, it is difficult to relate the observed increase in QUIN-IR with the genesis of clinical manifestations of neurological disease. It will be valuable to determine if substantial numbers of QUIN-IR cells are present in the brain during SIV infection. If that is found to be the case, then testing...
the effect of QUIN biosynthesis inhibitors on the development of the neurological symptoms of the disease will be worthwhile.

The mechanisms involved in the observed increase in QUIN-IR merit some discussion. Our recent studies involving in vitro infection of human peripheral blood MOs by HTLV-1 indicate that the increase in QUIN-IR is caused by some indirect mechanisms involving cytokines, such as IFNγ, but not by the virus per se (Venkateshan et al., 1996). We believe 1–2% of T-cells (infected and uninfected) will be viable under our present culture conditions in spite of the absence of exogenous IL-2 in the culture medium. The IFNγ released by the infected T-cells are likely to act on the MOs to increase QUIN production by induction of the first and rate-limiting enzyme IDO. Further, activated MOs also may produce IFNγ in an autocrine fashion which in turn may also contribute to the increase in QUIN-IR (Young, 1995).

Until recently, the focus of QUIN research was restricted to its potential role as an excitotoxin. However, recent studies suggest that QUIN may have cellular regulator properties, especially in the initial phase of the immune response. In addition to MOs, other antigen-presenting cells including dendritic cells, have been shown to produce increased amounts of QUIN in the initial phase of activation (Espey et al., 1995, 1996). This suggests that during the early phase of an immune response, QUIN may be involved in antigen presentation and related immune functions. This hypothesis merits further investigation.

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References


