Adapted focal experimental autoimmune encephalomyelitis to allow MRI exploration of multiple sclerosis features

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ABSTRACT

We aimed to determine an optimal protocol for inducing a focal inflammatory lesion within the rat brain that could be large enough for an easier MRI monitoring while still relevant as a multiple sclerosis (MS) like lesion. We adapted a two-hit model based on pre-sensitization of the Lewis rat with myelin oligodendrocyte protein (MOG) followed by stereotaxic injection of pro-inflammatory cytokines (TNFα + IFNγ) within the internal capsule. We compared the following two strategies to increase focal lesion development for an easier MR translation: (1) a higher sensitization step (MOG50) or (2) a higher cytokine step with lower sensitization (MOG25). Control animals were administered only cytokines without MOG pre-sensitization. Animals were followed with T2, diffusion and T1 post gadolinium weighted images at 1, 3 and 7 days following cytokine injection. Immunostaining was performed at the same time points for macrophages (ED1), myelin (MBP and Luxol Fast Blue) and blood brain barrier integrity (IgG). At day 1, the focal lesions depicted with T2-weighted images were very similar among groups and related to vasogenic edema (high apparent diffusion coefficient (ADC), gadolinium enhancement and IgG extravasation) induced by cytokines irrespective of the pre-sensitization step. Then, at day 3, MOG50 rats developed statistically larger T2 lesions than MOG25 and control rats that were correlated with inflammatory cell accumulation. At day 7, MOG50 rats also showed larger T2 lesions than MOG25 and control rats, together with loss of anisotropy that were correlated with demyelination. In contrast, MOG25 and control rats developed similar MR lesions decreasing over time and almost undetectable at day 7. We conclude that with a high pre-sensitization step, the focal lesion can be monitored by MRI whose signal reflects some features of a MS-like lesion, i.e. edema, inflammatory cell accumulation and later demyelination.

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Introduction

Magnetic resonance imaging (MRI) is playing an increasing role in the investigation and clinical management of multiple sclerosis (MS) (Bakshi et al., 2008); MRI has helped in diagnosis, provided *in vivo* parameters for better understanding of the pathophysiology and has allowed monitoring of treatment efficacy in clinical trials (Bar-Zohar et al., 2008).

Animal models of MS are needed to further explore mechanisms of disease initiation and progression and to test therapeutic and restorative approaches (Gold et al., 2006; Steinman and Zamvil, 2006). Experimental autoimmune encephalomyelitis (EAE) is the most widely used animal model of MS because it shares several clinical and histological similarities with MS (Denic et al., 2011). However, the multifocal and disseminated nature of the lesions in EAE models makes it challenging to investigate a lesion at an early stage before it becomes clinically significant. The nature of these EAE lesions also makes it difficult to characterize the evolution and resolution of a given lesion. One solution is to use a focal lesion model, targeting a single EAE lesion that reflects a prototypic MS lesion (Kerschensteiner et al., 2004b). This model consists of a subthreshold sensitization of the Lewis rat with myelin oligodendrocyte protein (MOG) followed 20 days later by the induction of focal inflammation by microinjection of pro-inflammatory cytokines (tumor necrosis factor α [TNFα] and

interferon γ [INFγ]). Focal lesions share histological similarities with those seen in MS (Kerschensteiner et al., 2004b; Merkler et al., 2006) and this methodology could provide several advantages, including knowledge of the exact onset and location of the lesion.

As with humans, MR endpoints could be of great interest in rodent studies, allowing *in vivo* longitudinal monitoring that could be better than the five step clinical scale, which is relatively crude and does not correctly quantify and correlate with structural damage (Wujek et al., 2002). The following two conditions are required when using MRI for rodent inflammatory lesion exploration: (*i*) the MR signal should reflect the features of a MS lesion, with development of the entire lesion spectrum (including edema, blood brain barrier [BBB] damage and demyelination); (*ii*) the lesion should be large enough to fall within MRI resolution. MR exploration of EAE lesions is challenging because of their small size and selective location within small anatomic structures such as the brainstem, optic nerve or spinal cord (Sakuma et al., 2004). The focal model, induced into a well-defined anatomic structure within the brain, can facilitate MRI detection. However, the initial protocol has to be more severe to ensure that it is detectable with the lower resolution of MRI. Cytokine doses can be increased (Rausch et al., 2009; Serres et al., 2009a; Serres et al., 2009b) but come with the risk that lesions may appear more cytokine-like than MS-like. This distinction is a key point because the combination of MRI and focal lesion induction could be used as a platform for therapeutic tests, implying that this is a relevant model.

Consequently, considering the focal EAE model as a "two-hit" model (first sensitization and then cytokine injection), we compared two strategies to increase lesion severity for better MR application. In one strategy, we balanced toward a higher sensitization step; in another strategy, we balanced toward a higher cytokine step.

Materials and methods

To compare the high sensitization and high cytokine strategies, we followed two experimental groups over time: MOG50 (balanced toward sensitization) and MOG25 (balanced toward cytokines). We also followed two control groups: complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA; Fig. 1).

Animals

We used adult (150 to 210 g) female Lewis rats (RT1[1] genetic background), obtained from Janvier laboratories (France) and housed in groups under a 12:12-hour light/dark cycle with food and water *ad libitum*. All experiments were performed in accordance with European Union (86/609/EEC) and French National Committee (87/848) recommendations (animal experimentation permission, France 33/00055).

Sensitization procedure

Rats were anesthetized by inhalation of 1.5–2.0% isoflurane and were injected subcutaneously at the base of the tail with a total volume of 100 μl.

For the MOG25 group, the solution consisted of a mixture of 25 μg of the N-terminal sequence of rat MOG (amino acids 1 to 125, AnaSpec, Fremon, CA, USA) emulsified in IFA (Sigma-Aldrich, France). The corresponding control group was injected subcutaneously with 100 μl of saline emulsified in IFA (1:1). For the MOG50 group, the solution consisted of a mixture of 50 μg of 1–125 MOG emulsified in CFA (Sigma-Aldrich, France) containing 200 μg of heat-killed *Mycobacterium tuberculosis* (H37Ra; Difco, Detroit, USA). The corresponding control group was injected subcutaneously with 100 μl of saline emulsified in CFA + 200 μg of H37Ra (1:1).

Clinical evaluation

MOG-sensitized rats were weighed and scored daily for clinical signs of EAE as follows: 0, no sign; 1, tail weakness or tail paralysis; 2, hind leg paraparesis or hemiparesis; 3, hind leg paralysis or hemiparalysis; 4, complete paralysis (tetraplegy); and 5, moribund or death, as previously described (Weissert et al., 1998). Animals from MOG50 groups that died due to EAE before cytokine injection (n = 13) were replaced with additional rats.

Stereotactic injection of cytokines

From 18 to 22 days post sensitization (mean = 19.8 days, SD = 1.6 days), focal EAE was induced by stereotactic injection of cytokines. Rats were anesthetized by intraperitoneal (*i.p.*) injection of pentobarbital (1 ml/kg *i.p.*) and were immobilized in a stereotactic frame (David Kopff). Injection coordinates were measured from bregma to target the right internal capsule (2 mm posterior, 3.5 mm lateral and 6 mm deep), which is the largest white matter tract within the rat brain. A small hole was drilled in the skull and cytokines were infused through a glass-capillary pipette with calibration marks every 1 μl (Drummond Scientific company, Broomall, PA, USA) and with a tip
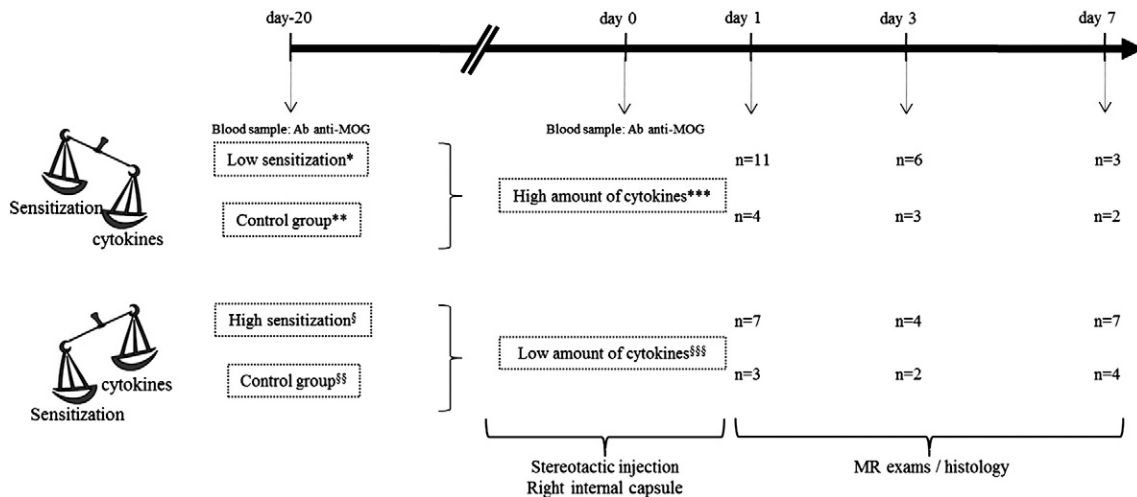

Fig. 1. Experimental protocol. Two strategies were tested, either balancing the model toward a higher sensitization step (bottom row) or toward a higher cytokine injection (top row). After completion of the model, animals were followed by MR at days 1, 3 and 7. *: 25 μg MOG + IFA; **: saline + IFA; ***: TNFα 720 ng + INFγ 160 ng; §: 50 μg MOG + CFA + H37Ra; §§: saline + CFA + H37Ra; §§§: TNFα 360 ng + INFγ 80 ng. See Materials and methods for details.