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Human mesenchymal stem cells abrogate experimental allergic encephalomyelitis after intraperitoneal injection, and with sparse CNS infiltration

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Abstract

Multiple sclerosis is a currently incurable inflammatory demyelinating syndrome. Recent reports suggest that bone marrow derived mesenchymal stem cells may have therapeutic potential in experimental models of demyelinating disease, but various alternative mechanisms, ranging from systemic immune effects to local cell replacement, have been proposed. Here we used intraperitoneal delivery of human mesenchymal stem cells to help test (a) whether human cells can indeed suppress disease, and (b) whether CNS infiltration is required for any beneficial effect. We found pronounced amelioration of clinical disease but profoundly little CNS infiltration. Our findings therefore help confirm the therapeutic potential of mesenchymal stem cells, show that this does indeed extend to human cells, and are consistent with a peripheral or systemic immune effect of human MSCs in this model.

Keywords

Mesenchymal stem cells; Multiple sclerosis; Experimental allergic encephalomyelitis

Multiple sclerosis is an acquired inflammatory demyelinating syndrome of unknown cause, and which is currently incurable. In many individuals, progressive disability occurs during the course of the disease, as a consequence of irreversible CNS damage. The ineffectiveness of current therapies has emphasised the importance of novel treatment approaches, and stem cells are widely held as having particular promise.

Bone marrow derived mesenchymal stem cells can proliferate substantially, and can differentiate into cells of all three germ cell layers, including neural cells; moreover they are relatively accessible, could be used for autologous therapy, and are capable of entering the CNS (particularly when damaged) from the circulation [6,11]. They are therefore considered good candidates for early clinical stem cell therapeutic studies.

Recently, reports have appeared suggesting that bone marrow-derived cells can ameliorate toxic and inflammatory experimental demyelinating disease following intravenous delivery [1,3,5,7,13–16]. However, whether this effect is achieved through cell replacement and

promotion of remyelination, axon protection, or through immunological effects, is not clear, and even the degree to which CNS infiltration occurs is uncertain. Here we explore an immune model of CNS demyelination, experimental allergic encephalomyelitis (EAE) and use intraperitoneal delivery of human mesenchymal stem cells to help test (a) whether human cells can indeed suppress disease, and (b) whether CNS infiltration is required for any beneficial effect.

Adult human bone marrow was obtained with informed consent from patients undergoing hip replacement surgery, and human mesenchymal stem cells (hMSCs) prepared as previously described [4]. Some were labelled using EGFP-CMV34 lentivirus construct, again as described elsewhere [4].

Sixteen C57/bl6 mice were immunised by subcutaneous tail vein injection of adjuvant together with and MOG peptide (35–55); they were also injected intraperitoneally with 200 ng pertussis toxin per mouse. Six days later, mice were injected with either human mesenchymal stem cells or phosphate-buffered saline (as a sham), via the intra-peritoneal route. Mice were divided into six experimental groups (Table 1): four groups were inoculated to induce EAE, and of these four, one group was treated subsequently with (sham) PBS, two with normal human MSCs (6th or 3rd passage), and one with GFP-transfected hMSCs; of the remaining two (non-EAE) groups, one received (sham) PBS, the other GFP-labelled hMSCs.

Mice were examined daily thereafter, and clinical EAE scores recorded using a standard scoring system [10] (0 – Normal; 1 – Tail flaccidity or hind limb weakness; 2 – Partial hind limb paralysis; 3 – Complete hind limb paralysis, spastic paresis, impaired righting reflex; 4 – Complete hind and fore limb paralysis; 5 – Dead). On Day 50, mice were sacrificed; brain and spinal cord, together with liver, were extracted for analysis. CNS tissue was fixed in 4% paraformaldehyde solution and 15 μ m sections were cut. Solochrome cyanine staining was used to identify demyelinated lesions in the brain and spinal cord, and immunofluorescence staining with anti-human nuclear antigen (HuNu), or GFP detection used to identify human cells at lesion sites.

We found substantial amelioration of clinical disease in mice treated with human mesenchymal stem cells (Fig. 2). The benefit seen in mice treated with ‘wild-type’-hMSCs did not reach statistical significance, in contrast with the results (Fig. 1; top) using GFP-labelled hMSCs, though we do not believe this apparent difference is likely to be meaningful biologically, other comparisons of GFP-labelled and normal hMSCs having failed to disclose any difference in properties [4].

Examination of both brain and spinal cord revealed the presence of disseminated demyelinated lesions, particularly in the cerebellum and brain stem and in the spinal cord. Exhaustive study of these areas, and of normal or unaffected CNS tissue, however, revealed that only extremely small numbers of human cells could be identified either using GFP expression (Fig. 2), or in the case of treatment with ‘wild-type’ hMSCs, using immunofluorescence labelling with anti-human nuclear antigen specific antibodies (results not shown).

Others have previously reported the amelioration of EAE using bone marrow derived cells [1,3,5,7,13–16]. Some studies propose and indeed help define systemic immune effects [3,5,7,13,14]; some find there to be CNS infiltration (following intravenous delivery of cells) [1,3,5,16] with certain groups proposing not immune ‘therapeutic’ activity but rather differentiation into microglia and/or oligodendrocyte lineage cells [1,5,16]. Alternatively, local neuroprotective effects dependent on local infiltration also have been suggested as the therapeutic mechanism [5,16]. Any and all of these possible activities could of course be

therapeutically relevant to MS [16], but whether one or other are more important functionally is a vital question if any cell therapy paradigm is to be optimised.

Our approach of delivering human MSCs intraperitoneally may have helped minimise access of these cells to the CNS [2]. Certainly using either antibody staining for human nuclear antigen or the fluorescence of GFP-labelled cells we found barely detectable levels of CNS infiltration, and yet observed a pronounced therapeutic impact of these cells on EAE severity. Others have shown that hMSCs delivered intraperitoneally do indeed infiltrate lymph nodes [9], and this of course may be highly relevant to their mode of action.

In our study, all of the animals were sacrificed at day 50, and we have not therefore excluded the possibility that, over this period, hMSC's infiltrated the CNS but also exited before day 50. What is perhaps most pertinent, however, is that inflammatory demyelinated lesions were obvious throughout the CNS at this stage (day 50) and yet few or no hMSCs were disclosed. Rejection would be unlikely in view of the recognised immune suppressive effects of these cells [12]; others have likewise found no rejection of human MSCs in rodent recipients [8,17].

Our findings therefore help confirm the therapeutic potential of mesenchymal stem cells, and that this does indeed extend to human cells, and finally our results are consistent also with a peripheral or systemic immune effect of human MSCs in this model.

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Fig. 1.

The clinical profile of EAE mice given active treatment with intraperitoneal human mesenchymal stem cells or sham PBS treatment. The top panel shows mean daily clinical scores following intraperitoneal PBS treatment (pink triangles) or active treatment with 1×10^6 6th passage hMSCs transduced with the CMV34-GFP construct at a multiplicity of infection of 10 (blue squares). Error bars shown. The bottom panel shows the cumulative score, time of disease onset, and maximum clinical score attained, all as indicated.

Fig. 2.

Human cells are found extremely rarely in the cerebellum of EAE-mice treated with GFP-labelled hMSCs given intra-peritoneally. A and B in the immunofluorescence photographs on the right refer to areas A and B in the left solochrome cyanine panel.

Table 1

Experimental design.

Treatment	No. of mice	Virus (MOI)	Cells/mouse
EAE-GFP hMSC	5	CMV34 (10)	7.5×10^5
EAE-PBS	5	n/a	n/a
EAE-hMSC (6P ^a)	4	n/a	1.1×10^6
EAE-hMSC (3P ^a)	2	n/a	1.0×10^6
Naïve-GFP hMSC	2	CMV34 (20)	1.2×10^6
Naïve-PBS	2	n/a	n/a

^a 6th or 3rd passage cells.