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Microglia activation by SIV-infected macrophages: alterations in morphology and cytokine secretion

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Abstract

HIV infection in brain and the resultant encephalitis affects approximately one-third of individuals infected with HIV, regardless of treatment with antiretroviral drugs. Microglia are the resident phagocytic cell type in the brain, serving as a “first responder” to neuroinvasion by pathogens. The early events of the microglial response to productively-infected monocyte/macrophages entering the brain can best be investigated using *in vitro* techniques. We hypothesized that activation of microglia would be specific to the presence of SIV-infected macrophages as opposed to responses to macrophages in general. Purified microglia were grown and stimulated with control or SIV-infected macrophages. After 6 hours, aliquots of supernatant were analyzed for 23 cytokines using Millipore non-human primate-specific kit. In parallel experiments, morphologic changes and cytokine expression by individual microglia were examined by immunofluorescence. Surprisingly, the presence of macrophages was more important to the microglial response rather than whether the macrophages were infected with SIV. None of the cytokines examined was unique to co-incubation with SIV-infected macrophages compared with control macrophages, or their supernatants. Media from SIV-infected macrophages, however, did induce secretion of higher levels of IL-6 and IL-8 than the other treatments. As resident macrophages in the brain, microglia would be expected to have a strong response to infiltrating innate immune cells such as monocyte/macrophages. This response is triggered by incubation with macrophages, irrespective of whether or not they are infected with SIV, indicating a rapid, generalized immune response when infiltrating macrophages enter brain.

Keywords

cytokine; chemokine; microglia; HIV; neuroinflammation; neuropathogenesis

1. INTRODUCTION

Approximately one-third of HIV-infected individuals develop HIV-associated neurocognitive disorders [1]. HIV, and the ancestral virus (SIV), enter the brain during routine immune surveillance [2] within monocyte-derived macrophages, a so-called “Trojan Horse” [3]. Additional monocytes are recruited to this initial site of infection, where they can also become infected [4].

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The environment within the parenchyma is sampled by ramified microglia [5], which are capable of facilitating a targeted immune response [6]. There is a change in the phenotype of microglia, from ramified to amoeboid morphology within four hours [7]. Activated microglia secrete pro-inflammatory cytokines and chemokines [8–11], selectively recruiting leukocytes to the site of infection. In SIV/HIV infection it is not known if the microglial response is a general response to macrophages or is specific to SIV-infected macrophages.

Previous studies have shown SIV-infected microglia secrete increased cytokines [12]. However, infiltrating monocyte-derived macrophages would also be expected to induce pro-inflammatory signaling cascades. We hypothesized that SIV-infected macrophages would induce secretion of a specific “barcode” of cytokines and chemokines in purified microglia [13]. These studies were therefore undertaken to differentiate a pathogen-specific, from generalized macrophage ingress into brain.

Morphology of microglia was observed following co-incubation with SIV-infected or control macrophages and their supernatants. Additionally, we monitored the secretion of cytokines and chemokines previously shown to be important in AIDS neuropathogenesis [14, 15]. Cytokines and chemokines that were found to be differentially regulated by SIV in microglia were examined at the individual cellular level by immunofluorescent microscopy.

Cultures of microglia were shown to have increased CCL2, GM-CSF, and TNF- α when cultured with either control or SIV-infected bone marrow-derived macrophages, and increased levels of IL-6, IL-8 and VEGF in the presence of SIV-infected macrophage supernatants. These data suggest that microglia rapidly respond to both direct interaction with macrophages, and to factors secreted by SIV-infected macrophages.

2. MATERIALS AND METHODS

2.1. Morphological examination of microglia *in situ*

A complete necropsy of SIV-infected macaques at the Tulane National Primate Research Center includes collection of a complete set of brain tissues including multiple blocks of cerebral cortex, subcortical grey and white matter, thalamus, basal ganglia, cerebellum and spinal cord.

2.1.1. Golgi staining—Tissues were stained using the Golgi-Cox procedure developed by Gibb and Kolb [16], with the following modifications: tissue blocks (approximately 5mm) were fixed in paraformaldehyde, and immersed in Golgi-Cox solution for two weeks, followed by immersion in sucrose solutions as described. Sections were then cryostat sectioned (100 μ m), and mounted onto gelatin-coated slides. Golgi-Cox stain was developed as per the published method.

2.1.2. Immunofluorescent staining—Formalin-fixed, paraffin-embedded tissues were sectioned at 6 μ m and mounted onto positively charged glass slides. Sections were baked for 1 h at 60°C, deparaffinized in xylene, and then rehydrated in graded concentrations of ethanol. Antigen retrieval was carried out for 20 min using a microwave on high power and a citrate-based antigen unmasking solution (Vector Labs, Burlingame, CA). Tissues were blocked in a 10% normal goat serum solution (GIBCO/Invitrogen, Carlsbad, CA) for one hour at room temperature before AIF-1 antibody (1:50 dilution, Wako, Taiwan) was applied overnight at 4°C, washed three times with PBS with 0.2% fish skin gelatin (PBS/FSG), and then incubated in the dark for 60 min at room temperature with secondary antibodies directly conjugated with Alexa 488 (green) (Molecular Probes/Invitrogen, Carlsbad, CA). Sections were washed three times in PBS/FSG, and coverslipped with anti-quenching reagent (Molecular Probes/Invitrogen).

2.2. Culture of microglia

Mixed glia cultures were prepared as previously described [4]. In brief, frontal cortices were aseptically obtained from juvenile normal Indian-origin rhesus macaques at necropsy. Meninges were removed, the tissue diced and incubated with Trypsin (0.25%, Invitrogen, Carlsbad, CA) and DNase (4 U/ml, Sigma, St. Louis, MO) for one hour at 37°C. The resulting slurry was triturated and passed through a 110µm pore filter and centrifuged at 1000rpm for 5 minutes. Pellets were washed twice and resuspended in M199 (Mediatech, Manassas, VA) supplemented with 10% fetal calf serum, 10mM glucose and 4ml of 7.5% sodium bicarbonate (Invitrogen). As cultures approached confluence, microglia were removed from cultures by vigorous shaking. These microglia were then plated at high density and cultured in the presence of GM-CSF (0.5ng/ml, Peprotech, Rocky Hill, NJ). Microglia cultures were used 4 days after plating.

2.3. Culture and infection of macrophages

Bone marrow-derived macrophages (BMDM) were cultured as previously described [17]. Bone marrow was obtained from juvenile normal macaques aseptically at necropsy, vortexed, and filtered through 70µm pore filters. Macrophages were grown in IMDM media (Mediatech) supplemented with 10% FCS and antibiotics (Invitrogen). BMDM were infected by incubating with SIVmac251 (a neuropathogenic strain) using 100 TCID₅₀ per T25 flask for four hours before washing and culturing for a further 48 hours. At that point, media were harvested, pooled, and macrophages trypsinized. The macrophages were resuspended in fresh macrophage media.

2.4. Stimulation of glial cultures

To model the early events following SIV neuroinvasion, purified microglia were washed twice with PBS and incubated for six hours with one of the following: control BMDM supernatant (CMS), SIV-infected BMDM supernatant (SIMS), control BMDM cells, or SIV-infected BMDM cells.

2.5. Quantification of cytokines secreted

Samples were taken immediately after addition of macrophages or conditioned media and again after 6 hours. Aliquots were frozen at -80°C until cytokines were measured by multiplex. Each aliquot underwent a single freeze-thaw cycle. Quantification of secreted cytokines was performed with the Luminex platform and Milliplex non-human primate-specific kits (Millipore, Billerica, MA) using the manufacturer's instructions. Each sample was measured in duplicate and averages plotted. For samples stimulated with conditioned media, the initial levels were subtracted from those measured 6 hours later. Thus, we are only reporting *de novo* secretion of cytokine by microglia.

2.5.1. Exclusion criteria—Each analyte in the kit is theoretically quantifiable from 1ng/ml through 10,000ng/ml. *De facto* ranges are smaller, and vary by analyte. For our analyses, any analyte where greater than 50% of the values were extrapolated beyond the range of the working standard curve were excluded. Analytes excluded as a result of this are: IFN-γ, IL-1ra, CCL4, CD40L, TGFβ & IL-18. Some analytes returned measured values at or near zero for each sample: it is known, for example, that IL-10 was not cross-reactive with rhesus macaque proteins. It was outwith the scope of these experiments to determine if other near zero values were accurate measurements, or the result of poor cross-reactivity. Analytes excluded for this reason are: IL-1b, IL-4, IL-5, IL-10, IL-12, IL-13, IL-15, IL-17, & CCL3.

2.5.2. Statistical analyses—To determine significant changes in cytokine levels, ANOVA with Tukey's post test was performed using GraphPad InStat version 3.0a for Macintosh (GraphPad Software, San Diego, California).

2.6. Blocking studies and image analyses

Parallel studies were performed using purified microglia cultured on chamber slides. As with the secretion studies, microglia were cultured for four days before washing twice with PBS and the addition of macrophages or their supernatants. In the final 2 hours of incubation, secretion of cytokines was blocked with Brefeldin A (5µg/ml, [8]). Cultures were fixed with 2% paraformaldehyde in PBS for 20 minutes before staining for immunofluorescent microscopy. Antibodies used were as follows: GFAP (Sigma, clone GA5, 1:1000 dilution), IL-6 (Chemicon, MAB1033, 1:2,000 dilution), CCL2 (Pharmingen, 5D3-47, 1:500 dilution), TNF-α (Santa Cruz, Goat, 1:250 dilution) and VEGF (Santa Cruz, Rabbit, 1:250 dilution). Cells were permeabilized with 0.1% Triton (in PBS with 1% BSA) and stained for relevant cytokine. Antibodies were detected with secondary antibodies directly conjugated with Alexa 488 or 568 (Molecular Probes/Invitrogen, Carlsbad, CA).

3. RESULTS AND DISCUSSION

These studies were undertaken to investigate acute microglial activation in the context of HIV neuroinvasion. The presence of inflammatory macrophages is one of the hallmarks of HIV infection of brain. Therefore we sought to determine if the lentiviral infection status of BMDM was a determinant in the activation status of microglia or if the secreted factors of infected macrophages was more important. As these studies used microglia in culture, we began by comparing the morphology of microglia *in situ* and *in vitro*.

3.1. Morphological examination of microglia *in situ*

Microglia alter their morphology from a ramified morphology (Figure 1A) to amoeboid (Figure 1B) in neuroinflammation. AIF-1, an actin binding protein, is considered to be a microglial-specific marker within normal brain [12, 18, 19]. As such, AIF-1 is ideally suited to examining morphological changes in microglia. Cells expressing AIF-1 in brain were ramified (C), changing to amoeboid—and considerably smaller—on activation (D).

Ramified microglia sample their environment using long processes. These processes retract on activation, allowing the microglia to migrate to the source of infection [20]. We were interested to determine if SIV infected macrophages would activate cultured microglia to alter from a ramified to amoeboid morphology.

3.2. Morphological examination of microglia *in vitro*

To confirm the microglial cells were pure, cultures were checked for contaminating astrocytes. No GFAP immunopositive cells were observed in the microglial cultures (25 non-overlapping fields, not shown). When stained with AIF-1 antibody, cultured microglia have a ramified morphology with many fine processes and a central nucleus (Figure 2A) similar to that observed in normal brain. When BMDM were coincubated with cultured microglia for 6 hours, the fine processes coalesce, or disappear entirely (Figure 2B), with orthogonal bands of AIF-1 staining common and intense peripheral staining. There was an apparent morphological difference if the macrophages were infected with pathogenic SIVmac251 (Figure 2C): the orthogonal bands of AIF-1 expression were not observed, with no discernible staining pattern. AIF-1 expression was not specific to any cellular region. Some cells had perinuclear AIF-1 expression: other cells towards the periphery. Microglia could be easily distinguished from co-incubated macrophages, as, in the six hour incubation, the recently adherent macrophages had yet to spread substantially (arrows).

Incubation of microglia with cell-free macrophage supernatants revealed more subtle changes in morphology. Control macrophage supernatant (CMS) induced a less severe phenotype than did BMDM with approximately half of microglia (7/13) retaining some processes (Figure 2D). SIV-infected macrophage supernatant (SIMS, Figure 2E) was noted to induce a more amoeboid phenotype, with few cells retaining any processes (3/14). AIF-1 expression was fairly evenly distributed across the cell with either CMS or SIMS.

Thus, microglia in culture appear to behave in a manner similar to that observed *in vivo*, with a ramified morphology that is rich in AIF-1 expression. Activation appears to cause these processes to coalesce in a stimulus-dependent manner.

3.3. Cytokine secretion in microglial cell cultures

To determine which interleukins and other cytokines/chemokines were differentially regulated in microglia in the context of HIV neuroinflammation, we cultured purified microglia with control or SIV-infected BMDM or their supernatants. For each treatment, the initial level of cytokine was subtracted from final levels. Therefore, we are reporting the *de novo* secretion of cytokine induced within 6 hours by BMDM or cell-free macrophage supernatant.

There was a low level of secretion of interleukin 2 (IL-2, Figure 3A) when macrophages were present, with a larger increase in the presence of control BMDM (29.3pg/ml) compared to SIV-infected BMDM (19.93pg/ml). This difference was not, however, statistically significant ($p>0.05$). SIMS was sufficient to induce an increase in IL-2 secretion to a level approaching that of SIV-infected BMDM (14.81pg/ml). This was higher than that observed for CMS (3.45pg/ml). The only statistically significant difference was between control macrophages and control media ($P<0.01$). These levels were still low, in agreement with previously published data [21]. SIVE/HIVE is characterized by the presence of inflammatory macrophages, with infrequent T cells [22]. It is therefore not surprising that we saw only a modest increase in a cytokine associated with T cell proliferation.

Substantial quantities of interleukin 6 (IL-6) secretion were noted in all four treatment groups (Figure 3B). SIV-infected BMDM (644pg/ml) induced secretion of IL-6 that was not significantly different from control BMDM (589pg/ml). The secretion was significantly higher when SIMS was added to the glia cultures compared to CMS ($p<0.001$). CMS induced a secretion of 330pg/ml. SIMS induced secretion of IL-6 greater than 6,000pg/ml which exceeded the working standard curve for the analyte. The infectious state of the BMDM made no difference to the quantity of IL-6 secreted ($p>0.05$). A factor secreted by SIV-infected BMDM induced a much higher level of IL-6 than the BMDM themselves. Microglia are therefore capable of rapid secretion of IL-6 in the presence of proinflammatory mediators, indicating IL-6 may be synthesized by microglia and stored awaiting a signal to induce the secretion. There are well characterized neurotoxicity manifestations associated with HIV infection [22], including increased IL-6 secretion of the neurotoxic IL-6 by glia in response to gp120 [23]. Therefore, rapid secretion of high levels of IL-6 by microglia would be anticipated to be a detrimental effect of SIV-infected macrophage infiltration into brain [24].

Interleukin 8 (IL-8, Figure 3C) was secreted at the same level by purified microglia regardless of whether BMDM were infected with SIV (1,095pg/ml) or uninfected (1,181pg/ml). CMS induced a significantly lower level of IL-8 (490pg/ml, $p<0.001$). Thus, contact with macrophages induced a secretion of IL-8 over and above that induced by supernatant alone. SIMS induced secretion of greater than 5,000pg/ml of IL-8, which exceeded the working standard curve for the analyte. Expression of IL-8 has been recently demonstrated to be increased in microglia in HIVE brain tissue [25], possibly in response to gp120 [26].

Our data suggest that the increased IL-8 expression observed in glial nodules by the Berman group may be largely due to a factor secreted by HIV-infected macrophages. IL-8 has been shown to have neurotoxic effects, and thus, plays a role in cognitive dysfunction associated with HIV [27].

Monocyte Chemoattractant Protein-1 (MCP-1, CCL2) was secreted at significantly higher levels in the presence of BMDM than supernatant (Figure 3D, $p < 0.01$), with SIV-infected BMDM inducing secretion of 3,513pg/ml and control BMDM inducing 3,593pg/ml. CCL2 has been reported to be increased in CSF of HIV [28] and SIV-infected individuals [29], but not correlated with levels in plasma in either humans [30] or macaques [31]. Additionally, CCL2 was not observed in brains of macaques with SIVE [32]. Therefore, the source of CCL2 has been open to debate in the context of HIV neuropathogenesis. However, SIMS did induce a marginally higher level of CCL2 (1,087pg/ml) than did CMS (687pg/ml, $p > 0.05$), indicating that microglia were secreting significantly higher levels of CCL2 in response to contact with inflammatory macrophages than secreted factors.

Control BMDM induced secretion of 47.72pg/ml granulocyte macrophage-colony stimulating factor (GM-CSF) compared with 33.91pg/ml by SIV-infected BMDM (Figure 3E, $p > 0.05$). This was higher than the levels of GM-CSF induced by media alone: SIMS induced 16.04pg/ml, and CMS induced 11.23pg/ml, which were not significantly different from each other ($p > 0.05$). Contact with BMDM induced significantly higher levels of GM-CSF compared their respective media ($p < 0.05$). It should be noted that the microglia had GM-CSF in the culture medium until washed with PBS immediately prior to experimental treatments. GM-CSF inhibits multinucleate giant cell (MNGC) formation [33]. MNGC are a hallmark of late stage HIV infection in brain [34]. It is probable that these late stage events are not mimicked in our model of acute HIV infection.

Tumor necrosis factor- α (TNF- α , Figure 3F) was found to be secreted at highest levels when microglia were incubated with control BMDM (138pg/ml) compared with SIV-infected BMDM (105pg/ml). SIMS induced secretion of 101pg/ml TNF- α , with CMS 75pg/ml. This, again, was surprising, especially as ourselves and other groups have shown TNF- α to be upregulated when SIV-infected macrophages are present in brain [14]. However, as there was no significant difference between secretion induced by control macrophages and SIV-infected macrophages ($p > 0.05$), it is apparent that the presence of macrophages may be just as important as that they are productively infected with SIV for the induction of TNF- α secretion by microglia. It is also possible, that microglia are not the primary producers of TNF- α in encephalitis: astrocytes and macrophages are also known to produce TNF- α [35].

Vascular endothelial growth factor (VEGF) was secreted (Figure 3G) following incubation with either SIV-infected BMDM (9.98pg/ml) or control BMDM (13.92pg/ml). This was approximately tenfold lower than levels secreted when microglia were incubated with SIMS (124pg/ml) or CMS (99pg/ml). This suggests that microglia secrete VEGF in response to a secreted factor from macrophages (whether SIV-infected or not). As the levels are low in response to BMDM, it is probable that this factor is secreted slowly, and is upregulated when BMDM are infected with SIV. VEGF secretion could lead to astrogliosis [36] and increased permeability of the blood-brain barrier [37].

Our data show a cytokine profile consistent with the hypothesis that, under inflammatory conditions, microglia behave similarly to activated macrophages [22]. Namely, expression of IL-6, IL-8, GM-CSF, and TNF- α all increase in the presence of SIV infected macrophages. Low levels of IL-2 were consistent with the nature of SIVE/HIVE as a predominately macrophage disease (*ibid*).

Surprisingly, co-incubation with uninfected BMDM resulted in cytokine secretion comparable to that of SIV-infected BMDM. This suggests that cell-to-cell contact is sufficient to induce an immune response. As perivascular macrophages are generally held within the basal lamina [38], microglia are unlikely to encounter macrophages under normal conditions in the brain, so it is possible that resting microglia respond to any contact with macrophages. Increased macrophages in brain have been associated with rapid disease progression [39] has also been shown to have an altered cytokine profile in the periphery [15, 40].

3.4. Visualization of select cytokines in microglial cultures

It is not possible to determine the cellular source of secreted cytokines with mixed cell cultures using multiplex. The BMDM are just as likely to be the source of cytokines as microglia. In order to address this limitation, we cultured microglia on chamber slides in the presence or BMDM or their supernatants, blocking secretion of cytokines with Brefeldin A. BMDM were differentiated from microglia based on morphology. We selected one each of interleukins (IL-6), chemokines (CCL2), angiogenic cytokines (VEGF), and TNF- α as a cytokine previously reported in SIV encephalitis [14].

IL-6 was detected at low levels within microglia in culture (Figure 4A) or when treated with either control (B), SIV-infected BMDM (C), or CMS (D), consistent with multiplex data (Figure 3C). There was considerably increased expression of IL-6 observed when microglia were incubated for 6 hours with SIMS (E).

Microglia in culture express a low level of CCL2 (Figure 4F) that is increased following incubation with either control (G) or SIV-infected (H) bone marrow-derived autologous macrophages. Incubation with CMS (I) or SIMS (J) induced a lower level of CCL2 than did BMDM. These data confirm the multiplex array (Figure 3G).

Control microglia had very low levels of TNF- α (Figure 4K), with increased expression following incubation with macrophages, regardless of whether the macrophages were infected with SIV (M) or not (L), consistent with multiplex data (Figure 3J). CMS (N) and SIMS (O) also increased the levels of TNF- α expressed over baseline, although there appear to be differences in the patterns of expression from numerous, disseminated, fine vesicles for SIMS treatment through very intense, but focal, staining with CMS.

VEGF was expressed by all microglia stained (P–T). Intense foci of staining were apparent with either CMS or SIMS treatment, with a more diffuse pattern evident when BMDM were incubated with microglia.

These data confirm the multiplex results (Figure 3), with similar levels of cytokines expressed by Brefeldin A-treated microglia following co-incubation with control or SIV-infected BMDM. The data also confirm that incubation with SIMS induced very high levels of IL-6. The BMDM added to the cultures were noted to express IL-6 (Figure 4B&C), CCL2 (G), TNF- α (L) and VEGF (Q). Therefore, it is probable that, in addition to microglia secreting cytokines, BMDM were contributing to the cytokine levels in Figure 3.

3.5. Conclusion

Microglia cultured from normal rhesus macaques have a morphology similar to those observed *in vivo* (Figures 1 and 2), and secrete low levels of pro-inflammatory cytokines (Figures 3 and 4). On incubation with either control or SIV-infected BMDM, microglia take on an amoeboid morphology (Figure 2 B, C) with alterations in AIF-1 expression reflecting alterations in actin cytoskeleton. This coincides with increased expression of pro-

inflammatory cytokines (Figures 3 and 4). There was no significant difference between levels of cytokines secreted by control BMDM and SIV BMDM.

Our data suggest that microglial activation in the brain may be due to contact with macrophages. This response is not changed by SIV infection of macrophages. However, SIV-infected macrophages appear to secrete factors that augment the microglial response. This suggests that the neuroAIDS response relies on a two-pronged initiation: a macrophage-microglia contact that is not related to the infection status of the macrophage. This is supplemented by microglia responding to soluble mediators released from SIV-infected macrophages.

Combined, these results suggest that further examination of cultured microglia cells will provide a useful tool to examine morphological changes, cytokine expression, and cell-to-cell interactions in glial cells. It will be of interest to determine if these changes are conserved in other neuroinflammatory diseases such as Lyme neuroborreliosis, multiple sclerosis, West Nile Virus or Eastern Equine Encephalitis.

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Figure 1. Morphological characterization of microglia *in situ*

Typical morphology of a ramified microglia in nonhuman primate cortex stained by the Golgi-Cox method (A). In brains with SIV encephalitis, microglia take on an amœboid, activated phenotype (B). This is mirrored using immunofluorescent staining for AIF-1 with ramified morphology (C) becoming amœboid in areas of inflammation (D).

Figure 2. Morphological characterization of microglia *in vitro*

Cultured microglia, have abundant processes radiating from the body when visualized with AIF-1 immunofluorescent staining (A). When incubated with noninfected macrophages (arrows), cultured microglia have an activated, amoeboid morphology (B), with evident stress fibers. When incubated with SIV-infected macrophages, the AIF-1 is localized to the periphery (C), with amoeboid morphology. Incubation with control BMDM supernatant induced an intermediary phenotype (D), with some process extension is still present, but not to the extent of ramified microglia. SIV-infected BMDM supernatant induced amoeboid morphology (E), with absence of processes, but with AIF-1 staining present throughout the cell.

Figure 3. Cytokine secretion by cultured microglia in the presence of SIV-infected macrophages and their supernatants

Incubation of microglia with control macrophages induced secretion equal or greater than that of SIV macrophages for most cytokines, consistent with the presence of macrophages as the inflammatory stimulus (A–G). SIV infected macrophages induced responses similar to the levels induced by control macrophages, often inducing a slightly lower response. SIV-infected BMDM supernatant induced the highest responses of IL-6, IL-8, and VEGF (B, C & G). Control BMDM supernatant induced the lowest values for most cytokines, with the exception of VEGF (G). Statistical significantly different levels of cytokines expression between: control BMDM supernatant and Control BMDM is represented by *; SIV-infected BMDM supernatant and SIV-infected BMDM by #; control BMDM supernatant and SIV-infected BMDM supernatant by ◊. There was no significant difference between levels of cytokines secreted by control BMDM and SIV BMDM.

Figure 4. Visualized expression of cytokines in cultured microglia

IL-6 production was detectable in all treatments (A–E). SIV-infected BMDM supernatant-treated (E) microglia produced abundantly higher levels, consistent with multiplex data. The presence of macrophages (arrows), either control (G) or SIV-infected (H) induced higher levels of CCL2 than did their respective supernatant (I and J, respectively), although there was increased expression over the untreated microglia (F). There was minimal expression of TNF- α by resting microglia in culture (K), which was increased in all four treatment groups. The most intense staining was in the macrophage treatment groups (L, M) with the lowest being control BMDM supernatant (N). VEGF was expressed in each of the conditions, including untreated microglia (P). The presence of macrophages (arrows), either control (Q) or SIV-infected (R) induced minimally increased expression of VEGF. Cell free supernatant from control (S) or SIV-infected (T) macrophages induced a punctate expression pattern indicative of increased secretion.