Autologous mesenchymal bone marrow stem cells: Practical considerations

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Abstract

A number of practical problems need to be addressed before any form of cell therapy can be widely applied in patients with multiple sclerosis. The choice of cell type is one considered elsewhere in this issue; others include the question of axon loss, that of continuing inflammatory disease activity, the mode of delivery of cells (bearing in mind the presence of innumerable lesions scattered throughout the CNS), the problem of measuring directly or indirectly the impact (if any) of an intervention, the timing of any treatment and perhaps above all the safety of the patient. All converge on the one increasingly relevant underlying question: when should stem cell treatments begin to be tested in patients?

Here we review the progress in various of these practical problems in order to explain how we have arrived at the conclusion that the clinical science has progressed to a stage where the ‘translation threshold’ can be safely and appropriately crossed, and therefore why we have already commenced in Bristol a small pilot/feasibility study of autologous bone marrow cell treatment in patients with multiple sclerosis.

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1. Introduction

Multiple sclerosis is a disorder which is, in theory, particularly attractive in relation to developing cell-based reparative therapies. The presumed immune process is targeted upon only a single cell type, the oligodendrocyte, so that we are not faced with the problem of replacing innumerable neural and glial elements. As a primary demyelinating disease, and notwithstanding the increasing emphasis on axon damage (to which we will return later) there is at least in early disease substantial and functionally useful preservation of axons: the overwhelming challenge of attempting therapeutically to reconnect neuronal circuitry does not apply. Also increasingly recognised of late is the occurrence of spontaneous, widespread though partial myelin repair in multiple sclerosis, which ‘relegates’ the challenge for cell therapy to that of supplementing a spontaneous process, rather than imposing repair de novo in an environment explicitly hostile to repair.

Finally, several decades of detailed research elucidating the fundamental and applied biology of oligodendrocytes, and in particular the great success of experimental myelin repair, much of which is outlined elsewhere in this issue, serve emphatically to prove the principle that therapeutic remyelination by delivery of exogenous cells can and does work in the demyelinated mammalian CNS.

Clearly though these manifest advantages (in relation to many other neurodegenerative diseases) have by no means led to the emergence of widespread therapies for patients. So what are the practical hurdles that remain to be addressed? How serious are they, and how might they be solved? Here we outline some of these difficulties (see Box 1), and some of the possible solutions proposed by those groups pursuing and developing such therapies. These include the questions of continuing inflammatory disease activity; the mode of
delivery of cells (bearing in mind the presence of innumerable lesions scattered throughout the CNS); measurement, directly or indirect, of the impact (good or bad, if any) of an intervention; the timing of any treatment, and perhaps above all the safety of the patient. Finally we will give a more personal perspective on the one increasingly relevant underlying question: when should stem cell treatments begin to be tested in patients? — and we will outline our own small pilot or feasibility study of autologous bone marrow cell treatment in patients with multiple sclerosis which commenced in October 2006.

First, however, given that the choice of cell type is also addressed elsewhere in this issue, we will concentrate on the occurrence of axon loss in multiple sclerosis and its impact on the practicalities of reparative cell therapy.

2. Axon loss and remyelinating cell therapies

That axon loss represents the principal pathophysiological cause of disability in chronic progressive disease is no longer questioned (or we believe, seriously questionable). Why does not the absence of axons wholly undermine the basis of for a remyelinating cell therapy? The answers are various.

First, it should be recalled that, while axon loss may be the most important cause of progressive disability, it is unlikely to be the exclusive cause. Residual deficit from relapses – probably the consequence of persistent demyelination – does make some contribution to chronic disability [1]. Second, the concept that disease processes in MS are primarily directed against oligodendrocytes and/or myelin remains unchallenged. Axons are relatively spared until later in the disease course [2,3].

But third and most significant is the mechanism of axon loss. Progressive axonal damage is likely to be (at least in part) a consequence of persistent myelin and oligodendrocyte loss [3–5], through loss of oligodendrocyte-derived trophic support [4,6,7], sustained demyelination-induced conduction block and electrical silence [8], and/or increased vulnerability of the exposed axon to injurious agents [9]. The restoration, by delivering exogenous cells, of a normal oligodendroglial environment should therefore help sustain previously demyelinated axons — and whilst not proving the mechanism, it is the case that bone marrow stem cells given in EAE reduce axon loss [10]. Note that we here are beginning to introduce something of a paradigm shift: the aim of cell therapy is evolving from one of repair to one that might be termed reparative neuroprotection.

3. Continuing disease activity

This question is closely linked to the timing of any cell therapeutic intervention — and indeed to the question of patient safety. Many practical and theoretical considerations point very clearly towards early treatment — quite apart from this being intuitively the better option. The importance and irreversibility of accumulating axon loss in secondary progressive disease [3,11] plainly support this both in the futility of attempting (late) to remyelinate absent axons, and the putative neuroprotective effect of implanted cells (see above). At the level of biology, changes in the cell surface expression of various molecules (e.g. PSA-NCAM) in axons occur some time after myelin loss, and these actively inhibit myelination [12]. Accumulated myelin debris also inhibits myelination [13], and chronic astrocytosis offers a profound inhibitory effect on the migration of remyelinating glia [14].

Is there an argument against early intervention? Both biological and practical, clinical objections have been raised; the latter perhaps more persuasive.

Exogenous cells arriving in early lesions would surely be exposed, along with any new myelin they elaborate, to ongoing inflammatory activity. There is less inflammatory activity in later disease: would not this therefore be a better time to deliver cells? Intuition might answer affirmatively, but in fact, spontaneous remyelination occurs maximally in acute inflammatory lesions [15,16], suggesting (paradoxically) that these offer an optimal reparative environment, and there is increasing experimental evidence, again discussed elsewhere in this issue, that reduced inflammation may impair myelin regeneration [17,18]. In addition, inflammation with consequent breakdown of the blood-brain barrier might facilitate access of intravenously delivered cells to lesions. However, the cardinal clinical rule primum non nocere might dictate that late MS is safest, particularly for early, pilot studies. While exacerbating disease is not logically an obvious risk of most forms of cell therapy, few would feel able to guarantee this. In late MS where progressive disability is already established, the possibility of doing damage or compromising spontaneous repair is remote. Contrariwise, in recent onset MS, little or no disability is present and therefore there is much to lose — and many such patients will of course never develop significant disability even without treatment. Perhaps therefore early clinical studies should target late disease partly as a means to an end: later studies might then move to early disease.

4. Mode of delivery

How to get cells where they are needed? Multiple inoculations of cells into innumerable and widely disseminated CNS lesions is neither attractive nor practically realistic. Two very different possibilities emerge. First, since many plaques are clinically silent, perhaps targeting a very small number of carefully selected, highly symptomatic (disabling) lesions for direct or stereotactic injection – for example, the optic nerves, the spinal cord, or the superior cerebellar peduncle – might yield a disproportionate therapeutic dividend [19], and also offer a vital proof-of-principle of the technique.

However, an increasingly attractive alternative, for multiple sclerosis perhaps above all neurological conditions, is to exploit therapeutically the homing behaviour of stem cells [20]. Not only do they distribute widely after intravenous infusion
[21], but there is much experimental evidence, studying bone marrow-and also brain-derived stem cells, pointing to their tropism for diseased or damaged tissue [20]. The inference is that disseminated (or perhaps also diffuse) disease, neurological or otherwise, may be rationally addressed by intravenous delivery of cells [22,23]. For a (very) multifocal disease of the central nervous system, this is self-evidently attractive, though concerns remain about whether a sufficient number of cells would find their way to, and take up residence in the brain or spinal cord.

Powerful evidence supporting this approach comes from a number of post mortem studies of individuals who have been treated for leukaemic (or other usually haematological conditions) using bone marrow transplantation, and who, more specifically have received marrow from donors who are HLA-matched but gender mis-matched. In these individuals, donor cells can readily be identified long after the procedure by sex chromosome labelling. In such individuals, the demonstration years or even decades after transplantation of small numbers of donor-derived cells in a number of tissues, most notably from a neurological perspective including brain [24,25] and muscle [26], often of highly differentiated morphology appropriate to their environment (for example, Purkinje cells in the cerebellum) and apparently fully integrated into that tissue, offers persuasive evidence of the functional pluripotentiality of intravenously delivered bone marrow-derived stem cells.

5. Assessing the effects of cell therapy intervention

Measuring any effects of an investigative cell therapy in multiple sclerosis is no easier than in any other therapeutic intervention in MS — and plainly we can learn from the valuable information emerging from other published trials, including those of ablative or immune-reconstituting bone marrow transplantation in multiple sclerosis [27,28]. However, new developments in magnetic resonance imaging do offer the prospect of more reliable detection of new myelin, including magnetisation transfer contrast [29,30], 3-dimensional MRI using multiple contrast [31], and radial diffusivity [32,33]. Axon loss and its prevention or amelioration might be disclosed by magnetic resonance spectroscopy measurement of N-acetyl aspartate [34,35].

Potentially remyelinating or reparative cells can be labelled to render them MRI-visible after injection [36–38], but from a safety perspective, even trivial manipulation of cells prior to implantation might best be avoided (and from a regulatory perspective this is certainly the case). Furthermore, graft survival cannot be inferred from migration, since dead cells remain visible [36]. Also, this method discloses cells, not new myelin formation; this is of course not only a limitation in itself, but is compounded by the fact that loading cells with paramagnetic baggage is very likely to impair the ability of other MR modalities to show myelin.

Clinical neurophysiological testing has over the last couple of decades been much overshadowed by MRI both as a diagnostic and an investigative tool in demyelinating disease. However, serial measurements may prove a valuable approach to the objective longitudinal study of multiple sclerosis, particularly if conduction in various motor and sensory pathways is assessed simultaneously and the results combined. Monitoring multimodal recordings of evoked potentials and conduction times may provide evidence of returning saltatory conduction in myelinated pathways, and may well prove extremely useful for interventions aimed at multifocal or more diffuse myelin repair [39,40]. If the intention were to target more focal lesions, the optic nerve has particular advantages; neurophysiological recordings can be combined with psycho-physical measurements of visual function and also with measurements of retinal nerve fibre thickness as an index of neuronal and axonal loss [41].

Finally, clinical methods of assessment are clearly more important than any paraclinical test. Robust, reproducible, and specific clinical outcome measures of function, disability, and handicap have improved substantially in recent years and are now well-validated: these too must be applied [42,43].

6. Safety issues

The histories of gene therapy, and of cell therapy in Parkinson’s disease, illustrate well that hazards in novel therapies are two-fold: the adverse events affect not only the individual patient(s) but also the enthusiasm with which regulatory, political and funding bodies might support continuing and future related clinical research endeavours. It is also the case that, following a period of predictably and boundlessly enthusiastic claims for stem cell therapies emanating from the scientific community, there has of late been an increasing appreciation of the potential adverse effects of (and practical reservations concerning) stem cells.

Apart from immune rejection by the recipient of allografted stem cells (more a foreseen but unintended consequence than a hazard per se), perhaps the two most obvious and serious adverse effects are tumour formation and infection — the latter applying particularly if stem cells are grown on non-human feeder cell layers, or if single, massively expanded cell lines are used to treat many patients. Neither of these potential dangers, however, appears to apply in the specific case of autologous bone marrow stem cell treatment. Transmissible infections plainly are not relevant to such an approach, while decades of delivering such cells to bone marrow transplant recipients has failed to provide evidence of donor-derived malignancy. This said, extensive in vitro proliferation or other manipulation of cells prior to infusion, whatever their source, may be presumed to carry a greater risk of tumourigenesis than the use of untreated cells, though such a risk has been shown more convincingly for embryonic stem cells [44–46] than for adult.

7. When?

When ought bone marrow stem cell treatments be tested in patients with multiple sclerosis? Clearly any answer must
be subjective – and as other reviews in this issue indicate, some such studies are already underway – but the brief consideration outlined above of the practical issues does allow a personal view. This is that, in the specific case of autologous infusion of unmanipulated bone marrow stem cells, we already have sufficient information to move into the clinical arena, and indeed we have commenced a small scale pilot or feasibility study in Bristol. The availability of practical techniques for treating patients, the likely safety of this approach, and more hypothetical considerations concerning the potential efficacy of these cells, underlie our own decision.

The extensive experience haematologists have accrued concerning the collection and delivery of bone marrow cells offers an invaluable endowment to this form of potential cell therapy — an efficient, effective and highly evolved clinical infrastructure, including practical techniques of cell harvest and infusion, dedicated transplant units, controlled and approved cell-handling facilities, and streamlined, user-friendly patient pathways — that we would be foolish to ignore. Not only this, but their immense experience also offers very reassuring long term safety data following autologous (if inadvertent) mesenchymal cell infusion, information which has now been much amplified by the rapidly increasing experience of cardiologists in using bone marrow cells to aid cardiac repair [47–49], and the longer term data emerging in other branches of (mostly oncological) medicine [50]. Provided intravenous rather than intraparenchymal delivery is first used, the cells are autologous and are not manipulated prior to re-infusion, and the treatment takes place in a clinical environment experienced in bone marrow transplantation, it is difficult to envisage any predictable adverse effects. Nonetheless, unpredictable consequences are by no means impossible, and early clinical work must surely commence with only very small scale pilot studies, arguably recruiting only individuals who have already accrued some measure of disability and have established progressive disease.

As outlined elsewhere, there is already a significant body of work attesting to the potential efficacy of these cells in models, both in remyelinating and preserving axons. The mechanisms are by no means clear but it can be argued that a complete understanding of modes of action is not an absolute prerequisite for the introduction of new treatments (we still do not know how interferons work in multiple sclerosis, or immunoglobulins in inflammatory neuropathies, etc — this list is long!). Questions of direct vs. intravenous delivery, cell numbers and preferred state of differentiation, the optimum phase of the disease can probably only be answered by clinical studies in patients, not experimental studies.

These considerations — above all the safety aspects — led us to commence a small study of intravenously delivered autologous bone marrow cells in 5–6 patients with chronic multiple sclerosis, with clinical, neurophysiological, MR and PET-based assessments before and after infusion.

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References


