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Platelet Lysate as Replacement for Fetal Bovine Serum in Mesenchymal Stromal Cell Cultures

Karen Bieback

Institute of Transfusion Medicine and Immunology, Medical Faculty Mannheim, Heidelberg University,
German Red Cross Blood Service Baden-Württemberg – Hessen, Mannheim, Germany

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Summary
Mesenchymal stromal cells (MSC) emerged as highly attractive in cell-based regenerative medicine. Initially thought to provide cells capable of differentiation towards mesenchymal cell types (osteoblasts, chondrocytes, adipocytes etc.), by and by potent immunoregulatory and pro-regenerative activities have been discovered, broadening the field of potential applications from bone and cartilage regeneration to wound healing and treatment of autoimmune diseases. Due to the limited frequency in most tissue sources, ex vivo expansion of MSC is required compliant with good manufacturing practice (GMP) guidelines to yield clinically relevant cell doses. Though, still most manufacturing protocols use fetal bovine serum (FBS) as cell culture supplement to isolate and to expand MSC. However, the high lot-to-lot variability as well as risk of contamination and immunization call for xenogenic-free culture conditions. In terms of standardization, chemically defined media appear as the ultimate achievement. Since these media need to maintain all key cellular and therapy-relevant features of MSC, the development of chemically defined media is still – albeit highly investigated – only in its beginning. The current alternatives to FBS rely on human blood-derived components: plasma, serum, umbilical cord blood serum, and platelet derivatives like platelet lysate. Focusing on quality aspects, the latter will be addressed within this review.

Platelet-Derived Factors for Cell Culture and Tissue Regeneration

Ex vivo/in vitro cell culture requires basal medium plus supplements providing growth factors, proteins, and enzymes to support attachment, growth, and proliferation. Fetal bovine serum (FBS) is commonly used to supplement cell culture media, because the fetal milieu is enriched in growth factors compared to the adult situation and poor in antibodies [1]. In contrast to plasma, serum contains a variety of growth factors, cytokines, and chemokines derived during blood coagulation and released by physiologically activated platelets [2]. Beside stop of bleeding, these factors mediate wound closure and healing. Studies in the 1980s defined growth-promoting effects of human platelet lysate (HPL) on various cell lines [3], tumor cells [4], and articular chondrocytes [5]. Especially the α-granule-derived factors such as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF-2/bFGF), hepatocyte growth factor (HGF), and epidermal growth factor (EGF) have been identified as cell mitogens with wound-healing capacity [6–8]. In conjunction with extracellular matrix molecules platelet factors, for instance TGF-β1, provide osteoinductive capacity for osteoblasts [6] (fig. 1). To utilize these physiological functions, platelet-derived factors have been applied as therapeutic agents for wound healing and bone regeneration. To concentrate the factors, platelet-rich plasma (PRP) has been developed. This is derived by centrifugation of anticoagulated whole blood, yielding plasma enriched in platelets. Further concentration can be achieved by a second centrifugation step [6, 9]. Subsequent coagulation with calcium forms a fibrin gel, which, in conjunction with the platelet released growth factors, serves as a therapeutic agent in plastic surgery, orthopedic interventions, chronic wound healing,

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Fax +49 761 4 52 07 14
Information@Karger.com
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PD Dr. Karen Bieback
Institute of Transfusion Medicine and Immunology
Medical Faculty Mannheim, Heidelberg University
German Red Cross Blood Service Baden-Württemberg – Hessen
Friedrich-Ebert Straße 107, 68167 Mannheim, Germany
Karen.bieback@medma.uni-heidelberg.de

Fig. 1. Diversity of biological activities of MSC. MSC have a broad spectrum of biological activities leading to a variety of potential clinical applications. Currently 307 clinical trials are found searching for 'mesenchymal stem cells' and 32 for 'mesenchymal stromal cells' (*http://clinicaltrials.gov*; assessed June 2013). Since the exact mode of action has not been revealed yet, the combination of biological activities appears to be advantageous for therapy success.

ophthalmology etc. [6, 9–13]. Despite the manifold interventions, evidence of effectiveness of PRP administration remains controversial: Sommeling et al. [10] reported a significant benefit in several indications, including wound healing as well as fat and bone grafting, whereas Martinez-Zapata et al. [13] found no evidence for a benefit of PRP in chronic wound treatment, similar to Sheth et al. [12], who state 'the current literature is complicated by a lack of standardization of study protocols, platelet separation techniques, and outcome measures'.

Certainly platelet-derived factors attracted attention as effective tool to supplement cell cultures replacing FBS. To improve safety for cell-based therapies Doucet et al. [14] initiated the use of HPL in supplementing mesenchymal stromal cell cultures.

Mesenchymal Stem/Stromal Cells

In the 1970s Friedenstein et al. [15, 16] described a population of non-hematopoietic progenitors isolated from the bone marrow. Although already described as being able to differentiate into a variety of mesodermal lineages such as bone, cartilage, fat, marrow stroma, tendon, muscle, dermis and connective tissues, further research rested until the 1990s [17, 18]. Only then, these non-hematopoietic cells were termed 'mesenchymal stem cells (MSC)', according to the hematopoietic stem cell (HSC) nomenclature [19]. Since later studies, however, failed to fulfill the self-renewal criterion of stem cells (1. self-renewal, 2. unspecialized, and 3. differentiation capacity to specialized cell types), it has been proposed to better name the cells 'mesenchymal stromal cells (MSC)' [20].

Of note, till now MSC are characterized as culture-adapted, ex vivo expanded cells. This population is still heterogeneous and contains progenitor cells at different maturation stages and also mature stromal cells [21]. In conjunction with the heterogeneity of cell preparations, inconsistencies in the use of different tissues as starting material and in isolation and cultivation protocols render comparability of results complicated. As an effort to standardize the term MSC, the International Society for Cell Therapy (ISCT) defined minimal criteria to be fulfilled [22]:

- adherence to cell culture plastic surfaces yielding cells of a fibroblastoid phenotype,
- expression of typical markers (CD105, CD73 and CD90) and lack of expression of CD45, CD34, CD14 (or CD11b), CD79α (or CD19) and HLA-DR surface molecules, and
- differentiation towards at least the three mesodermal chondrocyte, adipocyte and osteocyte lineages.

(for further overview see TRANSFUSION MEDICINE AND HEMOTHERAPY special issues Vol. 35, No. 3 and 4, 2008, and Vol. 37, No. 2, 2010 [23–41]).

Encouraged by this broad differentiation capacity, early clinical trials were initiated for a wide range of ailments [42, 43]. Based on the stromal origin within bone marrow, the stromal support capacity to facilitate HSC engraftment was assessed [44, 45]. Despite an unexpectedly low level of engraftment, a long-lasting therapeutic effect became apparent. To answer the question how MSC achieve this benefit without being actually present, MSC research returned 'back to bench' [46]. Now seminal studies identified strong immunomodulatory properties of MSC [47, 48]. In combination with their low immunogenicity based on the lack of HLA-DR and co-stimulatory molecules, MSC were rated well-suited for both autologous and allogeneic transplantation settings [49]. Continuatively the beneficial therapeutic effects could be attributed to the capacity of MSC to home to sites of inflammation and injury where MSC release a variety of pro-regenerative, anti-apoptotic and anti-fibrotic factors enabling endogenous repair processes [50].

Thus based on their differentiation capacity, hematopoietic support as well as their immunomodulatory and pro-regenerative features, MSC are increasingly applied in cell-based therapy: currently 307 clinical trials are found searching for 'mesenchymal stem cells' and 32 for 'mesenchymal stromal cells' (*http://clinicaltrials.gov*; assessed June 2013). The high number of clinical trials raise an intense controversy regarding the pace of translation [51]. Despite fascinating in vitro results and promising preclinical data, clinical data are often less prominent. The scientific basis for some clinical trials appears often rather weak, because, despite intense research, the mode of action of MSC remains elusive. Thus it is essential to carefully weigh the putative benefits with the risks.

Although, up to now, the majority of preclinical and clinical data fail to report severe adverse events after MSC application, suggesting that MSC can be applied safely [52], there have been reports of severe complications after stem cell infusions, including deaths [51, 53, 54]. In Korea, for instance, one patient died from pulmonary embolism after stem cell administration, and the International Cellular Medicine Society (ICMS) considered it 'likely to have been caused or triggered by the stem cell procedure' (*www.cellmedicinesociety.org*). Yet beyond these few cases possibly related to market-oriented companies that 'are setting up operations around the globe, and taking advantage of loopholes in other countries' regulations' [53], few adverse events have been reported. Calcifications were observed after transplantation of MSC or unfractionated bone marrow into infarcted hearts in a mouse model [55]. Tumor growth appeared facilitated after MSC infusion [56, 57]. Especially latent tumors, such as gliomas, sarcomas and melanomas, but also metastases became manifest after MSC infusion. This suggests that tumor surveillance may be impaired by MSC immunosuppressive activities or that direct integration into the tumor stroma and secretion of angiogenic factors may facilitate tumor growth [57]. It has been intensely debated whether or not the ex vivo expansion of MSC, which in most cases is needed to achieve clinically relevant cell numbers, provokes spontaneous transformations [58, 59]. The few cases reporting spontaneous transformation, however, had to retract their publications: 'spontaneous' transformation occurred due to laboratory cross-contamination with tumor cell lines. Thus yet consensus evolved that at least human MSC in general undergo replicative senescence instead of malignant transformation. Even documented events of aneuploidity, observed in few clinical-scale MSC preparations, in all cases caused progressive growth arrest and senescence [60]. Yet, by way of precaution, consensus on common standards and harmonized protocols need to be implemented to strengthen both efficacy and safety.

Towards Xenogenic-Free Manufacturing of Mesenchymal Stromal Cells

To ensure safety and efficacy, all steps within the MSC manufacturing process need to be standardized. Cellular quality and potency have to be reproducible. However, the manufacturing process for MSC is complex and composed of procurement, isolation, expansion, and quality control [37, 51, 61, 62]. Clinical success is linked to a sufficient number of vital and functional cells. Further, the cellular product shall bear no risks for infections, allergies, or malignancies. Thus within the manufacturing process, a number of considerations have to be taken into account [63]. Ancillary reagents pose a risk for the safety of the cellular product. FBS is typically used to isolate and expand MSC. It is a highly complex mixture of proteins and other factors and by nature ill-defined and variant from batch to batch [1]. Due to the high risk of contaminations (virus positivity reported to be as high as 20–50%), FBS is critically rated by the European Medicines Agency [64]. Because MSC internalize xenogenic proteins at high amounts, there is an additional risk of allergic reactions. FBS immunogenicity has already been demonstrated to compromise the therapeutic success [49, 65–67]. In view of these considerations xenogenic-free culture conditions appear desirable. Due to the numerous constituents of FBS, which positively (and negatively) affect adhesion (cell-cell and cell-matrix), mitosis, survival, apoptosis etc., a chemically defined medium needs an optimal composition of the few most essential factors to promote at least all key cellular features. By this it is hard to establish [68–70].

Although some regulatory agencies may tolerate xenogenic components, such as FBS, in phase I clinical trials, it is expected that later clinical trials including larger patient cohorts will require serum- or at least xenogenic-free cell preparations. Albeit washing procedures or sequential cultivation in human plasma or serum may help to reduce the content of xenogenic proteins, a residual risk is left over [65, 67].

To replace FBS, numerous studies now refer to 'humanized' culture conditions. 'Humanized' supplements include human serum (autologous or pooled allogeneic), cord blood serum as well as different platelet derivatives [69, 70]. Because these human blood components are in clinical use since years, can be derived from healthy blood donors and had been tested according to blood banking standards for infectious and immunological parameters, the potential risk is lowered. Similar to FBS, human blood component-derived supplements include a variety of essential factors capable of promoting cell growth. Human plasma, autologous and allogeneic serum as well as cord blood serum have been investigated as reviewed in [70]. Yet human platelet-derived factors emerge as the most intensely studied alternatives to FBS for MSC culture.

Platelet Releasate

As already stated, human platelets contain numerous factors to promote cell growth of cells and cell lines [3]. Early studies evaluated the MSC growth-promoting effects of platelet growth factors in PRP released by calcium and thrombin stimulation [71, 72]. Coagulated fibrin is subsequently removed by centrifugation and filtration. Both studies described accelerated expansion and migration but differed with respect to osteogenic differentiation potential. Thus platelet factors released by physiological stimuli might offer some advantages. Several substances have been shown to activate platelets, including thrombin, collagen, ADP/epinephrine and thrombin receptor-activating peptide (TRAP) [73]. Interestingly, own studies have shown that processed thrombin-activated platelet releasate in plasma (tPRP) and HPL promote different proliferation rates of bone marrow- and adipose tissue-derived MSC. Whereas HPL promoted a significant higher proliferation rate of bone marrow MSC than tPRP [74], adipose stromal cells exhibited, if at all, similar proliferative responses to HPL and tPRP [74, 75]. Differential proteomics of HPL and tPRP identified 20 differential proteins (Kinzebach et al. unpublished data). Identified proteins further denoted differences between bone marrow and adipose stromal cells: for example, fibrinogen significantly supported the expansion of adipose-derived stromal cells (ASC), and apolipoprotein A1 selectively reduced proliferation rate of bone marrow MSC.

Platelet Lysate

Platelet releasates contain only those factors released after platelet activation. Platelet lysates (HPL) in contrast contain all factors platelets are composed of. These can be easily derived by mechanical disruption of platelet concentrates via freezing and thawing. Subsequent centrifugation separates the platelet debris from the supernatant containing all bioactive platelet factors. Compared to chemical activation of platelets to gain the releasate, mechanical lysis to yield HPL appears preferable as being much easier, less time-consuming, and less cost-effective. It further avoids the use of additional substances such as thrombin which may cause side effects.

Platelet concentrates, used for HPL production, can be either frozen immediately after donation or used at the end of the shelf life (4–6 days after donation depending on the current local regulation) [76–78]. Thus those platelet concentrates not used for platelet transfusion can be allocated for HPL manufacturing, minimizing the decay of already donated units and consequently avoiding an additional donation from blood donors. Platelet concentrates can be then stored frozen, thawed, centrifuged, pooled, and sterile-filtered after quarantine storage (analogous to therapeutic fresh frozen plasma) to increase safety [77]. Final HPL can be stored at –20 °C for a prolonged time, maintaining a stable growth factor content [79].

Doucet et al. [14] have shown that PRP-derived HPL well supports MSC isolation and expansion whilst osteo-, adipo- and chondrogenic differentiation propensity is retained compared to FBS. A variety of studies followed, exploiting HPL as GMP-compliant substitute for FBS in MSC expansion, supporting even significantly enhanced proliferation of MSC [73, 74, 80–85]. For large-scale MSC expansion 50–100 conventional tissue culture flasks may be needed. These are hard to handle and prone to contamination. Thus a few studies evaluated the isolation and expansion within a bioreactor using HPL [86, 87] in order to define the risks of MSC therapy. The majority of these studies report a lower size of MSC and accelerated proliferation compared to FBS. Furthermore, HPL increased not only size but also numbers of colonies [82, 88]. Importantly, only in FBS-supplemented media clonal chromosomal instabilities were monitored but not under the humanized culture conditions [59, 82, 89].

Although MSC are considered to escape allo-recognition, mitogens such as FGF and PDGF-BB as well as inflammatory cytokines have been documented to induce HLA-DR expression in MSC and by this the stimulation of CD4 T cells. Importantly HPL appears not to cause HLA-DR expression [90]. Regarding the maintenance of immunomodulatory properties, contradictory data exist. Flemming et al. [91] directly compared bone marrow-derived MSC cultivated in FBS or HPL regarding their immunosuppressive capacities. The inhibitory effect on T-cell proliferation was similar, likewise the activation of cytomegalovirus-specific T cells. Similar data were presented by Bernardo et al. [83]. In contrast, Abdelrazik et al. [92] defined an alteration in surface protein expression relevant for immunomodulation and adhesion after culture in HPL. This corresponded to a reduced inhibition of T and NK cell proliferation. These authors compared MSC expanded in three different batches of FBS (including two commercially available MSC growth media) with HPL supplementation.

Microarray analyses highlighted the down-regulation of several gene families that are included in differentiation/development, cell adhesion / extracellular matrix-receptor interaction, TGF-β signaling and thrombospondin-1-induced apoptosis. Gene clusters associated with cell cycle, DNA replication, and purine metabolism were up-regulated concomitant to the enhanced proliferation in HPL as well as in human serum [84, 93]. Albeit low, these changes in gene expression may cause differences in MSC therapeutic potential. Homing and engraftment are prerequisites for most therapeutic interventions. Changes in cell adhesion molecules thus may have relevant consequences. We detected that the reduced expression of integrin α6 (CD49f) in adipose tissue-derived stromal cells cultivated in a humanized medium correlated to decelerated integrin signaling and reduced adhesion to laminin [94]. We also observed reduced adhesion to endothelial cells. Finally fewer cells cultivated in human serum were detected in the lungs of mice infused with MSC in contrast to higher numbers of those cells cultivated in FBS. Whether this diminished entrapment is related to the lower size of cells, reduced interaction with extracellular matrix molecules, changed homing specificities, or other reasons is a matter of further analyses. Although observed for cells cultivated in human serum, similar ideas are discussed by Lucchini et al. [95]. Thus the choice of supplement may play a critical role when balancing risk and efficacy: reduced adhesion to the lung may lower the potential risk of pulmonary embolism. It may, however, also reduce the release of immunomodulatory TSG-6 (TNF-stimulated gene 6 protein) secreted of lung-adhered MSC to provide protection, e.g., in myocardial infarction [96].

Combining the growth-promoting effect of HPL on MSC, the osteoinductive properties in bone, and the scaffold properties of fibrin, HPL has been used to promote bone tissue engineering. Dozza et al. [97] for instance demonstrated that HPL-expanded MSC applied as collagen or fibrin construct to an uncemented hip prosthesis significantly promoted new bone formation compared to the prosthesis alone. Furthermore, there are indications that HPL may induce osteogenic differentiation without any further osteogenic stimuli. Only ceramics seeded with MSC grown in HPL were able of ectopic bone formation [88, 98]. Based on this differentiation potential, these cells have been used to treat patients in various orthopedic conditions. Centeno et al. [99] reported the results of 339 patients having received autologous bone marrow-derived MSC expanded in autologous HPL. Adverse event surveillance revealed a few cases most probably related to the reimplant procedure and three cases possibly related to stem cell applications. These few cases were either self-limited or cured by small therapeutic interventions. Importantly, no neoplastic transformations were observed at the stem cell injection site. Although in total two patients developed tumors, the neoplasm rate was similar to the control population. 53.1% of the patients reported symptom relief in the follow-up period of 11 months.

Lange et al. [100] observed decreased adipogenic differentiation potential of MSC expanded in HPL. Amongst others, the reduced expression of lipocalin-type prostaglandin D2 synthase was related to this effect. The authors conclude that HPL might offer an option to prevent unwanted adipogenic differentiation.

Quality Criteria for Platelet Lysate

Human supplements, likewise FBS, still need to be considered ill-defined, and they share some safety concerns. Generally platelet concentrates are manufactured and released for therapeutic purposes to be transfused to patients with severely reduced platelet numbers and/or impaired platelet function before they are converted for use as MSC supplement. Having been released as blood product, stringent blood donor eligibility criteria as well as sensitive viral NAT testing have already been fulfilled, ensuring safety of the starting material for HPL manufacturing [77, 78].

Autologous or Allogeneic

Platelet transfusion practices consider the blood groups and rhesus factors to avoid adverse events for the patients. For preparation of autologous MSC, autologous HPL may be considered. However, in severely sick patients a whole blood donation or apheresis may be risky. Due to the restricted volume of HPL from one patient the cell number achieved within the expansion steps might be restricted as well. Furthermore, variations between individual autologous donors hamper standardization and increase the need to quality control each individual HPL batch. Thus the majority of current protocols rely on pooling platelet concentrates from up to 50 healthy blood donors [85]. The cryopreservation step prior to further pooling allows quarantine storage of individual platelet concentrates. These can be released for clinical-grade HPL production once the donors have been retested negative for all infectious markers after a consecutive second donation [77]. The resulting large batch is easy to be quality controlled according to blood banking standards and for protein and growth factor content. For patients at risk, e.g. patients with known antibodies, blood group/Rhesus factor-matching allogeneic platelet preparations could be considered [78]. Interestingly, donor age appears to be of impact for HPL quality: comparing umbilical cord with adult PRP revealed a higher concentration of mitogenic growth factors in the cord blood-derived preparations [101]. Similar HPL from younger donors (<35 years) was more proliferative than that from older donors which increased the expression of senescence markers [102].

Thrombocyte Concentration

Crucial for manufacturing MSC is the concentration of thrombocytes which is directly related to the growth factor concentration. Lange et al. [84] evaluated different thrombocyte concentrations as 5% supplement in basal medium to evaluate the effect on MSC proliferation: 1.5, 1.0, 0.75, and 0.5×10^9/ml. It became obvious that a platelet concentration below 1.5×10^9/ml significantly reduced the pro-proliferative effect.

Shelf Life of Platelet Concentrates prior to HPL Production

To reduce the risk of transfusion-transmitted bacterial infections, in Germany the shelf life of platelet concentrates is 4 days. A variety of laboratories allocate the platelet concentrates at the end of this shelf life for HPL production. Bacterial screening complemented by subsequent donor testing is taken as safety measures. Fekete et al. [77] compared HPL prepared from platelet concentrates 2 days or 6 days after donation without any change in quality. Consequently, the platelet concentrate does not need to be discarded when not used for the initial intended therapeutic use but can be converted to HPL production after the shelf life has been exceeded.

Plasma or Thrombocyte Additive Solution

Although most critical, data regarding growth factor concentrations significantly differed in the various publications [79]. These discrepancies are predominantly due to different preparation methods to manufacture platelet concentrates, using either plasma or plasma additive solution. Plasma additive solution has been introduced to reduce the adverse effects of plasma [78]. Initially convinced that plasma components in conjunction with the platelet factors comprise the optimal MSC supplement, we for instance evaluated a pool of freshly prepared buffy coat-derived platelet concentrates prepared in human AB plasma of one of the blood donors [73, 74]. Later we and others tested outdated 'routine' platelet concentrates. As pool of 8–50 donors, no divergence from freshly prepared platelet lysate was obvious, suggesting that outdated platelet concentrates – instead of autoclaving – can be cryopreserved to manufacture HPL [77, 85]. For instance the group of Dirk Strunk filed an US-patent entitled 'Plasma-free platelet lysate for use as a supplement in cell cultures and for the preparation of cell therapeutics' (Pub. No.: US 2009/0305401 A1; Dec 10, 2009).

Buffy Coat- or Apheresis-Derived Platelet Concentrates

Within their study Fekete et al. [77] furthermore compared GMP-grade HPL obtained from pooled whole blood-derived buffy coats with those from apheresis-derived platelet concentrates. There were no significant differences regarding the cytokine content (bFGF, sCD40L, PDGF-AA, PDGF-AB/BB, sVCAM-1, sICAM-1, RANTES, TGF-β1) fitting to the similar support of MSC proliferation.

Specific Cytokines

As already mentioned, HPL contains a plethora of cytokines, chemokines, and soluble adhesion molecules which have been intensely studied by proteomic approaches [8, 82, 103]. However, concentrations between the groups differ significantly [77, 79]. And those growth factors essential for MSC isolation and expansion have not been defined yet. It needs to be established whether the composition and concentration of platelet-derived factors can serve as quality control parameters for HPL.

FGF2/bFGF, PDGF, EGF, IGF, and TGF, among others, have been studied but failed to support MSC growth when supplemented solitary or in combination to serum-free medium [104]. Yet added to FBS-supplemented media, these growth factors were capable of fostering proliferation and differentiation. This effect, however, may not necessarily hold true in 'humanized' culture systems. Similar to our own yet unpublished data, bFGF appears to support expansion of FBS-supplemented bone marrow MSC cultures, but fails to do so in HPL-supplemented systems [105]. Neutralization of PDGF-AB/BB, TGF-β1 and FGF significantly diminished the proliferative effect of HPL, with the strongest effects seen by neutralizing FGF alone or in combination with PDGF-BB [77]. Although these factors appear to be essential, it was not possible to induce proliferation by a cocktail of these growth factors in serum-free medium. Providing extracellular matrix molecules as attachment factors in addition likewise failed to mimic HPL. Obviously, additional components are necessary to fully support MSC proliferation.

Pathogen Reduction Strategies

Combining a number of advantages, human supplements still pose the risk of transferring infectious agents. Hemovigilance plus quarantine storage can in part overcome the risk of the diagnostic window [77]. However, there is still a residual risk due to pathogens which are currently not routinely tested (especially viruses). Then, being stored at room temperature, platelet concentrates bear the risk of undetected bacterial contamination. Thus various pathogen reduction or inactivation strategies became investigated for erythrocyte and platelet concentrates [106]. Pathogen inactivation protocols based on photochemical treatment have been established and pathogen-inactivated human serum showed no differences regarding the quality of MSC compared to control serum [107]. A virally inactivated HPL was introduced by Shih et al. [108]. Here the HPL was treated by solvent/detergent, then extracted by soybean oil, and further purified by C18 chromatography and sterile filtration. Via a semiquantitative human cytokine antibody array cross-reacting with some bovine proteins, the growth factor cocktail was compared. 22 cytokines showed a higher concentration in the virally inactivated HPL than in FBS, and only two cytokines (angiopoietin-2 and bFGF) were found at lower concentrations. Virally inactivated HPL induced massive proliferation compared to FBS. The typical MSC characteristics, phenotype, immune phenotype, and differentiation were maintained, indicating the feasibility of this approach.

Clinical Trials with Mesenchymal Stromal Cells Expanded in Human Supplements

A few studies already applied 'humanized' culture conditions to expand MSC for clinical trials. The study presented by von Bonin et al. [109] evaluated bone marrow MSC expanded in human HPL in patients with refractory graft-versus-host disease (GvHD). Two out of 13 patients treated in total benefitted from the treatment. After a second dose, 5 out of 11 patients responded with a mitigation of their symptoms. Similar findings were obtained in a study where 11 pediatric patients suffering from acute or chronic GvHD have been treated with MSC expanded in HPL. Some patients received up to 5 MSC infusions with no acute and late side effects. A complete response was observed in 23.8% and a partial remission reported in 47.6% of patients [95]. In contrast, a higher rate of complete response has been reported analyzing MSC expanded in FBS. Here a complete response in 30 from a total of 55 patients has been obtained [110]. Further, 9 patients profited with an improvement of GvHD symptoms. It is discussed whether differences in the mode of MSC expansion may cause different migration towards chemotactic stimuli and by this favor one or the other organ affected by GvHD. By this, the discussion fits to the described changes in gene expression related to adhesion and homing.

Conclusion

For standardized MSC manufactured on a routine basis, chemically defined media approved for GMP and clinical use are regarded as ultimate endpoint. Until achieving this, objective supplements derived from human blood products (serum or platelet lysate) emerged as reasonable alternatives to FBS. Rauch et al. [79] defined quality criteria for HPL: a 10- to 20-fold enrichment of α-granule factors compared to serum and a reduced overall protein content, including immunoglobulins and albumin achieved by washing.

Any change of culture conditions can be of major impact on cellular quality. Switching from FBS to human supplements induces measurable changes. Although main characteristics of MSC, as defined by the ISCT, appear retained, continuative studies indicated that the choice of supplement for instance affected gene and protein expression. Adhesion as well as homing are changed by humanized culture systems compared to FBS. It is thus recommended to study the cellular effects in detail before – simply acting on the assumption that FBS and HPL have similar effects – changing culture conditions between preclinical and clinical trials. This is, however, complicated by the fact that the mode of action has not yet been defined comprehensively for FBS-cultivated cells. Thus the effects of a changed culture condition cannot be compared. These effects are expected to be even more drastic when developing chemically defined media, composed of only few factors and lacking the manifold other undefined factors present in HPL or serum. Otherwise, identifying the impact of defined media components may allow generating designed MSC for specific therapeutic applications, similar to the differentiation media already applied for targeting differentiation of MSC.

Of note, culture medium is only one building block in the complex structure of the GMP-compliant MSC manufacturing process. The establishment of standardized manufacturing protocols, quality control parameters, and assays is of utmost importance. This becomes even more important given the rapid pace in which clinical trials are initiated, often based on only weak scientific evidence. Despite the intensified research work in the translational field, only agreement on standardized protocols in conjunction with a strictly regulated environment performed by experts in the field of GMP manufacturing is expected to enable meaningful comparative multicenter studies assessing feasibility, safety, and efficacy.

The fact that the transition to clinical trials employing MSC is evolving rapidly, even without knowing the mode of action, complicates this demand. To not slow down clinical MSC evolution, an intense interaction between basic and clinical research is demanded to deepen our knowledge and to develop safe but efficacious MSC-based novel therapies.

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