Distinguishing Inflammation from Tumor and Peritumoral Edema by Myeloperoxidase Magnetic Resonance Imaging

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Abstract

**Purpose:** Inflammation occurs routinely when managing gliomas and is not easily distinguishable from tumor regrowth by current MRI methods. The lack of noninvasive technologies that monitor inflammation prevents us from understanding whether it is beneficial or detrimental for the patient, and current therapies do not take this host response in consideration. We aim to establish whether a gadolinium (Gd)-based agent targeting the inflammatory enzyme myeloperoxidase (MPO) can selectively detect intra- and peritumoral inflammation as well as glioma response to treatment by MRI.

**Methods:** We carried out serial Gd-bis-5-HT-DTPA (GdO) MRI before and after treating rodent gliomas with different doses of oncolytic virus (OV) and analyzed animal survival. The imaging results were compared with histopathologic and molecular analyses of the tumors for macrophage/microglia infiltration, virus persistence, and MPO levels.

**Results:** Elevated MPO activity was observed by MRI inside the tumor and in the peritumoral cerebrum at day 1 post–OV injection, which corresponded with activation/infiltration of myeloid cells inhibiting OV intratumoral persistence. MPO activity decreased, whereas tumor size increased, as the virus and the immune cells were cleared (days 1–7 post–OV injection). A 10-fold increase in viral dose temporally decreased tumor size, but augmented MPO activity, thus preventing extension of viral intratumoral persistence.

**Conclusions:** MPO-Gd MRI can distinguish enhancement patterns that reflect treatment-induced spatiotemporal changes of intratumoral and intracerebral inflammation from those indicating tumor and peritumoral edema. This technology improves the posttreatment diagnosis of gliomas and will increase our understanding of the role of inflammation in cancer therapy.

Introduction

Management of brain tumors induces inflammatory responses that interfere with tumor imaging and monitoring the treatment course. Inflammation may also influence the outcome of the therapy in 2 opposite ways. It can lead to tumor control by killing cancer cells and establishing an anticancer immunity (1–8) or to tumor promotion by participating in glioma reoccurrence and progression (9–17). It is thus important to establish a noninvasive imaging technique that monitors intracerebral inflammation and distinguishes it from tumor to understand the clinical and physiologic consequences of this host response and to efficiently diagnose the outcome of cancer treatments that enhance or inhibit local inflammation.

Oncolytic viruses (OV) present a great potential for the treatment of malignant gliomas, due to their capacity to replicate in situ and reach peripheral invasive cancer cells. However, OVs are very immunogenic and, despite their replication capacity, they are rapidly cleared from the tumor by inflammatory cells that engulf virus-infected cancer cells (18–23). Because OV-induced inflammation is rapid and precisely localized, it is an optimal model to establish techniques for in vivo imaging of intracerebral inflammation during glioma treatment.

Myeloperoxidase (MPO) is an inflammatory enzyme present in myeloid cells (neutrophils, microglia, and macrophages). It is secreted during inflammation by activated, proinflammatory subsets of these cells (24). MPO utilizes hydrogen peroxide to catalyze the formation of hypochlorous acid, which is directly toxic to a variety of cell types.
Translational Relevance

Glioma management induces intracerebral inflammation undistinguishable from tumor regrowth with current MRI technologies. This poses a recurrent diagnostic dilemma that prevents the ability of the oncologists to provide the patients with a suitable treatment plan in a timely fashion. Moreover, it is accepted that inflammation influences the outcome of the treatment, but the role of this host response remains controversial, being described to control as well as promote tumor recurrence and progression. Because of the lack of means to monitor inflammation in vivo, this host response is not taken in consideration by current therapies, thus preventing further understanding of its clinical significance. Herein we address this diagnostic problem and establish a molecular MRI technology that tracks the spatiotemporal evolution of peri- and intratumoral inflammation while monitoring glioma response to treatment. Because this technology utilizes a stable gadolinium-based compound, it has a strong translational potential.

of reactive oxygen species that kill pathogens, covalently modify lipids, cause local damage, and further activate the inflammatory cascade (24, 25). Gd-bis-5-HT-DTPA (MPO-Gd) is a molecular MRI agent that reports MPO activity with high specificity and sensitivity (26–31). This agent has been validated in vivo to evaluate MPO activity and inflammation in atherosclerosis (32), experimental autoimmune encephalomyelitis (33), stroke (26), and myocardial ischemia (28). Imaging of MPO activity is possible because of a prolonged gadolinium (Gd) enhancement caused by MPO-mediated oxidation of the Gd-chelating agent that induces its polymerization and trapping in the tumor mesh (26, 27, 30, 34). Therefore, immediately after MPO-Gd administration, the MRI highlights areas of vessel leakage in the tumor and allows measurement of tumor size whereas prolonged enhancement observed 1 to 2 hours after injection of the agent reflects MPO activity.

We have investigated the possibility of using MPO-Gd MRI to analyze intratumoral and intracerebral inflammation during glioma treatment with OV and tested whether such inflammation was associated with improved therapeutic response. To do this, we examined the patterns of MPO-Gd MRI contrast enhancement in 2 different rodent glioma models (the rat D74-HveC and the mouse CT-2A gliomas) treated with different doses of the oncolytic herpes simplex virus hrR3 (35) and compared the imaging results with the extent of intratumoral/intracerebral infiltration/activation of inflammatory cells, viral load, MPO levels, and animal survival.

Our results indicate that MPO-Gd MRI reports the in vivo spatiotemporal evolution of the OV-induced inflammatory response and provides a powerful tool to understand the role of inflammation during glioma OV treatment.

Materials and Methods

Cells, tumor implantation, and treatment

D74/HveC rat glioma cells (36) were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum (FCS) and 7.5 μg/mL blasticidin S (Calbiochem; EMD Biosciences Inc.); CT-2A mouse glioma cells (provided by Dr. Thomas Seyfried, Boston College; ref. 37) were grown in DMEM with 10% FCS.

Male Fischer 344 rats (Taconic Farms Inc.) and C57BL/6j mice (National Cancer Institute, Frederick, MD) were kept according to the guidelines of the Subcommittee on Research Animal Care of MGH. Tumors were implanted using stereotaxy as described (36). Seven days after implantation, 103 or 105 plaque forming units (pfu) of hrR3 or equivalent volume of phosphate buffer (PBS) were injected in the tumor by using the same procedure and stereotactic coordinates as described for the cancer cells.

lacZ and MPO gene expression analysis

Total RNA from the anterior quarter of the tumor-bearing cerebrum was extracted with the RNeasy Lipid Tissue Kit (Qiagen), from animals treated with virus (105 or 107 pfu) or PBS (5 μL) for 1, 3, or 6 days. Three animals for each treatment group/virus dose/time point were used. cDNA was synthesized using the Omniscript Reverse Transcriptase Kit (#205113; Qiagen) and random primers (Invitrogen). TaqMan PCR was carried out with the ABI Prism 7000 HT Sequence Detection System and TaqMan PCR Master Mix (Applied Biosystems). PCR mix included a 25-μL aqueous solution containing each primer at 0.9 μmol/L, the probe at 200 nmol/L, and 2 μL of diluted cDNA. The PCR program included 1 cycle of 10 minutes at 95°C, 1 cycle of 10 minutes at 95°C, and 40 cycles comprising 15 seconds at 95°C, followed by 1 minute at 60°C. 18S rRNA was used as internal control. Relative quantification of gene expression was calculated as 2ΔΔCt, where ΔCt = difference between the numbers of cycles needed to reach saturation for the same gene in 2 different treatment groups. Primers were used as follows: LacZ forward-ttgctgcacctgtttaagtt, reverse-actcgcgcacatctgaact, probe-6FAM-cgtgcagcttgagc-TAMRA; 18S rRNA forward-ggacccaagcttact, reverse-ctcctactgctgtatcctca, probe-6FAM-aaagcgcagcggc-ggcg-TAMRA; MPO forward-ctgccacctgcgtttc, reverse-cgccacctgctggagc-TAMRA; 18S rRNA forward-ggacccaagcttact, reverse-ctcctactgctgtatcctca, probe-6FAM-aaagcgcagcggc-ggc-TAMRA; MPO forward-ctgccacctgcgtttc, reverse-cgccacctgctggagc-TAMRA; 18S rRNA forward-ggacccaagcttact, reverse-ctcctactgctgtatcctca, probe-6FAM-aaagcgcagcggc-ggc-TAMRA; MPO forward-ctgccacctgcgtttc, reverse-cgccacctgctggagc-TAMRA; 18S rRNA forward-ggacccaagcttact, reverse-ctcctactgctgtatcctca, probe-6FAM-aaagcgcagcggc-ggc-TAMRA; MPO forward-ctgccacctgcgtttc, reverse-cgccacctgctggagc-TAMRA; 18S rRNA forward-ggacccaagcttact, reverse-ctcctactgctgtatcctca, probe-6FAM-aaagcgcagcggc-ggc-TAMRA; MPO forward-ctgccacctgcgtttc, reverse-cgccacctgctggagc-TAMRA; 18S rRNA forward-ggacccaagcttact, reverse-ctcctactgctgtatcctca, probe-6FAM-aaagcgcagcggc-ggc-TAMRA; MPO forward-ctgccacctgcgtttc, reverse-cgccacctgctggagc-TAMRA; 18S rRNA forward-ggacccaagcttact, reverse-ctcctactgctgtatcctca, probe-6FAM-aaagcgcagcggc-ggc-TAMRA; MPO forward-ctgccacctgcgtttc, reverse-cgccacctgctggagc-TAMRA.

Immunohistochemistry

Rodent brains from animals treated with 105 pfu of virus or 5 μL PBS for 1, 3, or 9 days (3 animals/treatment group/time point) were frozen in an isopentane dry-ice bath and sectioned through the entire tumor volume. Every fifth section was collected for analysis. Tissue slides were fixed in ice-cold acetone and stained as follows. After blocking endogenous proteins and peroxidases with serum-free protein block (#X0090) and peroxidase-blocking reagent (#002428; DAKO-Cytomation), sections were incubated for 1 hour at room temperature with the following primary
antibodies (all antibodies were from Serotec, except for MPO antibody which was from Neomarkers): mouse anti-rat CD68 (MCA 341R) and CD163 (MCA 342R), rat antirabbit F4/80 (MCA497RT), and rabbit anti-human MPO (Ab-1). For bright-field staining, the slides were then incubated with a horseradish peroxidase–conjugated (HRP) secondary antibody (ECL anti-mouse IgG, NA931V, and anti-rat IgG, NA935V; Amersham Biosciences Ltd.; and anti-rabbit IgG BA-1000; Vector Laboratories), a Liquid DAB Substrate Chromogen system used for detection (#K3465; DAKO-Cytomation), and hematoxylin for counterstaining. For fluorescent staining, the slides were incubated with an anti-rat IgG-FITC secondary antibody (Jackson Immunology) and mounted with propidium iodide (PI) Vectashield mounting medium H-1300 (Vector Laboratories).

The paraffin-embedded human brain sections were deparaffinized, rehydrated, and treated with 1% SDS before staining. The rabbit anti-human MPO (Ab-1; Neomarkers), mouse anti-human CD68, and CD163 (AbD Serotec P34810 and Q86VB7) were used with the same secondary antibodies as described earlier.

Intratumoral viral spread was analyzed by detecting β-galactosidase activity with X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; Sigma-Aldrich).

Spectrophotometric assay for MPO activity

The anterior quarter of the tumor-containing cerebrum treated with 10⁵ pfu of virus or 5 μL of PBS for 1, 3, or 6 days (5 animals/group/time point) was extracted, weighed, and homogenized in 50 mmol/L potassium phosphate buffer at pH 6.0. After centrifugation, the pellet was resuspended in cetyltrimethylammonium bromide buffer (Sigma-Aldrich), homogenized again, and sonicated. After 3 cycles of freeze–thaw–sonication, the cell lysates were centrifuged and the supernatants were collected. Protein concentration was measured with the BCA Protein Assay Kit (#23225; Pierce, Thermo Fischer Scientific). The protein extracts were then used to measure MPO activity by detecting Amplitide ADHP (AAT Bioquant) oxidation through spectrophotometry at 535 nm. The units of activity were computed according to the following formula: activity (U/mL) = (AOD × Vₖ × 4)/(E × Δτ × Vₑ), where AOD is the change in absorbance, Vₖ is the total volume, Vₑ is the sample volume, E is the extinction coefficient = 3.9 × 10⁴ mol⁻¹ Ls⁻¹, and Δτ is the change in time. The resulting activity was normalized to 1 mg of protein or 1 mg of tissue to derive the specific activity. To measure the absorption, a 4-aminobenzoic acid hydrazide to the protein lysates before the spectrophotometric reading (38).

MRI

Rats treated with 10⁵ or 10⁶ pfu of virus or 5 μL PBS for 1, 3, or 7 days (3 animals/treatment group/viral dose/time point) were anesthetized with isoflurane (2.0% at 2 L/min) and imaged using a 4.7-T, 16-cm bore MRI system (Bruker Pharmscan). Rats were imaged 1 day before and 1, 3, and 7 days after injection of hrR3. MR images were acquired before and 2 hours after the intravenous administration of MPO-Gd, synthesized as previously described (29) from DTPA-Gd (Magnevist; Berlex Laboratories), and injected at 0.3 mmol/kg mouse and 0.1 mmol/kg rat, using a T₂-weighted rapid acquisition with refocused echoes (RARE) sequence [repetition time (TR) = 800 milliseconds, echo time (TE) = 13 milliseconds, matrix (MTX) = 192 × 192, slice thickness = 1.0 mm, number of excitations (NEX) = 8, field of view (FOV) = 2.5 cm for mouse and 3.5 cm for rats]. T₁-weighted imaging was also carried out (TR = 4217 milliseconds, TE = 60 milliseconds, MTX = 192 × 192, slice thickness = 0.8 mm, NEX = 8, FOV is the same as for T₂-weighted sequences). Standard DTPA-Gd was used in control animals to verify that MPO-Gd enhancement was specific for MPO activity.

MRI data analysis

Regions of interest (ROI) including the tumor, contralateral brain tissue, and muscle were selected using the freeware OsiriX (www.osirix-viewer.com). Contrast-to-noise ratios (CNR) were computed for each ROI according to the following formula: CNR = (postcontrast ROI_tumor−postcontrast ROI_muscle)/SD_noise−precontrast ROI_tumor−precontrast ROI_muscle)/SD_noise, where ROI_tumor is the ROI of the enhancing areas, and SD_noise is the SD of noise measured from an ROI placed in an empty area of the image. CNRs were normalized by dividing each CNR by the highest CNR to enable comparison between different animals. Activation ratios (AR) were computed by dividing the CNR of the late phase (75 minutes) over the CNR of the early phase (first postcontrast images, 5 minutes) to account for nonspecific enhancement from leakage. Tumor radius was computed by measuring the maximum transverse dimension.

Statistical analysis

Comparisons between multiple groups were carried out with 2-sided ANOVA test followed by means comparisons with post hoc Tukey’s test using the software Prism (Graphpad Software Inc.). Comparison between 2 groups was carried out using the Student t test. A value of P < 0.05 was considered to be statistically significant. All error bars indicate SEM.

Results

Kinetics of activation and recruitment of inflammatory cells

We have previously established that treatment of a rat glioma with hrR3 induces intratumoral recruitment of mature peripheral macrophages (CD68⁺/CD163⁺) and activation of brain inflammatory cells (CD68⁺/CD163⁻) that accumulate around the tumor borders (19, 20). To use OV treatment as a model for establishing in vivo imaging techniques of intracerebral/intratumoral inflammation, we analyzed the kinetics of CD68⁺ and CD163⁻ cells activation/recruitment during treatment of the D74-HveC rat glioma with hrR3.
Molecular MRI of Intracerebral Inflammation

Figure 1. Correlation of hrR3 clearance with activation/infiltration of CD68⁺ and CD163⁺ immune cells in the rat D74-HvEC glioma. Column 1 (LacZ), decrease of viral intratumoral spread ([β]-galactosidase staining (LacZ); blue) between days 1 and 9 post-hrR3 injection (black arrows indicate persisting OV clones). Columns 2 and 3, CD68⁺ cells (brown) in the tumor and peritumoral area (black arrows) in animals receiving hrR3 (CD68-hrR3) or PBS (CD68-PBS). Columns 4 and 5, CD163⁺ cells (brown) localized exclusively inside the tumor of hrR3- and PBS-treated animals (CD163-hrR3 and CD163-PBS). For each time point, adjacent slides are shown.

glioma with hrR3 beginning 1 day post–viral injection to a point when the animals appeared moribund (6 days from PBS injection in control animals, and 9 days from viral dosing in treated rats), which was considered as end of therapy. Because both these inflammatory cell subtypes engulfed hrR3-infected cancer cells (19, 20), we compared this kinetic profile with the kinetics of viral clearance.

The virus was gradually and completely cleared from the tumor between day 1 post–viral dosing and the end of therapy (Fig. 1). The intratumoral viral concentration matched with activation/infiltration of CD68⁺ and CD163⁺ cells, which were prominent at day 1 post–viral dosing (compared with animals receiving PBS), decreased at day 3, and were similar to control in moribund animals.

Because brain inflammatory cells produce high levels of MPO (31, 39), we hypothesized that MPO-Gd MRI could monitor OV-induced infiltration and activation of CD68⁺ and CD163⁺ cells. To test this hypothesis, we analyzed whether the kinetics of activation/infiltration of these inflammatory cells and viral clearance corresponded to changes in MPO mRNA levels and enzymatic activity (Fig. 2). For this assay, rats with established D74-HvEC gliomas were treated with PBS (controls) or hrR3 and sacrificed at days 1, 3, and 6 after virus injection. We chose 6 days as the last time point because of the high mortality of the control rats. We found that more than 90% of viral LacZ mRNA is cleared from the tumor between days 1 and 3 and the remainder is cleared by day 6 [undetectable by reverse transcription (RT)-PCR; Fig. 2A]. Accordingly, we observed the highest levels of MPO mRNA and activity at day 1 post–viral dosing and both decreased significantly by day 3 (Fig. 2B–D). No MPO mRNA levels were detected by RT-PCR in control animals and at day 6 after virus injection, however, we could still observe some MPO activity in both these situations (Fig. 2B and C). The decrease of MPO activity between days 3 and 6 was not very strong in OV-treated animals and showed a high variability among animals, suggesting that intratumoral decrease of macrophages is ongoing but not yet fully established as observed when rats appeared moribund (Fig. 2C). Because it is impossible to precisely dissect the tumor tissue from adjacent cerebrum, these assays do not distinguish between intratumoral and peritumoral MPO activities. However, immunohistochemical staining of rat brains harboring gliomas and treated with OV or PBS clearly shows the presence of MPO in both the intratumoral and peritumoral areas (Supplementary Fig. S1), thus matching the distribution of CD68⁺ and CD163⁺ cells observed in Figure 1.

We have previously reported on the similarity of inflammatory cellular responses in mice and rats carrying human or syngeneic gliomas treated with different types of OV (19, 20, 22, 23). Accordingly, comparison of hrR3 spread in the CT-2A mouse glioma between 6 and 72 hours from OV injection showed that most of the intratumoral viruses were cleared within 3 days of delivery, and this matched with infiltration of F4/80⁺ macrophages (Fig. 3A) and increased MPO mRNA levels and enzymatic activity (Fig. 3B). Moreover, the GBM from a patient treated with the oncolytic adenovirus ONX-015 (40) also presented infiltration of CD68⁺ and CD163⁺ cells (20) expressing MPO (Supplementary Fig. S2).

Altogether, these data indicate that the inflammatory response induced by OV is associated with MPO mRNA levels and enzymatic activity. This phenomenon is not species-specific and suggests the possibility to image inflammation in vivo with MPO-Gd MRI.

MPO-Gd MRI during OV treatment

We determined whether MPO-Gd MRI could detect the kinetics of CD68⁺ and CD163⁺ cells activation/infiltration observed by immunohistochemistry and distinguish the areas of inflammation from the bulk tumor tissue. We imaged rats carrying D74-HveC tumors 1 day previral dosing for tumor baseline signal, and 1, 3, and 7 days post–hrR3 injection (Fig. 4). Each imaging session included analysis of tumor size (we measured the diameter...
of the region enhancing 5 minutes after injection of the MPO-Gd contrast agent) and quantification of the MPO activation ratio (determined by analyzing the persistence of the contrast 75 minutes after injection of MPO-Gd). We could not image the animals after day 7 postviral dosing because of a rapid health decline.

Before viral dosing (day 0), the MPO-Gd enhancement faded over time during the imaging session, indicating low MPO activity (Fig. 4A). At days 1 and 3 post–viral dosing, the MPO-Gd contrast decreased comparatively slower inside the tumor center and increased in the parenchyma surrounding the tumor (Fig. 4A). Calculation of the MPO activation ratio from MPO-Gd MRI showed a 3-fold increase between days 0 and 1 and a trend of decrease from day 1 to 7 (Fig. 4C), which matched with the MPO activity measured through the enzymatic assays (Fig. 2C). At day 7, most of the enhancement in the center of the tumor faded 75 minutes after MPO-Gd injection (Fig. 4A and C) and few CD163+ macrophages were detected by histopathology in these tumors (Supplementary Fig. S3). However, there was persistence of MPO-Gd contrast at the periphery between the tumor and the brain parenchyma and histopathology revealed accumulation of CD68+ cells in the same regions (Supplementary Fig. S3). MRI carried out at day 1 after OV treatment, using standard DTPA-Gd as contrast agent, indicated rapid leakage of this agent from the tumor tissue and adjacent brain (Fig. 4B and D). These results show that MPO-Gd contrast enhancement comes from MPO secreted by 2 distinct cell populations, inside and surrounding the treated tumor, reflecting a difference in the host response to OV therapy.

Comparison of images obtained through MPO-Gd MRI with those obtained using dextran-coated iron oxide particles (MION), which are incorporated by phagocytes and confer superparamagnetic properties to these cells (41), on animals treated with hrR3 for 3 days indicates that these 2 technologies for in vivo imaging of immune cells overlap only partially. MION imaging did not detect the peritumoral area of inflammation visible through MPO-Gd MRI but had a broader distribution inside the tumor (Supplementary Fig. S3).

Analysis of tumor size (Fig. 4E) indicated tumor growth between days 0 and 1. This growth was slower between days 1 and 3 post–hrR3 injection and became pronounced again between days 3 and 7. The retardation of tumor growth observed between days 1 and 3 is likely due to the oncolytic activity of the virus and was not observed in animals treated with PBS. Notably, the overall trend of tumor size change after virotherapy, as reported on the diameters measured by MRI at 5 minutes after delivery of the contrast agent, is opposite of the trend of in vivo MPO activity measured from delayed images (75 minutes post–MPO-Gd injection; cf. Fig. 4C and E), underscoring that this technology can distinguish areas of inflammation from the tumor tissue.

We then imaged and measured hrR3-mediated induction of MPO activity with MPO-Gd MRI in mice carrying the
CT-2A glioma (Fig. 5). Also in this case, we detected a spatio-temporal change in the enhancement pattern between the early and delayed images, which differentiated tumor from inflammation. We found that increased \textit{in vivo} MPO activity reported by molecular MRI following viral treatment matched with the infiltration of F4/80$^+$ monocytic cells (Fig. 3) and with increased MPO activity measured through enzymatic assays on excised tumors (Fig. 3), suggesting that this technology is applicable to different species.

**Increased viral dose augments the inflammatory response and does not allow prolonged viral persistence**

To further match the MPO-Gd contrast enhancement with changes in virus-induced inflammation, we compared rats treated with 2 different doses of hrR3 ($10^5$ and $10^6$ pfu). Increasing viral load at the time of injection did not change the time frame with which the virus is completely cleared; that is, at 7 days, no virus was detected through RT-PCR in either treatment group (data not shown). One day after virus injection, there was 10-fold difference in LacZ between these 2 treatment groups that related to the difference in viral dose, but the MPO mRNA levels were similar (Fig. 6A and B). At day 3, the difference in LacZ mRNA between these 2 treatment groups was only about 2-fold and the group receiving the higher viral dose had increased MPO mRNA (Fig. 6A and B). This corresponded to increased MPO activity measured by MPO-Gd MRI (Fig. 6C) and smaller tumor size (Fig. 6D).

**Discussion**

We have shown that the myeloid cells activated in the brain and infiltrating the tumor upon OV treatment induce MPO, thus allowing MRI of their inflammatory activity through a gadolinium-based molecular imaging agent (27, 29). Our data indicate that the kinetics of intracerebral/ intratumoral inflammation, verified by histopathology of CD68$^+$ and CD163$^+$ cells in a rat orthotopic glioma model treated with an oncolytic herpes simplex virus, corresponds to the kinetics of MPO activity measured on excised brains by an enzymatic assay. These changes can be tracked \textit{in vivo} through MPO-Gd MRI. The specificity of the contrast induced by MPO-Gd was proved by comparing MPO-Gd MRI with standard DTPA-Gd MRI. Moreover, previously published works have shown that there is no detectable MPO-Gd MRI contrast in mice knocked out for MPO, thus emphasizing the specificity of this MRI agent (26, 28, 29).
31). The levels of viral LacZ and host MPO mRNAs display similar kinetics. However, whereas MPO mRNA is only detectable during the acute phase of this inflammatory response, baseline MPO activity is always present. This could be due to a general low abundance of MPO mRNA molecules or to a dual MPO regulation system: genetic and enzymatic (42). Because MPO-Gd MRI is reproducible in mouse and rat gliomas established in the respective syngeneic animal model and because we have shown that activation of CD68 + and CD163 + cells is not virus-, tumor-, or species-specific, and that these cells infiltrate the tumor of patients treated with OV (19, 20,
22, 23) and secrete MPO, there is high translational potential for this technology. This is strengthened by the lower toxicity of MPO-Gd than that of many clinically approved MRI agents (30).

An important advantage of MPO-Gd MRI is its ability to distinguish between inflammation and tumor. Inflammation decreases between days 1 and 7 post–viral dosing, whereas the tumor size increases. Moreover, comparison of animals treated with 2 different doses of virus shows stronger inflammation and smaller tumors for animals receiving the highest viral dose. Indeed, even though OVs induce other oxidases in cancer cells, MPO-Gd is specific to MPO, which is expressed only in myeloid cells. Current MRI strategies cannot distinguish between inflammatory areas from tumor regrowth. This is a recurrent diagnostic dilemma that prevents the ability of the oncologists to provide the timeliest and most suitable treatment plan to the patient. Thus, this MRI technology can solve a critical diagnostic problem for the treatment of brain tumors. To establish the broad diagnostic applicability of this technology, it is important to test its capacity to detect inflammation during other therapeutic strategies such as radiation, immunotherapies, and treatment with anti-inflammatory and antiangiogenic drugs.

Attempts to image intracerebral inflammation by MRI were previously made using MIONs (41), which detect all phagocytic cells. However, even though this strategy can detect OV-induced intratumoral macrophages (19, 20), it does not enhance the peritumoral inflammation, nor provides information on tumor size. Conversely, MPO-Gd MRI can identify spatiotemporal changes of active inflammatory cells infiltrating into the cancer and in the brain parenchyma surrounding the tumor. Fusion of MION and MPO-Gd MRI scans in the same tumor model suggests that these 2 strategies detect 3 different cellular subsets: (i) intratumoral phagocytic cells not making MPO; (ii) peritumoral MPO-secreting cells that are not phagocytic; and (iii) intratumoral phagocytic cells that also produce MPO. It is not clear whether the peritumoral cells are not enhanced by MION MRI because MIONs do not reach these cells or because they are not phagocytic. We have previously published that CD68+ microglia surrounding the tumor infiltrates into the tumor region and engulfs the infectious OV (20), suggesting that MIONs do not diffuse into the peritumoral parenchyma. However, the phagocytic activity of these cells may be acquired only after their infiltration into the tumor, indicating that the different

Figure 5. MPO-Gd MRI in the mouse CT-2A glioma. A, MRI scans 5 and 75 minutes after MPO-Gd injection in 1 mouse with CT-2A tumor 1 day before (day 0) and 3 days post-hrR3 injection. B, quantification of MPO activation rate in 3 mice before and after treatment.

Figure 6. Treatment diagnosis in animals receiving different doses of hrR3. A and B, changes in mRNA levels for the viral LacZ and host MPO genes between animals receiving 10^5 pfu of hrR3 (considered as baseline level 1) and those receiving 10^6 pfu of virus at 1 and 3 days after treatment (n = 4). C and D, differences in MPO activation rate and tumor radius measured through MPO-Gd MRI at 1 and 3 days post–hrR3 injection at 10^5 or 10^6 pfu (n = 3).
cellular subsets enhanced by MION and MPO-Gd MRI are the same cells at different stages of inflammatory activation. Further comparison and combination of these imaging technologies will allow a deeper understanding of the distinct role of individual innate immune cells in the inflammatory responses (31, 39, 43–46).

Imaging inflammation is also useful in ways other than simply recognizing it in a recurring tumor after a specific treatment. As our understanding of cancer biology advances, increased relevance is being given to this host defense response. Even though the role of inflammation in the ultimate therapeutic outcome is unclear (being described both as controlling and as promoting tumor recurrence and progression), it is accepted that it influences tumor treatment one way or the other. It is therefore crucial to establish in vivo techniques that allow understanding of the relationship between inflammation and the outcome of tumor treatment. Demand for these techniques is further emphasized by the current trend of examining drugs that modulate immune processes, such as COX-2 inhibitors and nonsteroidal anti-inflammatory drugs, as anticancer agents. Finally, inflammation poses a crucial dilemma in OV treatment. Even though it is established that host innate immunity is detrimental for OV lytic activity, inflammatory cells can also kill cancer cells and synergize with OV in tumor treatment (47, 48). Virotherapies aimed at increasing and suppressing OV-induced immunity were studied and both strategies presented positive results (47, 48). In this respect, our data indicate that the increase in the viral dose temporarily augments the infiltration/activation of MPO+ cells. Even though the higher viral dose transiently decreases the tumor size, it is impossible to establish from these data whether the temporal lysis of the tumor is mediated by OV or immune cells alone or by the combination of these 2 factors. Further studies comparing MPO-Gd MRI and MION MRI during OV treatment in the presence of anti-inflammatory or proinflammatory agents will bring more insights into the role that different innate immune cells may have in the therapeutic outcome.

In conclusion, MPO-Gd MRI will strongly improve our diagnostic ability of the effects of cancer treatment on the tumor versus tumor microenvironment. Imaging inflammation during proinflammatory and immunosuppressive therapies will allow understanding of its role in cancer treatment and on side effects of current therapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


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