Stem Cell Biology and Regenerative Medicine

Charles J. Malemud
Eben Alsberg *Editors*

Mesenchymal Stem Cells and Immunomodulation

Humana Press

Stem Cell Biology and Regenerative Medicine

Series Editor
Kursad Turksen

More information about this series at http://www.springer.com/series/7896

Charles J. Malemud • Eben Alsberg
Editors

Mesenchymal Stem Cells and Immunomodulation

Humana Press

Editors
Charles J. Malemud
Division of Rheumatic Diseases
Department of Medicine
Case Western Reserve University School of Medicine
Cleveland, OH, USA

Division of Rheumatic Diseases
Department of Medicine
University Hospitals Cleveland Medical Center
Cleveland, OH, USA

Eben Alsberg
Department of Biomedical Engineering
Case Western Reserve University
Cleveland, OH, USA

Department of Orthopaedic Surgery
Case Western Reserve University
Cleveland, OH, USA

ISSN 2196-8985 ISSN 2196-8993 (electronic)
Stem Cell Biology and Regenerative Medicine
ISBN 978-3-319-46731-3 ISBN 978-3-319-46733-7 (eBook)
DOI 10.1007/978-3-319-46733-7

Library of Congress Control Number: 2016955831

Printed on acid-free paper

This Humana Press imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Using mesenchymal stem cells for repairing specific injured tissues and organs has advanced to the stage where employing these cells constitutes a rational clinical alternative to other presently employed surgical procedures and/or medical and pharmacologic interventions. In that regard, recent discoveries provided compelling evidence indicating that mesenchymal stem cells, which can be expanded in cell cultures from a variety of adult tissues, including bone marrow, muscle, and adipose tissue, can be used in the treatment of a broad spectrum of human disease processes through a marshaling of their capacity to modulate immune cell-mediated inflammation, thus achieving impressive efficacy with respect to clinical response outcomes. To explore the role of mesenchymal stem cells in immunomodulation, this Springer E-Book in the "Stem Cell" series, entitled *Mesenchymal Stem Cells and Immunomodulation*, presents state-of-the-art reviews by esteemed scientists not only on the molecular and pathophysiological underpinnings for considering mesenchymal stem cells as modulators of immune-mediated cellular responses but also on the significant recent progress which has been achieved in deciphering the complexity of gene expressional events that underlie and serve as the linchpin for mediating immunomodulation. Thus, the paradigm for research in this field has generally followed a plan whereby basic preclinical research studies conducted primarily in organ and/or cell culture have been followed by an assessment of the immunomodulation achieved through the use of mesenchymal stem cells in well-validated animal models of human diseases. The design of these studies has led to a collection of compelling data which should ultimately provide direction for employing mesenchymal stem cell-based therapies for ameliorating the pathology of several diverse human conditions. These would include ameliorating chronic inflammation characteristic of arthritis and other autoimmune disorders such as systemic lupus erythematosus, type I diabetes, multiple sclerosis, uveitis, and scleroderma as well as asthma, cancer, injuries to the brain, and perhaps even amyotrophic lateral sclerosis. Drs. Malemud

and Alsberg, the co-guest Editors of this Springer E-Book, would like to acknowledge those contributors who accepted our invitation to write for this Springer E-Book as well as the support staff at Springer, particularly, Mr. Michael Koy, who worked diligently with us to bring this writing project to a successful conclusion.

Cleveland, OH, USA

Charles J. Malemud
Eben Alsberg

Contents

Contributors

Eben Alsberg, Ph.D. Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH, USA

Department of Orthopaedic Surgery, Case Western Reserve University, Cleveland, OH, USA

Tracey L. Bonfield, Ph.D. (ABMLI) Division of Pulmonary Medicine, Department of Pediatrics, Rainbow Babies and Children's Hospital, Case Western Reserve University School of Medicine, Cleveland, OH, USA

A.C. Bowles Center for Stem Cell Research and Regenerative Medicine, Tulane University School of Medicine, New Orleans, LA, USA

Department of Cell and Molecular Biology, Tulane University School of Science and Engineering, New Orleans, LA, USA

Hulya Bukulmez, M.D. Division of Pediatric Rheumatology & Pediatric Bone Health Program, Department of Pediatrics, MetroHealth Medical Center & Skeletal Research Center, Case Western Reserve University, Cleveland, OH, USA

B.A. Bunnell, Ph.D. Center for Stem Cell Research and Regenerative Medicine, Tulane University School of Medicine, New Orleans, LA, USA

Department of Pharmacology, Tulane University School of Medicine, New Orleans, LA, USA

Arnold I. Caplan, Ph.D. Department of Biology, Skeletal Research Center, College of Arts and Sciences, Case Western Reserve University, Cleveland, OH, USA

Benjamin D. Goldstein, M.D. Division of Pulmonary Medicine, Department of Pediatrics, Rainbow Babies and Children's Hospital, Case Western Reserve University School of Medicine, Cleveland, OH, USA

Lei Huang, Ph.D. Research Imaging Institute, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Peng Huang, M.D., Ph.D. Department of Laboratory Medicine and Pathology, Mayo Clinic, Jacksonville, FL, USA

Charles J. Malemud, Ph.D. Division of Rheumatic Diseases, Department of Medicine, Case Western Reserve University School of Medicine, Cleveland, OH, USA

Division of Rheumatic Diseases, Department of Medicine, University Hospitals Cleveland Medical Center, Cleveland, OH, USA

R.M. Wise Center for Stem Cell Research and Regenerative Medicine, Tulane University School of Medicine, New Orleans, LA, USA

Neuroscience Program, Brain Institute, Tulane University School of Science and Engineering, New Orleans, LA, USA

Zachary Wolff, M.D. Division of Rheumatic Diseases, Department of Medicine, Case Western Reserve University School of Medicine, Cleveland, OH, USA

About the Editors

Charles J. Malemud received his Ph.D. from George Washington University and completed his postdoctoral studies at the State University of New York at Stony Brook. Since then, Dr. Malemud has been a member of the faculty at Case Western Reserve University School of Medicine where he is presently Professor of Medicine and Anatomy in the Division of Rheumatic Diseases and Senior Investigator in the Arthritis Research Laboratory. He has published over 200 papers, chapters, and reviews primarily in the field of chondrocyte biology. Professor Malemud is on the editorial board of several rheumatology, immunology, and musculoskeletal journals and serves as editor-in-chief of two of them.

Eben Alsberg received his Ph.D. from the University of Michigan, Ann Arbor, and was a postdoctoral researcher at Harvard Medical School. He is currently a professor of Biomedical Engineering and Orthopaedic Surgery at Case Western Reserve University and also serves as Director of the Stem Cell and Engineered Novel Therapeutics Laboratory. He was a Lady Davis Fellow at the Technion (Israel), and he is currently an International Scholar (Visiting Professor) at Kyung Hee University (Korea). His lab focuses on the engineering of new technologies to regenerate tissues and treat diseases through the development of novel biomaterials and microenvironments. He has coauthored over 90 peer-reviewed papers and book chapters. His work has been recognized with the 2008 Ellison Medical Foundation New Scholar in Aging Award and the Crain's Cleveland Business 2009 Forty Under 40 Award, and he was elected to the American Institute for Medical and Biological Engineering (AIMBE) College of Fellows. He is on the editorial board of several tissue engineering and biomaterials journals, and multiple government agencies and foundations have supported his lab's research.

Mesenchymal Stem Cells and Immunomodulation: An Overview

Charles J. Malemud and Eben Alsberg

Mesenchymal stem cells (MSCs) are often principally considered cells suitable for use in repairing injury to various tissues and organs [1–3]. However, more recently, MSCs were shown to produce molecules, including cytokines and soluble mediators indicating robust function related to their roles in trophic signaling and immunomodulation [4–7]. This Springer E-Book in the "Stem Cell" series reviews how bone marrow-derived and adipose tissue-derived MSCs could serve as potential therapeutic alternatives to pharmaceutical drugs for the treatment of inflammatory conditions such as graft versus host disease, autoimmune disorders such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), multiple sclerosis (MS), type I diabetes, cancer, injuries to the brain, and asthma.

Chapter "Controversies in the Use of Mesenchymal Stem Cells for Treating Autoimmune Diseases": Compelling evidence has been presented indicating that MSCs possess potent immunomodulatory activity. In this review, Wolff and Malemud addressed several of the controversial issues which pertain to using MSCs to drive

C.J. Malemud, Ph.D. (✉)
Division of Rheumatic Diseases, Department of Medicine, Case Western Reserve University School of Medicine, Foley Medical Building, 2061 Cornell Road, Cleveland, OH 44106-5076, USA

Division of Rheumatic Diseases, Department of Medicine, University Hospitals Cleveland Medical Center, Foley Medical Building, 2061 Cornell Road, Cleveland, OH 44106-5076, USA
e-mail: cjm4@cwru.edu

E. Alsberg, Ph.D.
Department of Biomedical Engineering, Case Western Reserve University, Room 202, Wickenden Building, 10900 Euclid Avenue, Cleveland, OH 44106, USA

Department of Orthopaedic Surgery, Case Western Reserve University, Room 202, Wickenden Building, 10900 Euclid Avenue, Cleveland, OH 44106, USA
e-mail: exa46@cwru.edu

C.J. Malemud, E. Alsberg (eds.), *Mesenchymal Stem Cells and Immunomodulation*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-3-319-46733-7_1

immunomodulation. In that regard, MSCs were shown to house the major histocompatibility complex (MHC) class I antigens processing machinery and produce costimulatory molecules for T-cell activation and proliferation, such as B-7, as well as possibly expressing histocompatibility loci antigens. MSCs also synthesize a large array of cytokines and other trophic factors. However, the functional roles of various types of cytokines required for orchestrating immune responses, both positive and negative, could limit their clinical application for the treatment of inflammation associated with osteoarticular diseases, such as RA [8] and osteoarthritis (OA) [9].

MSCs are also being considered as a therapeutic alternative to pharmaceutical drugs for regulating immune-mediated inflammatory response because MSCs were shown to functionally alter deregulated immune responses in Type I diabetes, scleroderma, uveitis, SLE, irritable bowel disease, multiple sclerosis, and amyotrophic lateral sclerosis. However, Wolff and Malemud point out that the assessment of MSCs in various animal models of these human diseases will be required prior to mounting a full-scale testing of MSCs in human clinical trials, and even if MSCs prove to be clinically efficacious in animal models, results may not be predictive of their effectiveness in human diseases. For example, a potential roadblock for using MSCs as a therapy for SLE was evident in some of the confounding data regarding the extent to which allogeneic MSCs were capable of reversing the pathology in the lupus-prone mouse model of SLE [10].

Chapter "Mesenchymal Stem Cell Treatment in Mice Models of Systemic Lupus Erythematosus": SLE is considered the protean autoimmune disorder of man. In this chapter, Bukulmez provides compelling evidence from recent studies where the immunomodulatory function(s) orchestrated by MSCs was examined in several well-validated experimental mouse models of SLE [11–13]. In that regard, a robust peer-reviewed literature has emerged on this topic which shows that MSCs can alter (1) the abnormal function(s) of T-cells and natural killer cells, both of which are characteristic of SLE; (2) the aberrant activity of effector B-cells; (3) the elevated levels of proinflammatory cytokines; (4) the abnormalities in complement production and complement activity; and (5) the phagocytic activity of dendritic cells. Moreover, Bukulmez stressed that MSCs may facilitate immunomodulation by producing an impressive array of immunosuppressive molecules. To date several mouse models of SLE have been tested for their response to the administration of MSCs. The results lean mainly toward a partial remission, or in some cases, a regression of SLE disease pathology. However, important relevant information must be obtained for extending the results from animal models of SLE to human clinical trials. Thus, the ameliorative response in the SLE animal models to treatment with MSCs indicated that allogeneic or xenogeneic MSCs were more favorable compared to autologous MSCs, with the appropriate caveat [10] that MSCs may be unable to completely reverse SLE pathology.

Chapter "Anti-inflammatory Effects of Adipose-Derived Stem Cells (ASCs)": High-yield adipose-derived stem cells (ASCs) are easily obtained from adipose tissue. In this chapter, Bowles et al. review the results of those experimental studies that investigated the therapeutic efficacy of ASCs that make them highly valuable for future clinical applications, including the treatment of wounds,

ischemia–reperfusion/infarction, and autoimmune disorders, such as MS, RA, acute respiratory distress syndrome, and asthma, to name just a few. Apart from the fact that ASCs possess characteristics that are comparable to bone marrow-derived MSCs, ASCs also produce significant quantities of anti-inflammatory factors. These anti-inflammatory factors have been shown to modify immune cell function(s) and activity [14] and include a plethora of cytokines, chemokines, enzymes, and other soluble mediators that have been shown to alter the inflammatory milieu [15]. Of singular importance in this regard is accumulating evidence that ASCs have the capacity to induce T-regulatory (T_{reg}) cell production and to up-regulate T_{reg} cell function(s) [16] including the synthesis of IL-10 [17]. ASCs also possess the ability to rebalance the M1/M2 macrophage phenotype that is skewed toward M1 (proinflammatory phenotype) in many autoimmune disorders [18] as well as having both direct and indirect effects on regulatory B-cells [19].

Chapter "Similarities and Differences in Stem Cells Between Cancer, Normal and Injured Brain": MSCs have been shown to possess unique characteristics making them ideal for use in "homing" to sites of organ and tissue injury. In this chapter, L Huang and P Huang review emerging and compelling evidence that MSCs have significant activity in the treatment of brain injuries and brain tumors via their immunomodulatory function(s) which is characterized by the promotion of angiogenesis [20] as well as the synthesis of other trophic factors. The evidence that MSCs also appear to participate in in vitro neuronal *trans*-differentiation is also discussed. Finally, the concept that MSCs could be employed in future clinical applications to deliver therapeutic molecules is proposed with the long-range objective of using the "homing" property of MSCs to treat brain tumors.

Chapter "MSCs and Asthma": Asthma is a chronic inflammatory disease that appears to be independent of age, gender, and ethnicity. However, a recent analysis indicated an association between asthma and socioeconomic background, where asthma was especially prevalent in urban areas of middle- and low-level income countries [21]. Asthma is also the most common childhood chronic inflammatory disease of the pulmonary system. In this chapter, Goldstein et al. review the evidence from preclinical models of various pulmonary diseases which have demonstrated that MSCs have important therapeutic utility for reprogramming the inflamed lung to undergo repair of damaged lung tissue resulting from chronic inflammatory insults. Perhaps the foremost responses with respect to chronic asthma are the capacity of MSCs to reduce the production of leukotriene B4, to down-regulate mast cell degranulation, and to alter the phenotypes of T-cells and macrophages which are likely to produce an anti-inflammatory milieu.

There is now a general consensus that MSCs and ASCs possess potent immunomodulatory function(s). Thus, these cells may have the potential to correct immune dysfunction associated with autoimmune diseases and chronic inflammation. The extent to which clinical efficacy of MSCs and ASCs demonstrated in preclinical animal models can be extended to human clinical trials remains to be completely elucidated, although the future looks bright for employing stem cells for these clinical applications.

References

1. Ahmed TA, Hincke MT (2014) Mesenchymal stem cell-based tissue engineering strategies for repair of articular cartilage. Histol Histopathol 29:669–689
2. Herrmann M, Verrier S, Alini M (2015) Strategies to stimulate mobilization and homing of endogenous stem and progenitor cells for bone tissue repair. Front Bioeng Biotechnol 3:79
3. Mamidi MK, Das AK, Zakaria Z, Bhonde R (2016) Mesenchymal stromal cells for cartilage repair in osteoarthritis. Osteoarthritis Cartilage 24(8):1307–1316. doi:10.1016/j.joca.2016.03.003, pii: S1063-4584(16)01060-8
4. Hynes K, Bright R, Proudman S, Haynes D, Gronthos S, Bartold M (2016) Immunomodulatory properties of mesenchymal stem cell in experimental arthritis in rat and mouse models: A systematic review. Semin Arthritis Rheum 46(1):1–19. doi:10.1016/j.semarthrit.2016.02.008, pii: S0049-0172(16)00088-3
5. Najar M, Raicevic R, Fayyad-Kazan H, Bron D, Toungouz M, Lagneaux L (2016) Mesenchymal stromal cells and immunomodulation: a gathering of regulatory immune cells. Cytotherapy 18:160–171
6. Gao F, Chiu SM, Motan DA, Zhang Z, Chen L, Ji HL, Tse HF, Fu QL, Lian Q (2016) Mesenchymal stem cells and immunomodulation: current status and future prospects. Cell Death Dis 7:e2062
7. de Witte SF, Franquesa M, Baan CC, Hoogduijn MJ (2016) Toward development of mesenchymal stem cells for immunomodulatory therapy. Front Immunol 6:648
8. González MA, González-Rey E, Rico L, Büscher D, Delgado M (2009) Treatment of experimental arthritis by inducing immune tolerance with human adipose-derived mesenchymal stem cells. Arthritis Rheum 60:1006–1019
9. Freitag J, Bates D, Boyd R, Shah K, Barnard A, Huguenin L, Tenen A (2016) Mesenchymal stem cell therapy in the treatment of osteoarthritis: reparative pathways, safety and efficacy - a review. BMC Musculoskelet Disord 17:230
10. Youd M, Blickarz C, Woodworth L, Touzjian T, Edling A, Tedstone J, Ruzek M, Tubo R, Kaplan J, Lodie T (2010) Allogeneic mesenchymal stem cells do not protect NZBxNZW F1 mice from developing lupus disease. Clin Exp Immunol 161:176–186
11. Liu K, Mohan C (2006) What do mouse models teach us about human SLE? Clin Immunol 119:123–130
12. Sang A, Yin Y, Zheng YY, Morel L (2012) Animal models of molecular pathology systemic lupus erythematosus. Prog Mol Biol Transl Sci 105:321–370
13. Bukulmez H, Bilgin A, Caplan AI, Jones O (2014) Prevention of late stage renal failure in BXSB SLE mouse model with human bone marrow derived mesenchymal stem cell treatment. Arthritis Rheumatol 66(S3):S149
14. Maria AT, Maumus M, Le Quellec A, Jorgensen C, Noël D, Guilpain P (2016) Adipose-derived mesenchymal stem cells in autoimmune disorders: state of the art and perspectives for systemic sclerosis. Clin Rev Allergy Immunol
15. Gimble JM, Katz AJ, Bunnell BA (2007) Adipose-derived stem cells for regenerative medicine. Circ Res 100:1249–1260
16. Mohammadzadeh A, Pourfathollah AA, Shahrokhi S, Hashemi SM, Moradi S l, Soleimani M (2014) Immunomodulatory effects of adipose-derived mesenchymal stem cells on the gene expression of major transcription factors of T cell subsets. Int Immunopharmacol 20:316–321
17. Ivanova-Todorova E, Bochev I, Dimitrov R, Belemezova K, Mourdjeva M, Kyurkchiev S, Kinov P, Altankova I, Kyurkchiev D (2012) Conditioned medium from adipose tissue-derived mesenchymal stem cells induces CD4+FOXP3+ cells and increases IL-10 secretion. J Biomed Biotechnol 2012:295167
18. Adutler-Lieber S, Ben-Mordechai T, Naftali-Shani N, Asher E, Loberman D, Raanani E, Leor J (2013) Human macrophage regulation via interaction with cardiac adipose tissue-derived mesenchymal stromal cells. J Cardiovasc Pharmacol Ther 18:78–86

19. Park MJ, Kwok SK, Lee SH, Kim EK, Park SH, Cho ML (2015) Adipose tissue-derived mesenchymal stem cells induce expansion of interleukin-10-producing regulatory B cells and ameliorate autoimmunity in a murine model of systemic lupus erythematosus. Cell Transplant 24:2367–2377
20. Merino-González C, Zuñiga FA, Escudero C, Ormazabal V, Reyes C, Nova-Lamperti E, Salomón C, Aguayo C (2016) Mesenchymal stem cell-derived extracellular vesicles promote angiogenesis: potential clinical application. Front Physiol 7:24
21. Lundbäck B, Backman H, Lötvall J, Rönmark E (2016) Is asthma prevalence still increasing? Expert Rev Respir Med 10:39–51

MSCs and Asthma

Benjamin D. Goldstein, Arnold I. Caplan, and Tracey L. Bonfield

Introduction

Mesenchymal Stem Cells (MSCs) have been utilized as a source of regenerative and medicinal therapeutics in over 560 clinical trials to date (Clinicaltrials.gov) [1]. In these trials, the MSCs are utilized to either reconstruct tissue or to promote the host to manage its own repair. Although the mechanisms of how the MSCs participate in these processes are poorly understood, the end result is nothing less than impressive when the patient has a positive response [2]. When MSCs are given intravenously, as in most clinical trials, their first stop depending on the method of administration is the lung and its vasculature [3]. The cells dwell within the lung interstitial tissue and then can subsequently migrate to areas of injury. It may be that MSCs go to the lung, instruct macrophages which then have the capacity to perpetuate the therapeutic effects. How and why the MSCs track to sites of injury is still under investigation. The fact that the major initial site of MSCs impact is the pulmonary system, treating pulmonary disease seems obvious. Preclinical models of a variety of pulmonary syndromes have shown promise for the use of MSCs as a potential therapeutic tool, not necessarily to regenerate lung tissue, but to "signal" to the lung how to manage its own corrective process [4].

B.D. Goldstein, M.D. • T.L. Bonfield, Ph.D. (ABMLI) (✉)
Division of Pulmonary Medicine, Department of Pediatrics, Rainbow Babies and Children's Hospital, Case Western Reserve University School of Medicine, 11100 Euclid Avenue, Cleveland, OH 44106, USA
e-mail: Benjamin.goldstein@uhhospitals.org; tracey.bonfield@case.edu

A.I.Caplan, Ph.D.
Department of Biology, Skeletal Research Center, College of Arts and Sciences, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106-7080, USA
e-mail: arnold.caplan@case.edu

C.J. Malemud, E. Alsberg (eds.), *Mesenchymal Stem Cells and Immunomodulation*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-3-319-46733-7_2

Asthma is a chronic inflammatory disease, prevalent across age, sex, and ethnic background. According to the most recent data from the Center for Disease Control (CDC) National Asthma Control Program, more than 25 million adults are affected by asthma, which is roughly 1 in 12 people [5]. In children, specifically, asthma is the most common chronic condition, afflicting more than seven million people under the age of 18, which is roughly one in ten children [6]. The economic burden of asthma is large; asthma is the most common reason children required hospitalization in the developed world and is also the most common cause of missed school days as well as missed work days. In 2008, there were over 10 million missed days of school and over 14 million missed days of work. In the United States alone, asthma costs around $56 billion dollars each year with an average cost of over $1000 dollars per child [7].

Symptoms and Causes of Asthma and Potential Avenues for MSC Intervention

Asthma can be classified as allergy (atopic) or nonallergic (nonatopic). Both types of classification can impact lung function; symptoms are characterized as repeated episodes of wheezing, chest tightness, breathlessness, as well as nighttime or early morning coughing [8]. Wheeze is the most frequent symptom reported due to the variable small airway obstruction in asthma. There are many triggers and/or irritants that supply the inflammatory response that promotes the phenotypic signs of asthma. The most common triggers for asthma exacerbations have been associated with a virus etiology; those viruses that are implicated include rhinovirus, respiratory syncytial virus (RSV), metapneumovirus, parainfluenza virus, and influenza virus [5]. Lemanske and colleagues [9] investigated the risk of wheezing later in childhood if there was evidence of previous infection with RSV or rhinovirus within the first year of life. Their results show that those patients who wheezed with RSV in the first year of life had an increased association between exposure to virus and wheezing in the third year of life was increased 3.5-fold. Similar findings were seen in patients with a history of wheezing due to Rhinovirus in the first year of life; the adjusted odds of wheezing in the third year of life were increased tenfold [10].

Other triggers, including bacterial and atypical pneumonia, as well as inhalational injury, either due to smoking or inhalation of other aerosolized toxins such as chlorine, pollution, or dust mite antigens, can also cause direct injury to the epithelium and promote localized inflammation [11]. Atopic disease, such as allergic rhinitis, can lead to inflammation and the symptoms associated with an asthma exacerbation, either by stimulating an IgE-specific response causing mast cell and basophil degranulation, or by the rhino-bronchial reflex [12]. In the rhino-bronchial reflex, localized irritation in the nasal mucosa by allergens and cold air can cause bronchopulmonary disease characterized by cough, asthma-like bronchoconstriction and variable wheezing, and bronchopulmonary infections [13, 14]. This could be due to postnasal drip

of inflammatory cells and mucus into the lower airways causing irritation, but also localized activation of the eosinophils within the nasal passages can release mediators in the lower airways as well (described later). Other known triggers include nonsteroidal anti-inflammatory drugs (NSAIDs), aspirin, exercise, and stress can lead to bronchoconstriction and airway inflammation [15, 16].

In preclinical studies using a variety of antigens, asthma can be easily induced using antigens against ovalbumin, Der P1 (house dust mite allergen), or cockroach antigen. The use of the animal models to study the pathophysiology of asthma has been going on for years with measurable parameters including goblet cell hyperplasia, infiltration of lymphocytes and eosinophils, upregulation of cytokines such as IL-5 and IL-13, and increased deposition of extracellular matrix components [17, 18]. MSCs have been shown to attenuate many of these processes in asthma models, suggesting that they may have an important therapeutic niche [19, 20]. The issue with MSC therapeutics is the cell-based approach which in the current pharmaceutical explosion is not generally well accepted as a potential therapeutic tool for patients [21]. However, in a subset of patients that have been shown to be refractory to current medications including steroids, autologous or allogeneic MSCs maybe a viable approach [5, 22].

Pathophysiology and MSCs Therapeutics

In asthma, clinically airflow limitation is recurrent, and due to numerous changes within the airway, including bronchoconstriction, airway edema, airway hyperresponsiveness, and airway remodeling [7, 15]. These changes are variable and can often overlap to produce symptoms on a continuum; these symptoms can be intermittent or persistent as well as be episodic with acute phenotype ultimately transitioning into components associated with chronicity.

The inflammatory response to asthma is due to an infiltrative cell response to a number of triggers and/or antigens. The most common infiltrative cell includes eosinophils if due to an allergen, but lymphocytes (specifically T-cell lymphocytes), neutrophils, and mast cells also play a role [23, 24]. The interplay between the innate immune response and the adaptive immune response is responsible for the phenotypic features of asthma [25]. Dendritic cells within the airway are the primary antigen-presenting cells in the lung [26]. There are multiple proposed mechanisms of action that initiate the immune response seen in asthma. At the epithelial cell level, toll like-receptors, or TLRs, and protease-activated receptors, or PARs interact with allergens or endotoxin within the lumen of the airway [27, 28]. The TLRs and PARs lead to increased thymic stromal lymphopoietin expression, which augments dendritic cell, mast cell, and eosinophil cell recruitment to the site of inflammation [29]. A second mechanism proposed includes dendritic cell direct sampling and interaction with bacteria, viruses, fungi, parasites, or enzymatically active antigens within the lumen of the airway and through extensions between epithelial cells [30]. Pattern recognition receptors, or PRRs, on the surface of the

dendritic cells recognize pathogen-associated molecular patterns, or PAMPs, on the surface of the stimulant, which in turn releases interleukins, tumor necrosis factor (TNF)-α, and interferons [31, 32]. These inflammatory mediators stimulate recruitment of eosinophils to the airways, as well as migration of dendritic cells to the lymph nodes, which stimulates a Th2 immune response. The Th2 helper T-cells from the lymph nodes will then play a major role in changes to the epithelium and blood vessels in asthma, based on which cytokines are released. Interleukin (IL)-4, IL-5, IL-9, IL-13, and TNF-α induce most of the features of asthma [33, 34]. Within the bronchial smooth muscle cell, these inflammatory factors promote bronchial hyper-reactivity characterized by both hypertrophy and hyperplasia of these cells, resulting in bronchoconstriction [35, 36]. Other inflammatory changes include goblet cell hyperplasia and hypersecretion of mucus into the small airways, epithelial cell damage, and activation/survival of eosinophils [37].

MSCs have been shown to suppress and redirect T-cell activity in a variety of models [38, 39]. In our own models, we have been able to demonstrate the effectiveness on decreasing IL-5 and eosinophil recruitment as well as T-cell activity [3, 20]. As a therapeutic for acute asthmatic episodes, it is clear that MSCs may provide some benefit; however, the sustainability of the clinical impact may not be reflected in defined benefit without periodic infusions. In addition, MSCs have been shown to alter dendritic cell activation with the potential of changing how the dendritic cell communicates the adaptive immune needs [40, 41]. Since the dendritic cells are responsible for activation as well as resolution of inflammation, the contribution of the MSCs maybe related to the ability to aid in redirecting the communicating signals of the allergic response toward tolerance instead of activation.

There are two phases of inflammation, an early phase response and a late phase response [42]. During the early phase response, IgE is formed from the interaction of T-cells and B-cells (more specifically plasma cells) in response to IL-4 and IL-13 [43]. The IgE then binds to and cross-links FcεRI receptors on mast cells and basophils. This interaction and cross-linking causes mast cell degranulation and release of multiple preformed mediators including histamine, proteases, proteoglycans, tryptase, and matrix metalloproteases, or MMPs [44, 45]. Other cytokines and chemokines are released from mast cells including IL-4, IL-5, granulocyte macrophage-colony stimulating factor (GM-CSF), TNF-alpha, transforming growth factor beta (TGF-β), eotaxin-1, macrophage inflammatory factor-1 alpha (MIP-1a), and macrophage chemoattractant protein-1 (MCP-1) [46, 47]. Basophils also express FcεRI receptors and degranulate after IgE cross-linking in a similar fashion to mast cells. They release histamine, eicosanoids, leukotrienes, IL-4, and IL-13 [48, 49]. During the late phase response, eosinophils are attracted by eotaxin and IL-5 to the site of inflammation to the lung and release many other proinflammatory markers including major basic protein (MBP), leukotrienes LTC4 and LTD4, eosinophil cationic protein (ECP), and platelet activating factor (PAF) [50–52].

MSCs have been shown to have an active role in downregulating proinflammatory mechanisms in a variety of animal models. The published literature supports MSC downregulation of MMPs, GM-CSF, TNFα, as well as shifting chemokine production [2, 4, 53]. In our research, we have demonstrated that MSCs have the

capacity to downregulate local cytokines to potentially decrease eosinophilic recruitment while increasing systemic cytokines involved in cellular recruitment associated with resolution of the inflammatory response [54, 55].

Treatment of Acute Asthma Exacerbation and MSCs

The symptoms seen during an acute exacerbation of asthma are subjective shortness of breath, chest tightness, cough, and wheezing. These symptoms are due to bronchospasm and usually respond to bronchodilator therapy [56].

Treatment of acute asthma exacerbations targets relieving smooth muscle bronchoconstriction as well as decreased inflammation. The cornerstones of therapy for acute exacerbations include inhaled short acting beta-adrenergic agonist medication (SABAs) such as albuterol, levalbuterol, and historically pirbuterol, and systemic corticosteroid therapy [56]. The smooth muscle cells in the airway contain beta-2 adrenergic receptors that respond to beta-2 agonists. When the medication binds to the receptor, adenylate cyclase is activated and increases cyclic AMP, which produces functional antagonism of bronchoconstriction. Inhaled beta-2 agonists have a faster onset, less adverse side effects, and are more effective than systemic beta-agonists [57]. Other selective beta-2-agonists such as isoproterenol, metaproterenol, isoetharine, and epinephrine are not recommended due to excessive cardiac effects [58, 59]. Systemic corticosteroids are initiated for moderate to severe exacerbations as adjunctive therapy to SABAs to speed recovery and to prevent recurrence of symptoms in the near future [60]. They work by decreasing, controlling, and potentially reversing airway inflammation. Other adjunctive therapies include supplemental oxygen is hypoxemia is present, which may be utilized concurrently with inhaled anticholinergic medications such as ipratropium bromide, and systemic magnesium sulfate [61, 62].

Treatment of acute asthma with MSCs is suggested by preclinical studies and by others showing improvement in methacholine challenge responses as well as inflammatory outcome measures of the acute response to antigenic challenge [19, 63, 64]. The main issue with using MSCs as a therapeutic for treating asthma is the unpredictable nature of the acute response. In this case, it might be reasonable to suggest that in scenarios of uncontrolled asthma, whether it is atopic or nonatopic, infusions might need to be repeated to get a beneficial response of attenuating the overt acute response.

Chronic Asthma, Airway Remodeling, and MSCs

If long-standing inflammation is continued within the small airways of asthma and unable to be repaired properly, remodeling can occur [65]. This process can be irreversible. Based on histologic and immunohistochemical specimens, the studies showed that airway remodeling included epithelial damage and detachment,

subepithelial fibrosis, increased myofibroblast proliferation and hyperplasia, increased smooth muscle mass and number, diffuse edema, goblet cell hyperplasia and hypersecretion of mucins, and proliferation of blood vessels, or angiogenesis [66, 67]. Underneath the basement membrane of the airway epithelium, a dense network of structural compounds is formed which includes fibronectin, proteoglycans, as well as collagen type I and type III [68–70]. As stated previously, the stimulus for airway remodeling is based on a Th2 inflammatory response and eosinophil chemotaxis. More specifically, eosinophils are produced in response to GM-CSF, IL4, and IL13 and they in turn produce profibrotic mediators, especially TGF-β [71–73].

MSCs have been shown to decrease many of the components associated with remodeling, but what is the definition of "remodeling" [63, 74, 75]. In the context of asthma, it is excessive would healing and the associated repair that can lead to scar formation. Depending on the mechanisms governing the scar formation MSCs can have an impact on the pathophysiology. In our own studies, MSCs are capable of reproducibly decreasing the scar associated with the competition between repair and remodeling. MSCs have the capacity to decrease the expression of collagen genes and change the activity levels of MMPs further contributing to the potential of altering the progression of chronic asthma-induced airway remodeling associated with excessive scaring [76–78].

Chronic Asthma Control

The goal of chronic asthma control is to reduce impairment by preventing symptoms (coughing, breathlessness, exercise-induced shortness of breath and wheezing, etc.), reduce short-acting beta agonists (SABA) use, maintain normal activity level, and meet family and patient expectations and satisfaction with asthma care. This is accomplished as an outpatient, with assessment of lung function, assessment of subjective symptoms, as well as providing all patients with asthma action plans to organize current care and contingency plans if an exacerbation were to occur. The other goal of chronic control is to reduce future risk by preventing exacerbations and hospitalizations, preventing loss of lung function, and providing optimal pharmacotherapy with minimal adverse side effects. This is accomplished by emphasizing education to patients and families regarding trigger avoidance, medication adherence, and proper technique of technologic devices for better medication deposition.

Pharmacotherapy is used to control asthma symptoms and prevent exacerbations while improving quality of life. Long-term control medications include inhaled corticosteroids (ICSs), leukotriene modifiers, cromolyn, methylxanthines (theophylline), and immunomodulators [58, 59]. Because the basis of asthma severity is due to eosinophilic and lymphocytic inflammation, the most effective long-term therapy decreases inflammation. The addition to pharmacotherapeutic intervention in asthma is the role of cell-based therapeutics and the overall additional support they may bring to the regimes available for patients. MSCs have the potential of contributing therapeutically

Table 1 MSCs as surrogate therapeutics in asthma

Pharmacotherapy	MSC impact
Long-Acting Beta Agonist/Inhaled Corticosteroids Note: LABAs not used as single agents to control asthma symptoms	MSCs have been found to be effective in the resolution of steroid-resistant GVHD [88]. The main mechanism remains unknown; however, previous literature suggests upregulation of regulatory T-cells, release of soluble factors, decreased and repair of damaged tissue. Other mechanisms include inhibition of T-cell proliferation [89]. Decreased inflammation in chronic asthma stimulated by OVA peptide, shown as decreased epithelial cell thickness, decreased smooth muscle thickness, decreased basement membrane thickness, and decreased goblet cell hyperplasia [90–92].
Leukotriene receptor antagonists	MSCs contain cysteinyl leukotriene receptors (LTRA) [83]; LTRA have been found to affect MSC differentiation and can contribute to local inflammation regulation. CysLT1 receptor antagonists inhibit the airway remodeling processes, including eosinophil trafficking to the lungs, eosinophil degranulation, T_H2 cytokine release, mucus gland hyperplasia, mucus hypersecretion, smooth muscle cell hyperplasia, collagen deposition, and lung fibrosis [93].
Mast cell modulators	Bone marrow-derived mesenchymal cells produce low level of thymic stromal lymphopoietin (TSLP) under steady-state conditions [84], which is markedly increased by stimulation with proinflammatory cytokines IL-1 and TNF or IgE-activated MCs [94]. MSCs suppressed MC degranulation, proinflammatory cytokine production, chemokinesis, and chemotaxis.

GVHD graft versus host disease, *LTRA* leukotriene receptor antagonists, *LABA* long-acting beta agonist, *IL-1* interleukin 1, *MSCs* mesenchymal stem cells, *MCs* mast cells, *TNF* tumor necrosis factor

in a similar manner to traditional pharmacotherapy (Table 1) [20, 41, 79–85]. Since MSCs have the ability to produce bioactive factors that can manipulate their microenvironment, they can be utilized to optimize the milieu for improved pharmacotherapeutic efficacy and potency [86, 87].

Inhaled Corticosteroids

The most effective of these medications are the inhaled corticosteroids which has been shown to reduce the severity of symptoms, improved quality of life, improved spirometry and PEF, reduced systemic steroid usage, reduced hospitalization, and most importantly reduced death due to asthma [95, 96]. Corticosteroids suppress the generation of cytokines, reduce eosinophil recruitment, and decrease the release of inflammatory mediators. Patients with mild or moderate persistent asthma that are treated with ICS vs. any other single agent showed improved prebronchodilator FEV1, reduced airway hyper-responsiveness, improved symptom scores, decreased exacerbation rates and symptom frequency, decreased usage of SABA, fewer courses of oral corticosteroids, and less hospitalizations.

The asthmatic population that should be a primary focus of MSC therapeutics is the group of patients that are refractory to corticosteroid therapy. Even with the caveats of cell-based therapeutics, the disproportionate cost of treating asthmatic patients who do not respond to conventional anti-inflammatory therapies makes delineation of the mechanism for glucocorticoid resistance an important field of asthma research. Unbiased cluster analysis indicates that asthma is a syndrome with a number of distinct phenotypes and 5–10 % of asthmatics fall into this category of relative glucocorticoid insensitivity [5]. Novel treatments will consist of either developing new anti-inflammatory treatments targeting pathways aberrantly activated in these patients or of suppressing signaling pathways that attenuate glucocorticoid receptor function and thereby restoring glucocorticoid sensitivity such as might be gained with MSC therapeutics [92, 97]. MSCs have the capability of secreting a spectrum of soluble mediators which have the potential to alter a variety of pathophysiological outcomes associated with chronic unrelenting asthma either through improving airway flow or inflammation [1]. Conventional therapies combined with MSCs may provide a unique treatment profile with the potential of providing therapeutic impact [54, 98]. Further, additional add-on treatments using drugs directed against aberrantly expressed inflammatory pathways or mediators along with an inhaled glucocorticoid are likely to prove the most effective new therapies in the future.

Long-Acting β-Agonists

Long-acting β-adrenergic receptor agonists (LABAs) are medications used in the control of chronic asthma symptoms and not acute asthma exacerbation management [96, 99]. MSC therapeutics may be beneficial in the setting of COPD since the MSCs secrete molecules that impact both SABA and LABA mechanisms. These studies obviously should be done in vivo prior to studies in humans. Studies have suggested that MSCs have the capacity to impact Beta-2 adrenergic signal transduction through their production of RANKL and potentially osteoprotegerin [2, 100]. MSCs can also be regulated by Beta-2-adrenergic signaling [100, 101]. Activation of the B2-AR signal in MSCs suppresses their osteogenic differentiation potential and modulates their chemokine expression for regulating the homeostasis of hematopoietic stem cells. Although not directly tested, it is possible that the MSCs not only may provide an alternative therapeutic for steroid resistant asthma but at the same time may provide a supplemental therapeutic to enhance LABA effectiveness.

Leukotriene Receptor Antagonists

One of the newer asthma controller medications is a leukotriene receptor antagonist such as BIIL 284, montelukast, and zafirlukast [102, 103]. This type of medications works in the arachidonic acid pathways of inflammation by blocking the

leukotriene receptors. Activation of the leukotriene pathway is through metabolic modification of arachidonic acid. In this pathway, arachidonic acids are metabolized to 5-HPETE by 5-lipoxygenase, and eventually metabolize to leukotriene A4, B4, C4, D4, and E4 [102]. These proteins promote vasoconstriction, bronchospasm, and increased vascular permeability and inflammatory cell recruitment. Montelukast (Singulair) blocks the action of leukotriene D4 (LTD4) and its secondary ligands LTC4 and LTE4 by antagonizing the CysLT1 receptor in the lungs and bronchi, which subsequently reduces bronchospasm and inflammation [6, 102, 104]. Another example of a leukotriene receptor antagonist is zafirlukast (Accolate) but is dosed twice daily and not used often [105, 106]. Zileuton (Zyflo) is an oral medication that works by inhibiting the enzyme 5-lipoxygenase [107]. Zyflo is available in two forms: the immediate release tablet is taken four times daily and the extended release tablet is taken twice daily. Overall, LTRA have shown improvement in lung function when used as monotherapy in adults and children greater than 5 years of age [106].

MSCs are anti-inflammatory, decreasing proinflammatory cytokines and increasing anti-inflammatory cytokines [53, 108]. Additionally, MSCs have been shown to decrease leukocyte migration due to suppression of chemokines and leukotriene B4 [109]. This enhances the immunomodulatory effectiveness of the MSCs supplementing the decreased expression of IL-1B, IL-6, TNF-α, and TSG-6 [110, 111]. Further the downregulation of PGEs and PGE2 are also part of the suppression process. Many of these anti-inflammatory effects are thought to be mediated through suppressing NFκB [112].

Mast Cell Modulators

Mast cell modulators act by blockade of chloride channels and stabilize the membrane on mast cells, thereby decreased mediator release and eosinophil recruitment [45, 113]. Cromolyn and nedocromil have been shown to provide between control of symptoms versus placebo in some, but not all clinical trials [59].

MSCs are a promising tool for the therapy of immune disorders; however, their efficacy and mechanisms in treating allergic disorders are less well defined [94]. Certainly mast cells and their degranulation products are important in the pathophysiology of the allergic disease [44, 45, 114]. MSCs have been shown to exert a cell-to-cell contact independent suppressive effect on mast cell degranulation through an increased production of prostaglandin E2 [84, 115]. Additionally, TGF B1 production from the MSCs in response to stimulation with factors such as IL-4 contributes to the attenuation of mast cell degranulation by downregulating FcER1 expression in mast cells [85, 116]. These potential activities of the MSCs imply their potential therapeutic efficacy in scenarios of allergy as well as anaphylaxis which may be concurrent with severe asthma [85].

Immunomodulators

Many different pharmaceutical agents have been developed with the attempt to control asthma symptoms long term and spare using steroid-containing medications. There are many different types of immunomodulators, such as omalizumab (anti-IgE antibody), methotrexate, soluble IL-4, anti-IL-5 antibodies, recombinant IL-12, cyclosporin A, intravenous immunoglobulin (IVIG), clarithromycin [117–120]. Unfortunately, only omalizumab has been shown to be beneficial in the control on chronic asthma symptoms.

Omalizumab (Xolair) is a recombinant DNA-derived human monoclonal antibody against the Fc portion of the IgE antibody, which prevents the binding of IgE to a specific receptor (FcεRI) on mast cells and basophils [62, 121]. The goal is that in response to an allergen exposure, decreased IgE will result in decreased mast cell mediator production and release. Omalizumab has been shown to decrease FcεRI expression on basophils and airway submucosal cells decreased eosinophils in sputum, and decreased CD3+, CD4+, and CD8+ T-cells in bronchial biopsy [118, 122].

MSCs would also fall within the immunomodulatory group [76, 108, 123]. MSCs main therapeutic strength is the capacity to redirect the immune response to instituting resolution of the inflammatory phenotype whether it is redirecting the proinflammatory mediators associated with the chronic asthmatic lung or through redirecting the remodeling process [124–126]. The MSC capacity to downregulate LTB4, mast cell degranulation, and change the directionality of T-cell phenotypes and macrophages may provide enough preclinical data to support an investigator-initiated clinical trial to explore the use of MSCs to treat severe unrelenting steroid-resistant asthma. If clinical efficacy can be found in this population then it is reasonable to think that the greater asthma patient population could benefit from the MSCs therapeutics.

Summary

MSCs have the capacity to provide a unique therapeutic intervention for asthma as outlined in Fig. 1 and summarized throughout this chapter. There is accumulated information for its potential impact on both the acute and chronic phases of the disease. In Fig. 1, we highlighted a simplified version of the sequence of events that occur in the initiation of the inflammatory profile associated with asthma and the capacity of the MSC to redirect the phenotypic response that is associated with this initiation of airway reactivity and inflammation [86, 109, 127]. As the inciting irritation continues, the asthma will take on a different phenotype associated with more of a chronic inflamed airway including lymphocyte redirection, activation of the airway smooth muscle cells, and goblet cell hyperplasia [6, 65, 84, 128, 129]. In the context of MSC therapeutics, literature has shown the capacity of stem cell therapy to attenuate both the remodeling aspects as well as the hyperplasia all which would improve lung function [3, 34, 85, 94, 130, 131]. All along the way there are points

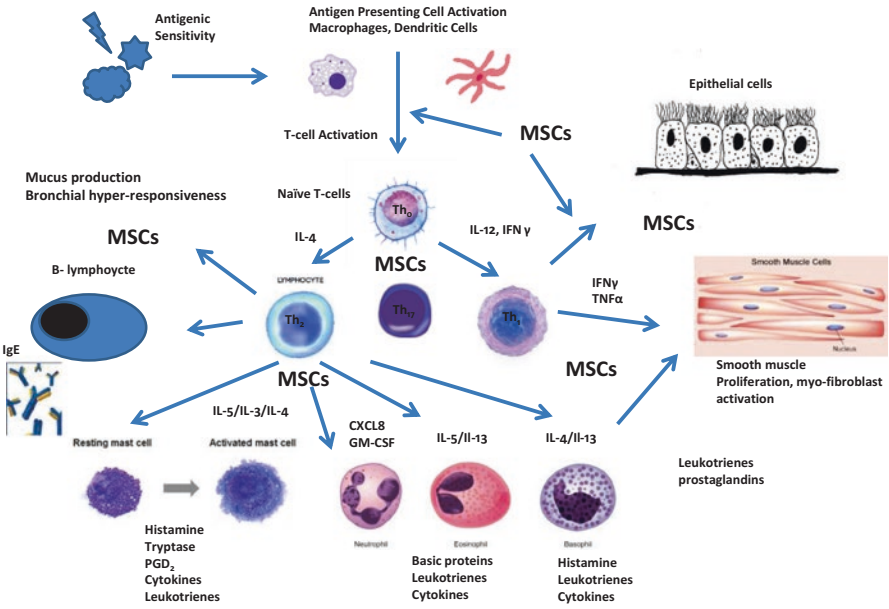

Fig. 1 Asthma and MSCs. A simple outline of the pathophysiological mechanisms associated with asthma and the areas which MSCs may intervene in the disease process. The initiation of asthmatic pathophysiology starts a cascade which in its earlier phases may benefit from the protective effects of the MSCs. The earlier activation and sustained changes in the airway epithelium and smooth muscle cells also have the capacity to benefit from the immunomodulatory effect of the MSCs. Because asthma is associated with dysregulated immunity, MSCs have been shown to impact a variety of immune cell functions emphasizing the multifaceted therapeutic potential of the MSCs in scenarios like asthma. In all, the mechanistic approaches to asthma therapy may include MSCs utilizing their potential as an adjunct or in some cases an "instead" of therapeutic with the potential of ameliorating the pathophysiological mechanisms of airway disease associated with asthma

at which MSCs have the capacity to impact the outcome depending on the severity and duration of the disease. Future studies will outline the specifics of MSC therapeutics in this disease setting.

Acknowledgments The authors would like to thank the insight of the contributing references in this review as well as the support of Rainbow Babies and Children's Hospitals, and the David and Virginia Baldwin Fund.

References

1. Caplan AI (2015) Adult mesenchymal stem cells: when, where, and how. Stem Cells Int 2015 Article ID 628767, 6 pages
2. Caplan AI, Hariri R (2015) Body management: mesenchymal stem cells control the internal regenerator. Stem Cells Trans Med 4:695–701

3. Bonfield TL, Nolan Koloze MT, Lennon DP, Caplan AI (2010) Defining human mesenchymal stem cell efficacy in vivo. J Inflamm (Lond) 7:51
4. Caplan AI, Sorrell JM (2015) The MSC curtain that stops the immune system. Immunol Lett 168:136–139
5. Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. American Thoracic Society. 2000. Am J Respir Crit Care Med 162:2341–2351
6. Aujla SJ, Ross KR, Chmiel JF, Holguin F (2011) Airway molecular phenotypes in pediatric asthma. Curr Opin Allergy Clin Immunol 11:122–126
7. Cohn L, Elias JA, Chupp GL (2004) Asthma: mechanisms of disease persistence and progression. Annu Rev Immunol 22:789–815
8. Bhakta NR, Woodruff PG (2011) Human asthma phenotypes: from the clinic, to cytokines, and back again. Immunol Rev 242:220–232
9. Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, Printz MC, Lee WM, Shult PA, Reisdorf E, Carlson-Dakes KT, Salazar LP, DaSilva DF, Tisler CJ, Gern JE, Lemanske RF (2008) Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. Am J Respir Crit Care Med. 178(7): 667–672
10. Helms PJ (2009) Recent advances in pediatric asthma treatment. Expert Rev Respir Med 3:165–175
11. Ross K, Chmiel JF, Ferkol T (2012) The impact of the Clean Air Act 16. J Pediatr 161:781–786
12. Barnes P (1995) Air pollution and asthma: molecular mechanisms [Review]. Mol Med Today 1:149–155
13. Hallstrand T, Ault K, Bates P, Mitchell J, Schoene R (1998) Peripheral blood manifestations of T_{H2} lymphocyte activation in stable atopic asthma and during exercise-induced bronchospasm. Ann Allergy Asthma Immunol 80:424–432
14. Kacmarek RM, Ripple R, Cockrill BA, Bloch KJ, Zapol WM, Johnson DC (1996) Inhaled nitric oxide. A bronchodilator in mild asthmatics with methacholine-induced bronchospasm. Am J Respir Crit Care Med 153:128–135
15. Li J (1997) Mechanisms of asthma [Review]. Curr Opin Pulm Med 3:10–16
16. Cho JY (2011) Recent advances in mechanisms and treatments of airway remodeling in asthma: a message from the bench side to the clinic. Korean J Intern Med 26:367–383
17. Coffer PJ, Schweizer RC, Dubois GR, Maikoe T, Lammers JW, Koenderman L (1998) Analysis of signal transduction pathways in human eosinophils activated by chemoattractants and the T-helper 2-derived cytokines interleukin-4 and interleukin-5. Blood 91:2547–2557
18. Corren J (2011) Cytokine inhibition in severe asthma: current knowledge and future directions. Curr Opin Pulm Med 17:29–33
19. Inamdar AC, Inamdar AA (2013) Mesenchymal stem cell therapy in lung disorders: pathogenesis of lung diseases and mechanism of action of mesenchymal stem cell. Exp Lung Res 39:315–327
20. Bonfield TL, Koloze M, Lennon DP, Zuchowski B, Yang SE, Caplan AI (2010) Human mesenchymal stem cells suppress chronic airway inflammation in the murine ovalbumin asthma model. Am J Physiol Lung Cell Mol Physiol 299:L760–L770
21. Mullane K (2011) Asthma therapeutics: recent strides, new hurdles. Mol Interv 11:103–106
22. Berry MA, Hargadon B, Shelley M, Parker D, Shaw DE, Green RH, Bradding P, Brightling CE, Wardlaw AJ, Pavord ID (2006) Evidence of a role of tumor necrosis factor alpha in refractory asthma. N Engl J Med 354:697–708
23. Kips JC, Tournoy KG, Pauwels RA (2001) New anti-asthma therapies: suppression of the effect of interleukin (IL)-4 and IL-5. Eur Respir J 17:499–506
24. Humbles AA, Lloyd CM, McMillan SJ, Friend DS, Xanthou G, McKenna EE, Ghiran S, Gerard NP, Yu C, Orkin SH, Gerard C (2004) A critical role for eosinophils in allergic airways remodeling. Science 305:1776–1779
25. Robinson DS (2010) The role of the T cell in asthma. J Allergy Clin Immunol 126:1081–1091

26. Cravens PD, Lipsky PE (2002) Dendritic cells, chemokine receptors and autoimmune inflammatory diseases. Immunol Cell Biol 80:497–505
27. Blease K, Kunkel SL, Hogaboam CM (2001) IL-18 modulates chronic fungal asthma in a murine model; putative involvement of toll-like receptor-2. Inflamm Res 50:552–560
28. Genuneit J, Cantelmo JL, Weinmayr G, Wong GW, Cooper PJ, Riikjarv MA, Gotua M, Kabesch M, von Mutius E, Forastiere F, Crane J, Nystad W, El-Sharif N, Batlles-Garrido J, Garcia-Marcos L, Garcia-Hernandez G, Morales-Suarez-Varela M, Nilsson L, Braback L, Saraclar Y, Weiland SK, Cookson WO, Strachan D, Moffatt MF (2009) A multi-centre study of candidate genes for wheeze and allergy: the International Study of Asthma and Allergies in Childhood Phase 2. Clin Exp Allergy 39:1875–1888
29. Ying S, O'Connor B, Ratoff J, Meng Q, Mallett K, Cousins D, Robinson D, Zhang G, Zhao J, Lee TH, Corrigan C (2005) Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. J Immunol 174:8183–8190
30. Walker C, Bauer W, Braun RK, Menz G, Braun P, Schwarz F, Hansel TT, Villiger B (1994) Activated T cells and cytokines in bronchoalveolar lavages from patients with various lung diseases associated with eosinophilia. Am J Respir Crit Care Med 150:1038–1048
31. Netea MG, van der Meer JW, Kullberg BJ (2006) Recognition of pathogenic microorganisms by Toll-like receptors. Drugs Today (Barc) 42(Suppl A):99–105
32. Kapetanovic R, Cavaillon JM (2007) Early events in innate immunity in the recognition of microbial pathogens. Expert Opin Biol Ther 7:907–918
33. Barnes PJ (2001) Cytokine modulators for allergic diseases. Curr Opin Allergy Clin Immunol 1:555–560
34. Boyton RJ, Altmann DM (2004) Asthma: new developments in cytokine regulation. Clin Exp Immunol 136:13–14
35. Lauer ME, Cheng G, Swaidani S, Aronica MA, Weigel PH, Hascall VC (2013) Tumor necrosis factor-stimulated gene-6 (TSG-6) amplifies hyaluronan synthesis by airway smooth muscle cells. J Biol Chem 288:423–431
36. Rieder F, Nonevski I, Ma J, Ouyang Z, West G, Protheroe C, DePetris G, Schirbel A, Lapinski J, Goldblum J, Bonfield T, Lopez R, Harnett K, Lee J, Hirano I, Falk G, Biancani P, Fiocchi C (2014) T-helper 2 cytokines, transforming growth factor beta1, and eosinophil products induce fibrogenesis and alter muscle motility in patients with eosinophilic esophagitis. Gastroenterology 146:1266–1277
37. Careau E, Sirois J, Bissonnette EY (2002) Characterization of lung hyperresponsiveness, inflammation, and alveolar macrophage mediator production in allergy resistant and susceptible rats. Am J Respir Cell Mol Biol 26:579–586
38. Bai L, Lennon DP, Eaton V, Maier K, Caplan AI, Miller SD, Miller RH (2009) Human bone marrow-derived mesenchymal stem cells induce Th2-polarized immune response and promote endogenous repair in animal models of multiple sclerosis. Glia 57:1192–1203
39. Kebriaei P, Robinson S (2011) Treatment of graft-versus-host-disease with mesenchymal stromal cells. Cytotherapy 13:262–268
40. Le Blanc K, Ringden O (2007) Immunomodulation by mesenchymal stem cells and clinical experience. J Intern Med 262:509–525
41. Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, Lanino E, Sundberg B, Bernardo ME, Remberger M, Dini G, Egeler RM, Bacigalupo A, Fibbe W, Ringden O (2008) Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet 371:1579–1586
42. Bosnjak B, Stelzmueller B, Erb KJ, Epstein MM (2011) Treatment of allergic asthma: modulation of Th2 cells and their responses. Respir Res 12:114
43. Goleva E, Dunlap A, Leung DYM (2004) Differential control of TH1 versus TH2 cell responses by the combination of low-dose steroids with β2-adrenergic agonists. J Allergy Clin Immunol 114:183–191
44. Crimi E, Chiaramondia M, Milanese M, Rossi G, Brusasco V (1991) Increased numbers of mast cells in bronchial mucosa after the late-phase asthmatic response to allergen. Am Rev Respir Dis 144:1282–1286

45. Caughey GH (2011) Mast cell proteases as protective and inflammatory mediators. Adv Exp Med Biol 716:212–234
46. Kelly-Welch AE, Hanson EM, Boothby MR, Keegan AD (2003) Interleukin-4 and interleukin-13 signaling connections maps. Science 300:1527–1528
47. Ohkawara Y, Yamauchi K, Tanno Y, Tamura G, Ohtani H, Nagura H, Ohkuda K, Takishima T (1992) Human lung mast cells and pulmonary macrophages produce tumor necrosis factor-α in sensitized lung tissue after IgE receptor triggering. Am J Respir Cell Mol Biol 7:385–392
48. Maruyama N, Tamura G, Aizawa T, Ohrui T, Shimura S, Shirato K, Takishima T (1994) Accumulation of basophils and their chemotactic activity in the airways during natural airway narrowing in asthmatic individuals. Am J Respir Crit Care Med 150:1086–1093
49. Stone KD, Prussin C, Metcalfe DD (2010) IgE, mast cells, basophils, and eosinophils. J Allergy Clin Immunol 125:S73–S80
50. Kita H, Abu-Ghazaleh R, Sur S, Gleich G (1995) Eosinophil major basic protein induces degranulation and IL-8 production by human eosinophils. J Immunol 154:4749–4758
51. Lanz M, Leung D, McCormick D, Harbeck R, Szefler S, White C (1997) Comparison of exhaled nitric oxide, serum eosinophilic cationic protein, and soluble interleukin-2 receptor in exacerbations of pediatric asthma. Pediatr Pulmonol 24:305–311
52. Okamoto H, Kamatani N (2008) Platelet-activating factor, PAF acetylhydrolase, and anaphylaxis. N Engl J Med 358:1516–1517
53. Caplan AI, Dennis JE (2006) Mesenchymal stem cells as trophic mediators. J Cell Biochem 98:1076–1084
54. Bonfield TL, Ross KR (2012) Asthma heterogeneity and therapeutic options from the clinic to the bench. Curr Opin Allergy Clin Immunol 12:60–67
55. Bonfield TL, Caplan AI (2010) Adult mesenchymal stem cells: an innovative therapeutic for lung diseases. Discov Med 9:337–345
56. Rodrigo GJ, Neffen H, Colodenco FD, Castro-Rodriguez JA (2010) Formoterol for acute asthma in the emergency department: a systematic review with meta-analysis. Ann Allergy Asthma Immunol 104:247–252
57. Ortega VE, Peters SP (2010) Beta-2 adrenergic agonists: focus on safety and benefits versus risks. Curr Opin Pharmacol 10:246–253
58. Walsh GM (2006) Targeting airway inflammation: novel therapies for the treatment of asthma. Curr Med Chem 13:3105–3111
59. Walsh GM (2005) Novel therapies for asthma--advances and problems. Curr Pharm Des 11:3027–3038
60. Barrett TE, Strom BI (1995) Inhaled beta-adrenergic receptor agonists in asthma: more harm than good? Am J Respir Crit Care Med 151:574–577
61. Peters SP, Kunselman SJ, Icitovic N, Moore WC, Pascual R, Ameredes BT, Boushey HA, Calhoun WJ, Castro M, Cherniack RM, Craig T, Denlinger L, Engle LL, DiMango EA, Fahy JV, Israel E, Jarjour N, Kazani SD, Kraft M, Lazarus SC, Lemanske RF Jr, Lugogo N, Martin RJ, Meyers DA, Ramsdell J, Sorkness CA, Sutherland ER, Szefler SJ, Wasserman SI, Walter MJ, Wechsler ME, Chinchilli VM, Bleecker ER (2010) Tiotropium bromide step-up therapy for adults with uncontrolled asthma. N Engl J Med 363:1715–1726
62. Courtney AU, McCarter DF, Pollart SM (2005) Childhood asthma: treatment update 57. Am Fam Physician 71:1959–1968
63. Lin HY, Xu L, Xie SS, Yu F, Hu HY, Song XL, Wang CH (2015) Mesenchymal stem cells suppress lung inflammation and airway remodeling in chronic asthma rat model via PI3K/Akt signaling pathway 1. Int J Clin Exp Pathol 8:8958–8967
64. Martinez-Gonzalez I, Cruz MJ, Moreno R, Morell F, Munoz X, Aran JM (2014) Human mesenchymal stem cells resolve airway inflammation, hyperreactivity, and histopathology in a mouse model of occupational asthma 12. Stem Cells Dev 23:2352–2363
65. Al HS, McGovern TK, Martin JG (2011) Insights into asthmatic airway remodelling through murine models. Respirology 16:589–597
66. Hoshino M, Nakamura Y, Sim J, Shimojo J, Isogai S (1998) Bronchial subepithelial fibrosis and expression of matrix metalloproteinase-9 in asthmatic airway inflammation. J Allergy Clin Immunol 102:783–788

67. Xu J, Benyon RC, Leir SH, Zhang S, Holgate ST, Lackie PM (2002) Matrix metalloproteinase-2 from bronchial epithelial cells induces the proliferation of subepithelial fibroblasts. Clin Exp Allergy 32:881–888
68. Batra V, Musani AI, Hastie AT, Khurana S, Carpenter KA, Zangrilli JG, Peters SP (2004) Bronchoalveolar lavage fluid concentrations of transforming growth factor (TGF)-β1, TGF-β2, interleukin (IL)-4 and IL-13 after segmental allergen challenge and their effects on alpha-smooth muscle actin and collagen III synthesis by primary human lung fibroblasts. Clin Exp Allergy 34:437–444
69. Finotto S, Neurath MF, Glickman JN, Qin S, Lehr HA, Green FH, Ackerman K, Haley K, Galle PR, Szabo SJ, Drazen JM, De Sanctis GT, Glimcher LH (2002) Development of spontaneous airway changes consistent with human asthma in mice lacking T-bet. Science 295:336–338
70. Maekawa Y, Anzai T, Yoshikawa T, Sugano Y, Mahara K, Kohno T, Takahashi T, Ogawa S (2004) Effect of granulocyte-macrophage colony-stimulating factor inducer on left ventricular remodeling after acute myocardial infarction. J Am Coll Cardiol 44:1510–1520
71. Letterio JJ, Roberts AB (1998) Regulation of immune responses by TGF-β. Annu Rev Immunol 16:137–161
72. Nakao A (2001) Is TGF-β the key to suppression of human asthma? Trends Immunol 22:115–120
73. Rook GAW (2001) The TGF-β paradox in asthma. Trends Immunol 22:299–300
74. Atsma DE, Fibbe WE, Rabelink TJ (2007) Opportunities and challenges for mesenchymal stem cell-mediated heart repair. Curr Opin Lipidol 18:645–649
75. Petrie Aronin CE, Tuan RS (2010) Therapeutic potential of the immunomodulatory activities of adult mesenchymal stem cells. Birth Defects Res C Embryo Today 90:67–74
76. Dimarino AM, Caplan AI, Bonfield TL (2013) Mesenchymal stem cells in tissue repair. Front Immunol 4:201–210
77. Rowley JE, Johnson JR (2014) Pericytes in chronic lung disease 9. Int Arch Allergy Immunol 164:178–188
78. Ortiz LA, Gambelli F, McBride C, Gaupp D, Baddoo M, Kaminski N, Phinney DG (2003) Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. Proc Natl Acad Sci U S A 100:8407–8411
79. Wolf D, Wolf AM (2008) Mesenchymal stem cells as cellular immunosuppressants 32. Lancet 371:1553–1554
80. Firinci F, Karaman M, Baran Y, Bagriyanik A, Ayyildiz ZA, Kiray M, Kozanoglu I, Yilmaz O, Uzuner N, Karaman O (2011) Mesenchymal stem cells ameliorate the histopathological changes in a murine model of chronic asthma 5. Int Immunopharmacol 11:1120–1126
81. Ou-Yang HF, Huang Y, Hu XB, Wu CG (2011) Suppression of allergic airway inflammation in a mouse model of asthma by exogenous mesenchymal stem cells. Exp Biol Med (Maywood) 236:1461–1467
82. Ou-Yang HF, Han XP, Zhao F, Ti XY, Wu CG (2012) The role of bone marrow-derived adult stem cells in a transgenic mouse model of allergic asthma 2. Respiration 83:74–80
83. Akino K, Mineda T, Mori N, Hirano A, Imaizumi T, Akita S (2006) Attenuation of cysteinyl leukotrienes induces human mesenchymal stem cell differentiation 3. Wound Repair Regen 14:343–349
84. Allakhverdi Z, Comeau MR, Armant M, Agrawal R, Woodfolk JA, Sehmi R, Howie KJ, Gauvreau GM, Delespesse G (2013) Mast cell-activated bone marrow mesenchymal stromal cells regulate proliferation and lineage commitment of CD34(+) progenitor cells 1. Front Immunol 4:461–468
85. Brown JM, Nemeth K, Kushnir-Sukhov NM, Metcalfe DD, Mezey E (2011) Bone marrow stromal cells inhibit mast cell function via a COX2-dependent mechanism 43. Clin Exp Allergy 41:526–534
86. Caplan AI (2007) Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. J Cell Physiol 213:341–347
87. Caplan AI (2009) Why are MSCs therapeutic? New data: new insight. J Pathol 217:318–324

88. Bacigalupo A, Palandri F (2004) Management of acute graft versus host disease (GvHD) 17. Hematol J 5:189–196
89. Ringden O, Keating A (2011) Mesenchymal stromal cells as treatment for chronic GVHD. Bone Marrow Transplant 46:163–164
90. Li H, Fong C, Chen Y, Cai G, Yang M (2010) Beta-adrenergic signals regulate adipogenesis of mouse mesenchymal stem cells via cAMP/PKA pathway 4. Mol Cell Endocrinol 323:201–207
91. Baraldi E, Azzolin N, Zanconato S, Dario C, Zacchello F (1997) Corticosteroids decrease exhaled nitric oxide in children with acute asthma. J Pediatr 131:381–385
92. Wyles CC, Houdek MT, Wyles SP, Wagner ER, Behfar A, Sierra RJ (2015) Differential cytotoxicity of corticosteroids on human mesenchymal stem cells 7. Clin Orthop Relat Res 473:1155–1164
93. Faith A, Fernandez MH, Caulfield J, Loke TK, Corrigan C, O'Connor B, Lee TH, Hawrylowicz CM (2008) Role of cysteinyl leukotrienes in human allergen-specific Th2 responses induced by granulocyte macrophage-colony stimulating factor. Allergy 63:168–175
94. Belvisi MG, Hele DJ, Birrell MA (2004) New advances and potential therapies for the treatment of asthma. BioDrugs 18:211–223
95. Lin H, Sun Y, Lin RJ, Xu J, Li N (2010) [Influence of inhaled corticosteroids on distribution of throat flora in children with bronchial asthma]. Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 45:656–659
96. Chowdhury BA, Seymour SM, Levenson MS (2011) Assessing the safety of adding LABAs to inhaled corticosteroids for treating asthma. N Engl J Med 364:2473–2475
97. Tolar J, Villeneuve P, Keating A (2011) Mesenchymal stromal cells for graft-versus-host disease. Hum Gene Ther 22:257–262
98. Abdallah BM, Kassem M (2008) Human mesenchymal stem cells: from basic biology to clinical applications. Gene Ther 15:109–116
99. Dorfmüller P, Zarka V, Durand-Gasselin I, Monti G, Balabanian K, Garcia G, Capron F, Coulomb-Lherminé A, Marfaing-Koka A, Simonneau G, Emilie D, Humbert M (2002) Chemokine RANTES in severe pulmonary arterial hypertension. Am J Respir Crit Care Med 165:534–539
100. Dasu MR, Ramirez SR, La TD, Gorouhi F, Nguyen C, Lin BR, Mashburn C, Stewart H, Peavy TR, Nolta JA, Isseroff RR (2014) Crosstalk between adrenergic and toll-like receptors in human mesenchymal stem cells and keratinocytes: a recipe for impaired wound healing 2. Stem Cells Trans Med 3:745–759
101. Deans RJ, Moseley AB (2000) Mesenchymal stem cells: biology and potential clinical uses. Exp Hematol 28:875–884
102. Fireman E, Schwartz Y, Mann A, Greif J (2004) Effect of montelukast, a cysteinyl receptor antagonist, on myofibroblasts in interstitial lung disease. J Clin Immunol 24:418–425
103. Hon KL, Leung TF, Ma KC, Wong Y, Fok TF (2005) Brief case series: montelukast, at doses recommended for asthma treatment, reduces disease severity and increases soluble CD14 in children with atopic dermatitis. J Dermatolog Treat 16:15–18
104. Pieters WR, Wilson KK, Smith HC, Tamminga JJ, Sondhi S (2005) Salmeterol/fluticasone propionate versus fluticasone propionate plus montelukast: a cost-effective comparison for asthma. Treat Respir Med 4:129–138
105. Calhoun W, Lavins B, Minkwitz M, Evans R, Gleich G, Cohn J (1998) Effect of zafirlukast (accolate) on cellular mediators of inflammation: bronchoalveolar lavage fluid findings after segmental antigen challenge. Am J Respir Crit Care Med 157:1381–1389
106. Mastalerz L, Kumik J (2010) Antileukotriene drugs in the treatment of asthma. Pol Arch Med Wewn 120:103–108
107. Hasday JD, Meltzer SJ, Moore W, Wisniewski P, Hebel J, Lanni C, Dube L, Bleecker ER (2000) Anti-inflammatory effects of zileuton in a subpopulation of allergic asthmatics. Am J Respir Crit Care Med 161:1229–1236

108. Brooke G, Rossetti T, Ilic N, Murray P, Hancock S, Pelekanos R, Atkinson K (2008) Points to consider in designing mesenchymal stem cell-based clinical trials. Transfus Med Hemother 35:279–285
109. Conese M, Piro D, Carbone A, Castellani S, Di GS (2014) Hematopoietic and mesenchymal stem cells for the treatment of chronic respiratory diseases: role of plasticity and heterogeneity 18. ScientificWorldJournal 2014:859817–859828
110. Choi H, Lee RH, Bazhanov N, Oh JY, Prockop DJ (2011) Anti-inflammatory protein TSG-6 secreted by activated MSCs attenuates zymosan-induced mouse peritonitis by decreasing TLR2/NF-kappaB signaling in resident macrophages. Blood 118:330–338
111. Ge X, Bai C, Yang J, Lou G, Li Q, Chen R (2013) Effect of mesenchymal stem cells on inhibiting airway remodeling and airway inflammation in chronic asthma. J Cell Biochem 114:1595–1605
112. Novotny NM, Markel TA, Crisostomo PR, Meldrum DR (2008) Differential IL-6 and VEGF secretion in adult and neonatal mesenchymal stem cells: role of NFκB. Cytokine 43:215–219
113. Ajuebor M, Das A, Virag L, Flower R, Szabo C, Perretti M (1999) Role of resident peritoneal macrophages and mast cells in chemokine production and neutrophil migration in acute inflammation: evidence for an inhibitory loop involving endogenous IL-10. J Immunol 162:1685–1691
114. Cherwinski HM, Murphy CA, Joyce BL, Bigler ME, Song YS, Zurawski SM, Moshrefi MM, Gorman DM, Miller KL, Zhang S, Sedgwick JD, Phillips JH (2005) The CD200 receptor is a novel and potent regulator of murine and human mast cell function. J Immunol 174:1348–1356
115. Lee E, Yook J, Haa K, Chang HW (2005) Induction of Ym1/2 in mouse bone marrow-derived mast cells by IL-4 and identification of Ym1/2 in connective tissue type-like mast cells derived from bone marrow cells cultured with IL-4 and stem cell factor. Immunol Cell Biol 83:468–474
116. Oliveira SH, Lukacs NW (2003) Stem cell factor: a hemopoietic cytokine with important targets in asthma. Curr Drug Targets Inflamm Allergy 2:313–318
117. Aun MV, Bisaccioni C, Garro LS, Rodrigues AT, Tanno LK, Ensina LF, Kalil J, Motta AA, Giavina-Bianchi P (2011) Outcomes and safety of drug provocation tests. Allergy Asthma Proc 32:301–306
118. Tan RA, Corren J (2011) Safety of omalizumab in asthma. Expert Opin Drug Saf 10:463–471
119. Caimmi S, Caimmi D, Lombardi E, Crisafulli G, Franceschini F, Ricci G, Marseglia GL (2011) Antibiotic allergy. Int J Immunopathol Pharmacol 24:S47–S53
120. Erzurum SC, Leff JA, Cochran JE, Ackerson LM, Szefler SJ, Martin RJ, Cott GR (1991) Lack of benefit of methotrexate in sever, steroid-dependent asthma. Ann Intern Med 114:353–360
121. Rosenwasser LJ, Nash DB (2003) Incorporating omalizumab into asthma treatment guidelines: consensus panel recommendations. Pharm Ther 28:400–414
122. Grossman HL, Schlender A, Alperin P, Stanley EL, Zhang J (2010) Modeling the effects of omalizumab over 5 years among patients with moderate-to-severe persistent allergic asthma. Curr Med Res Opin 26:2779–2793
123. Caimi PF, Reese J, Lee Z, Lazarus HM (2010) Emerging therapeutic approaches for multipotent mesenchymal stromal cells. Curr Opin Hematol 17:505–513
124. Zeng SL, Wang LH, Li P, Wang W, Yang J (2015) Mesenchymal stem cells abrogate experimental asthma by altering dendritic cell function. Mol Med Rep 12:2511–2520
125. Zappia E, Casazza S, Pedemonte E, Benvenuto F, Bonanni I, Gerdoni E, Giunti D, Ceravolo A, Cazzanti F, Frassoni F, Mancardi G, Uccelli A (2005) Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. Blood 106:1755–1761
126. Yi T, Song SU (2012) Immunomodulatory properties of mesenchymal stem cells and their therapeutic applications. Arch Pharm Res 35:213–221

127. Caplan AI (2013) Adult mesenchymal stem cells and the NO pathways. Proc Natl Acad Sci U S A 110:2695–2696
128. Alam R, York J, Boyars M, Stafford S, Grant JA, Lee J, Forsythe P, Sim T, Ida N (1996) Increased MCP-1, RANTES, and MIP-1a in bronchoalveolar lavage fluid of allergic asthmatic patients. Am J Respir Crit Care Med 153:1398–1404
129. Alving K, Weitzberg E, Lundberg JM (1993) Increased amount of nitric oxide in exhaled air of asthmatics. Eur Respir J 6:1368–1370
130. Ballow M (2006) Biologic immune modifiers: trials and tribulations--are we there yet? J Allergy Clin Immunol 118:1209–1215
131. Cho KS, Park MK, Kang SA, Park HY, Hong SL, Park HK, Yu HS, Roh HJ (2014) Adipose-derived stem cells ameliorate allergic airway inflammation by inducing regulatory T cells in a mouse model of asthma. Mediators Inflamm 2014:436476

Mesenchymal Stem Cell Treatment in Mice Models of Systemic Lupus Erythematosus

Hulya Bukulmez

Introduction

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune inflammatory disease in which vascular inflammation causes devastating organ damage such as end-stage renal disease, cardiovascular disease, and myocardial infarction [1]. Severe vasculitis in SLE results from inflammatory process affecting all type of vessels causing a diverse clinical spectrum of organ damage [2]. Current available therapies are mostly toxic and are not efficient in controlling disease progression. Innovation of nontoxic cellular therapies that target both, the vascular wall and the immune responses within the local microenvironment, is needed.

The control of the immunological deregulation and repair of the vascular integrity to prevent fatal organ damage (i.e., end-stage kidney disease) are keys for a successful treatment. Currently available chemotherapy options are toxic and are not efficient in controlling inflammation and vasculitis. There is a considerable amount of data showing that Mesenchymal Stem Cells (MSCs) have potent immunoregulatory properties, and therefore, they are an attractive alternative as a treatment for human diseases where tissue injury and/or inflammation dominate [3–23]. Exogenously introduced human MSCs (hMSCs) do not cause adverse effects, providing a remarkable safety and feasibility profile in clinical trials [24–29]. However, hMSCs' clinical efficacy remains an unresolved issue due to the variability of the results across different studies, which is attributed, to both the donor-to-donor variability, and to the cellular heterogeneity of MSC cultures.

H. Bukulmez, M.D. (✉)
Division of Pediatric Rheumatology & Pediatric Bone Health Program, Department of Pediatrics, MetroHealth Medical Center & Skeletal Research Center, Case Western Reserve University, 2500 Metro Health Drive, Cleveland, OH 44109, USA
e-mail: hbukulmez@metrohealth.org

C.J. Malemud, E. Alsberg (eds.), *Mesenchymal Stem Cells and Immunomodulation*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-3-319-46733-7_3

Nature of Immune Dysregulation in SLE

SLE is characterized by abnormalities in cellular and humoral autoimmunity. Pathogenic T-cells and B-cells recognize self-antigens resulting in immune hyperactivity and autoantibody production that culminates in a multisystem chronic inflammatory disease. Unfortunately there is still no uniformly effective treatment targeting both cellular and humoral autoimmunity for SLE. Many therapies targeting constituents of cellular or humoral immune system fail to induce persistent remission in disease activity in multicenter clinical trials. In order to design a new treatment that can control the cellular and innate immune activation and regenerate the damaged organs during active SLE, understanding of an immune dysregulation is necessary. Animal models of SLE have been very instrumental in understanding the highlights of the immune dysregulation in SLE etiopathogenesis which consist of abnormalities in CD4+ and CD8+ T-cells, dendritic cells (DC), B-cell overproduction of autoantibodies, T regulatory (Treg) cell dysfunction, and overproduction of Type I interferons (-α, -β, and -γ).

1. Abnormalities in T-Lymphocyte and Natural Killer (NK) Cells: There is evidence for a reduced numbers of CD8+ T-cells, functional defects, and sustained activation. Decreased numbers of T, B, and NK cells are common in SLE. CD8+ T-cells and NK cells have decreased cytotoxic activity. These two cell populations increase IgG production in lupus patients, which has been attributed partly to defective production of transforming growth factors beta (TGF-β) and a reduced production of several other cytokines by T-cells. There is a general inability of TGF-β production, which in return accounts for sustained T- and B-cell hyperactivity and reduced Tregs activity and numbers [1, 19, 24]. Restoration of T-cell functions is important for disease control. Tregs consist of heterogeneous populations of CD4+, CD8+, and NK cells. In both human patients with SLE [24, 30] and in lupus-prone mice model [31] CD4+ CD25+ Foxp3+ Tregs are reported to be decreased during disease activity. There is strong evidence that when Tregs are increased SLE disease activity can be altered [24]. However, regulation of Tregs does not seem to be the only mechanism that could suppress the SLE disease activity by MSC treatment. CD4+ T helper cell subset (Th17 cells) are also increased in SLE in response to IL-17 activation. Blockage of IL-17 has also been suggested as a new treatment option [31, 32].
2. Abnormalities in B-lymphocytes and their action: The hallmark of SLE is the production of an array of IgG and IgM autoantibodies directed against one or more nuclear components, the most frequent of which are double-stranded (ds) DNA and/or single-stranded (ss) DNA. Both anti-ssDNA and anti-dsDNA are involved in disease development. Lupus-like autoimmunity can ensue due to B-cell hyperactivity, with either minimal or no contribution from T-lymphocytes.
3. Abnormalities in cytokines: (a) Reduced production of IL-2; (b) decreased production of TGFβ [33]; (c) high expression of IFN-γ [34, 35]; (d) increased IL10

production by blood B-lymphocytes. IL-10 is a potent stimulator of B-lymphocyte proliferation and differentiation; (e) increased IL4 production [36–38]. IL-4 promotes B-lymphocyte proliferation and thus enhances the production of autoantibodies.

4. Abnormalities in the complement system: Complement deficiency is responsible for about 5 % of all lupus patients, but at least 50 % of patients with homozygous deficiencies of the early classical complement pathway develop a lupus-like disease. The increased susceptibility to SLE, associated notably with C1q, C1r, C1s, and C1 deficiency which is due to immune complex clearance impairment. C1R which binds C3B and C4B is decreased in lupus patients and levels correlate with disease activity [39].
5. Abnormalities in phagocytes: There is defective clearance of the immune complexes due to abnormal phagocytosis.
6. Abnormalities in Type I Interferons: There is overproduction of type I interferons (INF). Type I interferons regulate dendritic cell (DC) maturation into immunogenic antigen-presenting cell (APCs). APCs contribute to B-cell hyperactivity and induce a Th1 response and in return maintain T-cell activation. Type I interferon overproduction partially explained the deficiency in Tregs. It is suggested that an imbalance between the immunogenic and tolerogenic DCs during SLE activity limits the expansion of Tregs. The remaining Tregs are not sufficient to overcome the strong T-cell activation [40].
7. Abnormalities in Dendritic Cells (DC): DCs play a pivotal role in determining the balance between responsiveness and tolerance in the immune system [41]. While persistence of host DCs following bone marrow transplantation correlate with the development of severe, acute, and chronic GVHD [42–44]. Persistent, chronic DC activation leads to autoimmunity [45]. In SLE, immune complexes induce interferon-α secretion from plasmacytoid dendritic cells that stimulates the myeloid dendritic cells to further activate T- and B-cells. Thus, any therapy that alters the DC activation might alleviate the autoimmune activity in SLE [45]. BAFF (B-cell survival factor) blockage suppresses DC maturation and has been shown to be a valid therapeutic target in SLE [46]. MSCs have been shown to decrease the DC secretion of proinflammatory cytokines such as INF-γ, IL-12, and TNF-α while increasing the production of IL-10, an immune suppressive cytokine in vitro. Thus, infusion of MSCs could be very successful in balancing the autoimmunity via their effect on DCs and indirectly increasing Tregs in SLE. In summary, current knowledge suggests that pathogenic T-cells that recognize self-antigens drive B-cell hyperactivity and play a central role in the pathogenesis of both human and murine lupus. When cellular components of the immune system are further dissected and analyzed, we come across with intriguing details and learn new information of cellular and humoral immunity.

Role of MSC Treatment in Immune Regulation of Autoimmune Diseases

There is considerable amount of data showing that exogenously introduced MSCs isolated from a variety of tissues have potent immune-regulatory actions without any observed adverse effects allogeneic or autologous [19, 24, 47–49]. These attributes make MSCs an attractive alternative for use in the treatment of human diseases, in which tissue injury and/or inflammation dominate. There are a number of publications that provide evidence that MSCs tend to home to sites of inflammation or tissue injury when infused into living organisms [50–54]. In this context, human MSCs (hMSCs) have been shown to be curative in a number of preclinical models and in human autoimmune inflammatory diseases such as SLE, scleroderma, and rheumatoid arthritis [30, 52, 54].

Potential Mechanisms of Action of MSCs in SLE Treatment

MSCs possess a collection of immunosuppressive molecules, which can be locally positioned, secreted to modulate inflammation according to the microenvironmental stimuli:

(a) MSCs have the ability to shift the balance from a proinflammatory Th1 phenotype-secreting interferon gamma (INF-γ) and tumor necrosis factor alpha (TNF-α) to a more anti-inflammatory profile-secreting Th2 phenotype. MSCs also modulate Th17 differentiation in favor of IL-4-producing Th2 cells [52].
(b) MSCs decrease IFN-γ production in vitro by T-cells subjected to Th1 cell-polarizing conditions [55]. hMSCs are able to modulate the cytokine-production profile of (in vivo) differentiated Th17 cells, as well as the production of the IL-17 and IL-22 [55]. MSCs promote the generation of antigen-specific Tregs in vitro directly or indirectly by modulating dendritic cells (DCs) [52].
(c) MSCs activate macrophages, down-regulating the production of TNF-α, IL-1α, IL-6, and IL-12p70 and increasing the production of anti-inflammatory molecule IL-10 and enhancing the phagocytic activity facilitating the resolution of inflammation [52].
(d) MSCs can interfere with the development and function of both conventional and DCs [52].
(e) Infused MSCs taking up the perivascular space behaving as pericyte-like cells could stabilize fragile vasculature observed in SLE vasculitis.

One key element of the possible effect of MSCs in SLE is that once MSCs enter the inflammatory environment in SLE-affected organs, their immune-modulatory phenotype could become activated by IFN-γ, TNF-α, and IL-1β. Furthermore, it has been shown that MSCs are chemotactically drawn toward a variety of wound-healing

cytokines in vitro, including IL-1 and TNF-α [56]. These data suggest that MSCs or endogenous cells resembling MSCs are likely to migrate to and participate in the response to tissue injury.

Two different types of MSCs have been described based on a differential immune-modulatory activity. These two types of MSCs (type 1 and type 2) trigger completely opposite responses based on the microenvironmental cues they sense in their corresponding locations (if such different locations exists), generating a pro- and anti-inflammatory effect, respectively [57, 58].

Interestingly, the phenotype diversification is acquired after treatment with certain "danger signals," such as lipopolysaccharide (LPS) and polyinosinic:polycytidylic acid (poly(I:C)), MSCs change their surface toll-like receptors (TLRs) and start different immune-modulatory activities, proinflammatory (MSC-1) or anti-inflammatory (MSC-2) [58]. This description is based on how these two populations sense the inflammatory environment and secondarily secrete immune-modulatory factors. Dependent on the danger signal that MSCs are exposed to, they express different cell surface markers and induce different signaling pathways. When already programmed/primed to induce certain pathways, MSCs or their subsets might provide better immune regulation and anti-inflammatory effects during autoimmune disease activity.

Mouse Models of SLE

NZB/NZW F1 and Mixed Derivatives

Mice from the first generation of cross between the strains New Zealand Black (NZB) and New Zealand White (NZW), known as NZB/NZW F1, develop human lupus-like syndrome characterized by lymphadenopathy, splenomegaly, elevated antinuclear antibody titers, and immune-complex-mediated glomerulonephritis mostly in females [59, 60]. NZB/NZW F1 mice are a widely used animal model for lupus; they mimic human lupus in several aspects including gender specificity, the appearance of circulating anti-dsDNA antibodies, renal deposition of immune complexes, and the development of fatal glomerulonephritis. They do not develop skin disease or hematologic manifestations and thus have been used primarily to study SLE nephritis. NZB/NZW F1 mice show development of clinical nephritis evident by proteinuria at a median age of 37 weeks and demonstrate the long-term presence of autoantibodies, thus representing a model of chronic lupus disease and die by the age of 52 weeks (1 year).

NZB/W F1 mice develop autoantibodies that also include antibodies against chromatin, histone H1, histone H2A, anti-Ro, -La, and -Sm, which all are also characteristic of human SLE except for antibodies for U1-snRNPs [61]. Neither of the parent strains develops overt pathology, although NZB mice develop mild autoimmune hemolytic anemia [62].

In order to search for causal loci in this model of SLE, investigators backcrossed NZB/NZW F1 mice to NZW, then used brother–sister matings to generate 27

substrains, which were called New Zealand mixed (NZM) mice [63]. Among these 27 substrains, NZM2410 was selected for further analysis because of the complete penetrance and severity of its pathology. Congenic mice derived from NZM2410 have shown the polygenic loci that were linked to different disease manifestations. B6.Sle1, a congenic strain on the C57BL/6 background which contains chromosome 1 derived from NZM2410, develops autoantibodies against subnucleosomes, and shows spontaneous T-cell activation, but no kidney involvement [64]. Another congenic strain, B6.Sle2, derived from chromosome 4, displays low B-cell activation that results in polyclonal IgM in the serum and absence of glomerulonephritis [65]. Interestingly, combining the two loci resulted in glomerulonephritis and enhanced mortality compared with the single congenic strains alone [66].

B6.SLe123

B6.Sle123 mice that possess three SLE susceptibility loci spontaneously develop highly penetrant severe systemic autoimmunity and fatal glomerulonephritis beginning at 6 months of age. Young mice are yet to develop autoimmunity but when treated with IFNα quickly developed renal immune complex deposition and nephritis. They also developed increased serum levels of proinflammatory cytokines such as TNFα and IL-6, activation of DCs, B-cells, and T-cells [67]. Interestingly, renal leukocyte infiltration was not affected by IFNα treatment [67].

MRL/lpr

MRL mice were derived from multiple crosses of inbred strains LG/J, C3H/Di, C57BL/6, and AKR/J [59]. A spontaneous mutation causing lymphoproliferation (lpr phenotype) was identified as a retrotransposon insertion that disrupts the Fas gene [68, 69], the gene that encodes the FAS death-inducing receptor that regulate and maintain number of lymphocytes. MRL/lpr animals demonstrate B-cell hyperactivity, circulating immune complexes, lymphoid hyperplasia, and glomerulonephritis [59] and present a severe form of SLE. It is suggested that presence of lpr mutation enhances disease severity, by inducing systemic lymphoproliferative disease and the mouse model was also suggested to be used studying autoimmune lymphoproliferative syndrome [70].

MRL/lpr mice produce a wide range of autoantibodies; antibodies against DNA [59], nucleosomes [71], RNA polymerase [72], cardiolipins [73], nucleolins [74], phospholipids [75], and antigens of brain [76]. However, it has been debated about how much the autoantibodies found in this mouse model induce disease activity. An example to this is the failure of MRL/lpr-derived anti-DNA antibodies inducing glomerulonephritis when injected into healthy control mice [77]. Furthermore, a particular mutant

MRL/lpr mouse B-cells were failed to secrete antibodies but did develop nephritis [78]. Thus, autoantibodies may not be the only responsible pathogenesis for nephritis. Another study showed that possibly TLRs are also responsible from disease onset in MRL/lpr mice, because a TLR7/TLR9 double mutant found to be protective against glomerulonephritis and autoantibody production [79]. Female mice develop higher serum IgG levels and increased ANA titers at 2–3 months of age [59] and show neuropsychiatric component of SLE [80].

Multiple cytokines have been linked to disease in MRL/lpr mice, including IFNγ [81, 82], IL-6 [83, 84], IL-1β [85, 86], and IL-18 [87, 88]. Regulatory or protective roles have been suggested for IL-10 [89] and IL-27 [90]. The humoral response in MRL/lpr mice is subject to regulation by IFN-I, which reduces antibody-mediated disease [91, 92], whereas IL-21 produced by activated T-cells drives autoantibody production [93]. A number of the regulatory mechanisms involved remain unclear and warrant further investigation using the MRL/lpr mouse model but has been widely utilized as a SLE mouse model.

MRL/MPJ Mouse Glomerulonephritis

BXSB.Yaa

The BXSB strain is derived from a C57BL/6 female and SB/Le male F1 backcrossed to SB/Le. BXSB manifests with hypergammaglobulinemia, high titers of serum anti-retroviral gp70 IgG, ANAs, secondary lymphoid tissue hyperplasia, and immune complex-mediated glomerulonephritis, which leads to death [94]. Males develop SLE more frequently, earlier and with increased severity than females [94, 95]. It is suggested that genetic disease-accelerating factor resides in the SB/Le Y chromosome. This genetic factor has been called as the Y-linked autoimmune accelerator (Yaa) [94]. NZW, MRL, and Sle1-3 lupus-susceptible strains all demonstrate exacerbated disease when they contain the BXSB Y chromosome [66, 96, 97]. FcγRIIB-deficient mice, which also develop spontaneous SLE-like disease [98], undergo a switch of autoantibody specificity from chromatin to nucleolar in the presence of the Yaa modifier [99]. The Yaa does not, however, induce autoimmunity on the C57BL/6 background. Thus, the Yaa genetic modifier is called an accelerator because by itself it does not initiate disease. However, it augments the severity in lupus-prone genetic backgrounds [100]. The Yaa is now known to be a 4-megabase translocation of the distal end of the X chromosome onto the pseudo autosomal region of the Y chromosome, which results in the duplication of over a dozen genes [101]. TLR7 is one of these dozen duplicated genes and is necessary for Yaa-mediated disease exacerbation: When TLR7 is deleted from the X chromosome abrogates Yaa-induced lupus phenotype [102]. TLR7 activation has been shown to affect antibody production by B-cells, inflammatory production by monocytes, and antigen presentation by dendritic cells [101].

NZW/BXSB Mice

NZW x BXSB F1 (W/BF1) male mice develop systemic lupus-like disease activity, and several autoantibodies, circulating immune complexes, vasculitis, and lupus nephritis. There is an abnormally high incidence of degenerative coronary vascular disease and thrombocytopenia because platelet-associated antibodies and antiplatelet antibodies W/BF1s. Disease manifestations start at 10 weeks of age with 50 % mortality by 20 weeks. W/BF1 mice develop end-stage kidney disease, autoimmune vasculitis, and secondary organ damage resembling human SLE disease [103, 104]. W/BF1 SLE mouse model provides a platform to study not only SLE-like disease but also autoimmune vasculitis.

Male W/BF1 mice carry two active copies of the TLR7 gene which in return cause development of anti-RNA and antiphospholipid autoantibodies. Female mice carry a single active copy of TLR7. Thus, they develop late onset nephritis, but not antiphospholipid syndrome. Male W/BF1 develops severe organ involvement such as severe inflammatory nephritis and antiphospholipid syndrome with thrombocytopenia, myocardial infarcts, and cardiomyopathy. Current understanding is that the inflammatory process in these mice is mainly due to interferon-α signaling. When treated prophylactically with anti-IFN α receptor antibody, survival of these mice is prolonged. IFNα-induced effects follow a significant increase in activated B- and T-cells in the spleen.

C57BL/6 Derivatives: Knockout and Transgenic Models

These mice have been generated in the C57BL/6 (B6) strain background, which does not develop spontaneous SLE unless they are induced by certain monogenetic mutations [105]. Some examples of these SLE mouse models are the one that show deficiencies in genes that prevent excessive lymphocyte activity or proliferation (i.e., FcγRIIB, Lyn, Fyn, CD22, PD-1, CD45 E613R, p21, and Bcl2 Tg). These genetic deficiencies may induce spontaneous activity of SLE-like disease in B6 strain.

MSC Treatment of SLE Mouse Models

Since MSCs have been found to be anti-inflammatory in human clinical trials of graft-versus-host disease attempts to use these cells in other inflammatory diseases have been increased. Initially, in preclinical trials mouse models of SLE were treated with MSCs from different sources (Table 1).

Most of these preclinical trials show favorable outcomes for mice regardless of the sources that MSCs are derived. In general, current understanding is that MSCs are able to suppress the SLE-like disease in mice and have immune regulatory

Table 1 Table for journal references that describe SLE mouse model MSC treatments

SLE mouse model	Syngeneic	Allogeneic	Xenogeneic (human MSC into mouse)
MRL/lpr	[77]	[19, 107, 114]	[19, 106, 113, 114]
(NZB/NZW)F1	[111, 112]	[114, 115, 119]	[114, 115, 120]
B6.Sle1.Sle3	[116, 117]	[118]	[118]

effects when they enter to the mouse with SLE disease activity. One exception to this is perhaps that the mouse MSCs driven from SLE mouse models. MSCs isolated from animals affected from SLE-like disease (SLE models) show limited anti-inflammatory activities as compared to the one isolated from healthy control animals in vitro.

MRL/lpr Mice

Mechanisms of SLE nephritis in this model are suggested to be mostly due to Fas-mediated apoptosis of active lymphocytes and T-cell-dependent production of autoantibodies that result in glomerulonephritis and severe vasculitis. Proinflammatory cytokines such as monocyte chemoattractant protein-1 (MCP-1) and high-mobility group box chromosomal protein 1 (HMGB-1) that are known to play roles in lupus nephritis were found to be decreased after xenogeneic (human umbilical cord-derived MSCs) and allogeneic (mouse bone marrow derived) MSC transplantation in MRL/lpr mice [19]. In addition, more recent studies demonstrated the efficacy of the multiple dose treatments in intervals in this model. Clinical and pathologic findings of lupus nephritis improved more when multiple injections of umbilical cord-driven MSCs were injected [106]. Suppression of HMGB-1 was particularly interesting since this molecule has been correlated with SLE disease clinical activity in human.

Dose effect of MSCs was also suggested in another study that mainly investigated the G1/S cell cycle transition of T-cells [107]. When allogeneic MSCs transplantation has been used it was shown that G1/S transition of the abnormal lupus T-lymphocytes was inhibited while expression of cell cycle regulatory proteins p21WAF1/CIP1 (suppress CDK2) and p27Kip1 was increased. Expression of cyclin-dependent kinase 2 (CDK2) was decreased. While high-dose MSCs (0.2×10^6 per 10 g of body weight) treatment had an inhibitory effect on G1/S transition of the abnormal lupus T-lymphocytes, low-dose MSCs (0.05×10^6 per 10 g of body weight) were not shown to make any changes. In addition, PI3K/Akt/GSK3β signaling pathway that plays a role in regulating lupus lymphocyte proliferation was also found to be inhibited when high-dose MSCs are used to treat MRL/lpr mice [107]. Low-dose allogeneic MSC treatment did not show improvement of the proteinuria and autoantibody production.

Using different tissue-derived MSC treatments in MLR/lpr mice did not show remarkable differences in anti-inflammatory effects and clinical outcomes [47].

In other words, MSCs were anti-inflammatory regardless of their originated tissue types. Most studies agreed upon the higher amount of MSC numbers to be used for increasing the efficacy of the treatments rather than suggesting a particular MSC tissue source.

More recently, efforts to make MSCs more potent and disease targeting showed enthusiastic results. MRL/lpr mice are known to develop spontaneous skin inflammation, dermatitis. In a recent study, dermatitis in the MRL/lpr mice was treated with adipogenic MSCs that overexpressed CTLA-4 Ig. Both human adipose tissue-derived MSCs and those overexpress CTLA-4 Ig were used to treat MRL/lpr mice and were found to be effective in preventing lupus dermatitis development [108]. MSCs with CTLA-4 Ig overexpression were found to be more effective as compared to those without gene overexpression.

Besides their anti-inflammatory effects in chronic diseases with active SLE-like disease models, MSCs are found to suppress acute inflammatory reactions such as those seen in rejection reactions (i.e., graft versus host disease). Thus, MSCs were investigated in transplant medicine for their effect in controlling the inflammatory reaction in GVHD. A recent project report demonstrated that transfusion-associated graft versus host disease in MRL/lpr mice model was suppressed when a combination of MSCs and hematopoietic stem cells (HSCs) was used together in a ratio of 5:1 (HSC:MSC). MRL/lpr mice also gained steady body weight and improved renal functions when compared to mouse transplanted alone with HSCs [109].

(NZB/NZW)F1 Mice

Earlier studies resulted in conflicting reports of effectiveness of MSC treatment in (NZB/NZW) F1 mice. In general, it is suggested that both allogeneic and xenogeneic MSC transplants diminished disease activity but did not completely stopped disease progress in this SLE mouse model.

Carlucci et al. [110] reported that there was improvement of the renal function when allogeneic bone marrow MSCs from C57/B6 mice were given to (NZB/NZW) F1 mice without any improvement in antibody production. It was suggested that allogeneic BM-MSCs affect B-cell receptor-dependent activation of both follicular and marginal zone B-cells from (NZB/NZW) F1 mice and this inhibitory effect was found to be IFNγ and cell contact dependent. However, allogeneic MSCs did not affect the production of autoantibodies, the level of proteinuria, or survival rates. Subsequently, another study by Youd e al. showed that MSCs driven from BALB/c mice bone marrow also showed no impact on disease activity in (NZB/NZW) F1 mice [110].

Furthermore, there are concerns whether syngeneic MSC treatments are as good as allogeneic MSC treatments. There have been several reports suggesting impaired MSC function when derived from lupus mice and lupus patients suggesting that syngeneic MSC treatment may not be optimal for transplantation [111, 112]. Effect of the active inflammatory disease on the bone marrow MSCs is largely unknown. MSCs isolated from human who has a particular chronic disease may not be efficacious as compared to the healthy human BM-driven MSCs.

Recent studies shed more light into the mechanisms of human bone marrow-derived MSCs in tissue cellular inactivation. When human bone marrow-driven MSCs were injected to (NZB/NZW)F1 mice the amount of follicular helper T (Tfh) cells decreased and diminished immune complex formation which are both responsible from trigger of widespread inflammatory damage including nephritis [113].

Differential effects of syngeneic, adipogenic, and allogeneic MSCs were tested in a study. Where, adipogenic stem cells (ASCs) from NZB/NZW F1 mice (syngeneic), BALB/c mice (allogeneic), or humans (xenogeneic) were isolated and tested on NZB/NZW F1 mice treatment. The same study also assessed the effect of transplanting human ASCs overexpressing CTLA-4 Ig. The results showed that regardless of the source of ASCs used there was improvement of (NZB/NZW) F1 survival rate about 6–7 weeks. Interestingly, the strongest humoral immune response was observed to be induced by xenogeneic transplantation, followed by allogeneic, CTLA4Ig-xenogeneic, and syngeneic transplantations.

Comparison of MRL/lpr and (NZB/NZW) F1: In a study of the differential effect of allogeneic versus syngeneic, mesenchymal stem cell transplantation was assessed in both MRL/*lpr* and (NZB/NZW) F1 mice models of SLE. In both mice, the MSCs driven from C57/B6 mice bone marrow (allogeneic) improved SLE-like disease. Splenic CD3 + CD4 + T-lymphocytes and CD19 + CD21 + B-lymphocytes have been found significantly decreased after treatment. However, when older (NZB/NZW) F1 mouse MSCs were used in these models there was not much improvement in the splenic cell counts or glomerulonephritis [114]. The end result of this study suggested that MSCs from (NZB/NZW) F1 mouse may not be as effective as allogeneic MSCs from healthy mouse due to the disease effect on MSCs but not necessarily due to an intrinsic pathology. It has been found that in both xenogeneic and allogeneic MSC treatment SLE-like disease improved because of CD4 + T-cell and naïve mature B-cell number decrease [115].

B6.Sle1.Sle3

The lupus mouse model, B6.Sle1.Sle3 injected with human kallikrein transduced MSCs (hKLK1-MSCs) showed diminished clinical and histopathologic findings of lupus nephritis. The mechanism for pathologic improvement was attributed to suppressed macrophage and T-lymphocyte infiltration into the kidney by suppressing the expression of inflammation cytokines. In addition, hKLK1 transduced MSCs were found to be more resistant to oxidative stress and induced apoptosis. These findings suggest genetically modified MSCs might be valuable for gene delivery and targeting to modulate inflammation and oxidative stress in lupus nephritis. Another mouse model called 129/svj mice which develop nephritis after anti-GBM antibody induction was transplanted with human kallikrein transduced murine mesenchymal stem cells and showed improved pathology and clinical manifestations [116].

In a recent study, possible deficiency of MSCs self-renewal abilities was tested. Pre-B-cell leukemia homeobox 1 (Pbx1)-d is a dominant-negative splice isoform of the gene Pbx1 that corresponds to the NZM2410 lupus susceptibility locus Sle1a1.

Pbx1 is necessary to maintain stem cell self-renewal. MSCs show immunosuppressive functions when they are successfully maintained. It was shown that Sle1a1 MSCs express high levels of Pbx1-d as compared with congenic C57BL/6J (B6) MSCs. Sle1a1 MSCs grew faster and differentiate significantly more rapidly into osteoblasts. There was an increase in the expression of genes associated with differentiation. Sle1a1 MSCs expressed a gene expression profile associated with an enhanced innate immunity and inflammation. This study highly suggested that Pbx1-d isoform expression causes defective MSC immune suppressive effect and promotes a proinflammatory environment [117].

Using the mouse models of SLE questions whether MSCs related with SLE etiology have been investigated. It is suggested that bone marrow-derived MSCs from lupus-like mice and SLE patients had an impairment in suppressing normal B-cell proliferation and differentiation, which was attributed to the decreased levels of CCL2. When CCL2 is overexpressed in MSCs their B-cell suppressive abilities were found to be restored. MSC-mediated B-cell inhibition was suggested to be dependent on MMP proteolytic processing of CCL2 [118].

Conclusions

To date in many SLE mouse models treated with MSC treatments at least a partial remission or regression of disease manifestations has been shown. Allogeneic or xenogeneic MSC treatments showed more favorable outcomes as compared to autologous MSC treatments. Furthermore, MSCs with particular gene overexpression have been more successful in controlling specific disease manifestations such as SLE dermatitis.

MSC therapies may provide us a novel approach to human SLE nephritis which may be at the level of end-stage organ failure in 20 % of the individuals affected with disease. Although we still do not understand the immune pathogenesis of SLE in human, there is enough evidence from SLE mouse models that MSCs regulate the abnormal immune activation in SLE. It is likely that human SLE manifestations can also be controlled with allogeneic human MSC treatment. Human MSC clinical trials need to be performed in advance for better understanding of MSCs effects in human SLE.

Table 1 shows the citations in this chapter for the syngeneic, allogeneic, and xenogeneic MSC treatments on different SLE mouse models.

References

1. O'Neill S, Cervera R (2010) Systemic lupus erythematosus. Best Pract Res Clin Rheumatol 24:841–855
2. Barile-Fabris L, Hernandez-Cabrera MF, Barragan-Garfias JA (2014) Vasculitis in systemic lupus erythematosus. Curr Rheumatol Rep 16:440

3. Ball LM, Bernardo ME, Roelofs H, van Tol MJ, Contoli B, Zwaginga JJ, Avanzini MA, Conforti A, Bertaina A, Giorgiani G, Jol-van der Zijde CM, Zecca M, Le Blanc K, Frassoni F, Egeler RM, Fibbe WE, Lankester AC, Locatelli F (2013) Multiple infusions of mesenchymal stromal cells induce sustained remission in children with steroid-refractory, grade III-IV acute graft-versus-host disease. Br J Haematol 163:501–509
4. Ennis J, Gotherstrom C, Le Blanc K, Davies JE (2008) In vitro immunologic properties of human umbilical cord perivascular cells. Cytotherapy 10:174–181
5. Freedman MS, Bar-Or A, Atkins HL, Karussis D, Frassoni F, Lazarus H, Scolding N, Slavin S, Le Blanc K, Uccelli A (2010) The therapeutic potential of mesenchymal stem cell transplantation as a treatment for multiple sclerosis: consensus report of the International MSCT Study Group. Mult Scler 16:503–510
6. Le Blanc K, Samuelsson H, Lonnies L, Sundin M, Ringden O (2007) Generation of immunosuppressive mesenchymal stem cells in allogeneic human serum. Transplantation 84:1055–1059
7. Ringden O, Le Blanc K (2011) Mesenchymal stem cells for treatment of acute and chronic graft-versus-host disease, tissue toxicity and hemorrhages. Best Pract Res Clin Haematol 24:65–72
8. Bonfield TL, Caplan AI (2010) Adult mesenchymal stem cells: an innovative therapeutic for lung diseases. Discov Med 9:337–345
9. Bonfield TL, Koloze M, Lennon DP, Zuchowski B, Yang SE, Caplan AI (2010) Human mesenchymal stem cells suppress chronic airway inflammation in the murine ovalbumin asthma model. Am J Physiol Lung Cell Mol Physiol 299:L760–L770
10. Singer NG, Caplan AI (2011) Mesenchymal stem cells: mechanisms of inflammation. Annu Rev Pathol 6:457–478
11. Gazdic M, Volarevic V, Arsenijevic N, Stojkovic M (2015) Mesenchymal stem cells: a friend or foe in immune-mediated diseases. Stem Cell Rev. doi:10.1007/s12015-014-9583-3
12. Good RA (2002) Cellular immunology in a historical perspective. Immunol Rev 185:136–158
13. Huang P, Gebhart N, Richelson E, Brott TG, Meschia JF, Zubair AC (2014) Mechanism of mesenchymal stem cell-induced neuron recovery and anti-inflammation. Cytotherapy. doi:10.1016/j.jcyt.2014.05.007
14. Ikehara S (2002) Bone marrow transplantation: a new strategy for intractable diseases. Drugs Today (Barc) 38:103–111
15. Le Blanc K, Ringden O (2007) Immunomodulation by mesenchymal stem cells and clinical experience. J Intern Med 262:509–525
16. Nasef A, Mathieu N, Chapel A, Frick J, Francois S, Mazurier C, Boutarfa A, Bouchet S, Gorin NC, Thierry D, Fouillard L (2007) Immunosuppressive effects of mesenchymal stem cells: involvement of HLA-G. Transplantation 84:231–237
17. Rakonczay Z Jr, Vag J, Foldes A, Nagy K, Nagy A, Hegyi P, Varga G (2014) Chronic inflammation in the pancreas and salivary glands--lessons from similarities and differences in pathophysiology and treatment modalities. Curr Pharm Des 20:1104–1120
18. Sensebe L, Krampera M, Schrezenmeier H, Bourin P, Giordano R (2010) Mesenchymal stem cells for clinical application. Vox Sang 98:93–107
19. Sun L, Akiyama K, Zhang H, Yamaza T, Hou Y, Zhao S, Xu T, Le A, Shi S (2009) Mesenchymal stem cell transplantation reverses multiorgan dysfunction in systemic lupus erythematosus mice and humans. Stem Cells 27:1421–1432
20. Zhang J, Zhao ZY (2014) Research advances on immunoregulation and anti-inflammation function of mesenchymal stem cells and their application in treatment of renal diseases. Zhejiang Da Xue Xue Bao Yi Xue Ban 43:372–378
21. Zhou H, Jin Z, Liu J, Yu S, Cui Q, Yi D (2008) Mesenchymal stem cells might be used to induce tolerance in heart transplantation. Med Hypothesis 70:785–787
22. Bai L, Lennon DP, Caplan AI, DeChant A, Hecker J, Kranso J, Zaremba A, Miller RH (2012) Hepatocyte growth factor mediates mesenchymal stem cell-induced recovery in multiple sclerosis models. Nat Neurosci 15:862–870

23. Tyndall A (2014) Mesenchymal stem cell treatments in rheumatology: a glass half full? Nat Rev Rheumatol 10:117–124
24. Carrion F, Nova E, Ruiz C, Diaz F, Inostroza C, Rojo D, Monckeberg G, Figueroa FE (2010) Autologous mesenchymal stem cell treatment increased T regulatory cells with no effect on disease activity in two systemic lupus erythematosus patients. Lupus 19:317–322
25. Zhou H, Guo M, Bian C, Sun Z, Yang Y, Zeng Y, Ai H, Zhao RC (2010) Efficacy of bone marrow-derived mesenchymal stem cells in the treatment of sclerodermatous chronic graft-versus-host disease: clinical report. Biol Blood Marrow Transplant 16:403–412
26. Koc ON, Gerson SL, Cooper BW, Dyhouse SM, Haynesworth SE, Caplan AI, Lazarus HM (2000) Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. J Clin Oncol 18:307–316
27. Devine SM, Peter S, Martin BJ, Barry F, McIntosh KR (2001) Mesenchymal stem cells: stealth and suppression. Cancer J 7(Suppl 2):S76–S82
28. Lombardo E, DelaRosa O, Mancheno-Corvo P, Menta R, Ramirez C, Buscher D (2009) Toll-like receptor-mediated signaling in human adipose-derived stem cells: implications for immunogenicity and immunosuppressive potential. Tissue Eng Part A 15:1579–1589
29. Wang P, Li Y, Huang L, Yang J, Yang R, Deng W, Liang B, Dai L, Meng O, Gao L, Chen X, Shen J, Tang Y, Zhang X, Hou J, Ye J, Chen K, Cai Z, Wu Y, Shen H (2014) Effects and safety of allogenic mesenchymal stem cell intravenous infusion in active ankylosing spondylitis patients who failed NSAIDs: a 20-week clinical trial. Cell Transplant 23:1293–1303
30. Casiraghi F, Azzollini N, Cassis P, Imberti B, Morigi M, Cugini D, Cavinato RA, Todeschini M, Solini S, Sonzogni A, Perico N, Remuzzi G, Noris M (2008) Pretransplant infusion of mesenchymal stem cells prolongs the survival of a semiallogeneic heart transplant through the generation of regulatory T cells. J Immunol 181:3933–3946
31. Singh RP, Dinesh R, Elashoff D, de Vos S, Rooney RJ, Patel D, La Cava A, Hahn BH (2010) Distinct gene signature revealed in white blood cells, CD4(+) and CD8(+) T cells in (NZBx NZW) F1 lupus mice after tolerization with anti-DNA Ig peptide. Genes Immun 11:294–309
32. Shah K, Lee WW, Lee SH, Kim SH, Kang SW, Craft J, Kang I (2010) Dysregulated balance of Th17 and Th1 cells in systemic lupus erythematosus. Arthritis Res Ther 12(2):R53
33. Ohtsuka K, Gray JD, Stimmler MM, Toro B, Horwitz DA (1998) Decreased production of TGF-β by lymphocytes from patients with systemic lupus erythematosus. J Immunol 160:2539–2545
34. Ronnblom L (2010) Potential role of IFNα in adult lupus. Arthritis Res Ther 12(Suppl 1):S3
35. Ronnblom L, Eloranta ML, Alm GV (2006) The type I interferon system in systemic lupus erythematosus. Arthritis Rheum 54:408–420
36. Tanaka Y, Saito K, Shirakawa F, Ota T, Suzuki H, Eto S, Yamashita U (1988) Production of B cell-stimulating factors by B cells in patients with systemic lupus erythematosus. J Immunol 141:3043–3049
37. Funauchi M, Yu H, Sugiyama M, Ikoma S, Ohno M, Kinoshita K, Hamada K, Kanamaru A (1999) Increased interleukin-4 production by NK T cells in systemic lupus erythematosus. Clin Immunol 92:197–202
38. Singh RR (2003) IL-4 and many roads to lupuslike autoimmunity. Clin Immunol 108(2):73–79
39. Truedsson L, Bengtsson AA, Sturfelt G (2007) Complement deficiencies and systemic lupus erythematosus. Autoimmunity 40:560–566
40. Horwitz DA, Zheng SG, Gray JD (2008) Natural and TGF-β-induced Foxp3$^+$CD4$^+$ CD25$^+$ regulatory T cells are not mirror images of each other. Trends Immunol 29:429–435
41. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K (2000) Immunobiology of dendritic cells. Annu Rev Immunol 18:767–811
42. Ferrara JL, Cooke KR, Pan L, Krenger W (1996) The immunopathophysiology of acute graft-versus-host-disease. Stem Cells 14:473–489
43. Ferrara JL, Cooke KR, Teshima T (2003) The pathophysiology of acute graft-versus-host disease. Int J Hematol 78:181–187

44. Chan GW, Foss FM, Klein AK, Sprague K, Miller KB (2003) Reduced-intensity transplantation for patients with myelodysplastic syndrome achieves durable remission with less graft-versus-host disease. J Blood Bone Marrow Transplant 9:753–759
45. Mehling A, Loser K, Varga G, Metze D, Luger TA, Schwarz T, Grabbe S, Beissert S (2001) Overexpression of CD40 ligand in murine epidermis results in chronic skin inflammation and systemic autoimmunity. J Exp Med 194:615–628
46. Ramanujam M, Davidson A (2008) BAFF blockade for systemic lupus erythematosus: will the promise be fulfilled? Immunol Rev 223:156–174
47. Zhou K, Zhang H, Jin O, Feng X, Yao G, Hou Y, Sun L (2008) Transplantation of human bone marrow mesenchymal stem cell ameliorates the autoimmune pathogenesis in MRL/lpr mice. Cell Mol Immunol 5:417–424
48. Tsukamoto H, Nagafuji K, Horiuchi T, Miyamoto T, Aoki K, Takase K, Henzan H, Himeji D, Koyama T, Miyake K, Inoue Y, Nakashima H, Otsuka T, Tanaka Y, Nagasawa K, Harada M (2006) A phase I-II trial of autologous peripheral blood stem cell transplantation in the treatment of refractory autoimmune disease. Ann Rheum Dis 65:508–514
49. Kean TJ, Lin P, Caplan AI, Dennis JE (2013) MSCs: delivery routes and engraftment, cell-targeting strategies, and immune modulation. Stem Cells Int 2013:732742
50. Kean TJ, Duesler L, Young RG, Dadabayev A, Olenyik A, Penn M, Wagner J, Fink DJ, Caplan AI, Dennis JE (2012) Development of a peptide-targeted, myocardial ischemia-homing, mesenchymal stem cell. J Drug Target 20:23–32
51. Lin P, Correa D, Kean TJ, Awadallah A, Dennis JE, Caplan AI (2014) Serial transplantation and long-term engraftment of intra-arterially delivered clonally derived mesenchymal stem cells to injured bone marrow. Mol Ther 22:160–168
52. English K (2013) Mechanisms of mesenchymal stromal cell immunomodulation. Immunol Cell Biol 91:19–26
53. Bukulmez H, Bilgin A, Bebek G, Caplan AI, Jones O (2014) Immunomodulatory factors produced by mesenchymal stem cells after *in vitro* priming with danger signals. Arthritis Rheumatol 66:S163
54. Bukulmez H, Bilgin A, Caplan AI, Jones O (2014) Prevention of late stage renal failure in BXSB SLE mouse model with human bone marrow derived mesenchymal stem cell treatment. Arthritis Rheumatol 66:S149
55. Ghannam S, Pene J, Moquet-Torcy G, Jorgensen C, Yssel H (2010) Mesenchymal stem cells inhibit human Th17 cell differentiation and function and induce a T regulatory cell phenotype. J Immunol 185:302–312
56. Jackson WM, Nesti LJ, Tuan RS (2012) Mesenchymal stem cell therapy for attenuation of scar formation during wound healing. Stem Cell Res Ther 3:20
57. Waterman RS, Tomchuck SL, Henkle SL, Betancourt AM (2010) A new mesenchymal stem cell (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an Immunosuppressive MSC2 phenotype. PLoS One 5:e10088
58. Waterman RS, Henkle SL, Betancourt AM (2012) Mesenchymal stem cell 1 (MSC1)-based therapy attenuates tumor growth whereas MSC2-treatment promotes tumor growth and metastasis. PLoS One 7:e45590
59. Andrews BS, Eisenberg RA, Theofilopoulos AN, Izui S, Wilson CB, McConahey PJ, Murphy ED, Roths JB, Dixon FJ (1978) Spontaneous murine lupus-like syndromes. Clinical and immunopathological manifestations in several strains. J Exp Med 148:1198–1215
60. Helyer BJ, Howie JB (1963) Renal disease associated with positive lupus erythematosus tests in a cross-bred strain of mice. Nature 197:197
61. Bender AT, Wu Y, Cao Q, Ding Y, Oestreicher J, Genest M, Akare S, Ishizaka ST, Mackey MF (2014) Assessment of the translational value of mouse lupus models using clinically relevant biomarkers. Translat Res 163:515–532
62. Sang A, Yin Y, Zheng YY, Morel L (2012) Animal models of molecular pathology systemic lupus erythematosus. Prog Mol Biol Transl Sci 105:321–370
63. Rudofsky UH, Evans BD, Balaban SL, Mottironi VD, Gabrielsen AE (1993) Differences in expression of lupus nephritis in New Zealand mixed H-2z homozygous inbred strains of

mice derived from New Zealand black and New Zealand white mice. Origins and initial characterization. Lab Invest 68:419–426
64. Mohan C (1998) Murine lupus: pathogenic mechanisms and genetic origins. Ann Acad Med Singapore 27:29–34
65. Mohan C, Morel L, Yang P, Wakeland EK (1997) Genetic dissection of systemic lupus erythematosus pathogenesis: Sle2 on murine chromosome 4 leads to B cell hyperactivity. J Immunol 159:454–465
66. Morel L, Croker BP, Blenman KR, Mohan C, Huang G, Gilkeson G, Wakeland EK (2000) Genetic reconstitution of systemic lupus erythematosus immunopathology with polycongenic murine strains. Proc Natl Acad Sci U S A 97:6670–6675
67. Fairhurst AM, Mathian A, Connolly JE, Wang A, Gray HF, George TA, Boudreaux CD, Zhou XJ, Li QZ, Koutouzov S, Banchereau J, Wakeland EK (2008) Systemic IFN-α drives kidney nephritis in B6.Sle123 mice. Eur J Immunol 38:1948–1960
68. Adachi M, Watanabe-Fukunaga R, Nagata S (1993) Aberrant transcription caused by the insertion of an early transposable element in an intron of the Fas antigen gene of lpr mice. Proc Natl Acad Sci U S A 90:1756–1760
69. Dixon FJ, Andrews BS, Eisenberg RA, McConahey PJ, Theofilopoulos AN, Wilson CB (1978) Etiology and pathogenesis of a spontaneous lupus-like syndrome in mice. Arthritis Rheum 21(5 Suppl):S64–S67
70. Wu J, Wilson J, He J, Xiang L, Schur PH, Mountz D (1996) Fas ligand mutation in a patient with systemic lupus erythematosus and lymphoproliferative disease. J Clin Invest 98:1107–1113
71. Amoura Z, Chabre H, Koutouzov S, Lotton C, Cabrespines A, Bach JF, Jacob L (1994) Nucleosome-restricted antibodies are detected before anti-dsDNA and/or antihistone antibodies in serum of MRL-Mp lpr/lpr and +/+ mice, and are present in kidney eluates of lupus mice with proteinuria. Arthritis Rheum 37:1684–1688
72. Stetler DA, Sipes DE, Jacob ST (1985) Anti-RNA polymerase I antibodies in sera of MRL lpr/lpr and MRL $^{+/+}$ autoimmune mice. Correlation of antibody production with delayed onset of lupus-like disease in MRL $^{+/+}$ mice. J Exp Med 162:1760–1770
73. Gharavi AE, Mellors RC, Elkon KB (1989) IgG anti-cardiolipin antibodies in murine lupus. Clin Exp Immunol 78:233–238
74. Hirata D, Iwamoto M, Yoshio T, Okazaki H, Masuyama J, Mimori A, Minota S (2000) Nucleolin as the earliest target molecule of autoantibodies produced in MRL/lpr lupus-prone mice. Clin Immunol 97:50–58
75. Greenwood DL, Gitlits VM, Alderuccio F, Sentry JW, Toh BH (2002) Autoantibodies in neuropsychiatric lupus. Autoimmunity 35:79–86
76. Moore PM, Joshi I, Ghanekar SA (1994) Affinity isolation of neuron-reactive antibodies in MRL/lpr mice. J Neurosci Res 39:140–147
77. Lake R, Staines N (1986) DNA-binding antibodies derived from autoimmune MRL mice fail to induce clinical changes when administered to healthy animals. Agents Actions 19:306–308
78. Chan OT, Hannum LG, Haberman AM, Madaio MP, Shlomchik MJ (1999) A novel mouse with B cells but lacking serum antibody reveals an antibody-independent role for B cells in murine lupus. J Exp Med 189:1639–1648
79. Pawar RD, Ramanjaneyulu A, Kulkarni OP, Lech M, Segerer S, Anders HJ (2007) Inhibition of Toll-like receptor-7 (TLR-7) or TLR-7 plus TLR-9 attenuates glomerulonephritis and lung injury in experimental lupus. J Am Soc Nephrol 18:1721–1731
80. Sakic B, Szechtman H, Denburg JA (1997) Neurobehavioral alterations in autoimmune mice. Neurosci Biobehav Rev 21:327–340
81. Haas C, Ryffel B, Le Hir M (1997) IFN-γ is essential for the development of autoimmune glomerulonephritis in MRL/Ipr mice. J Immunol 158:5484–5491
82. Santoro TJ, Benjamin WR, Oppenheim JJ, Steinberg AD (1983) The cellular basis for immune interferon production in autoimmune MRL-Ipr/Ipr mice. J Immunol 131:265–268

83. Cash H, Relle M, Menke J, Brochhausen C, Jones SA, Topley N, Galle PR, Schwarting A (2010) Interleukin 6 (IL-6) deficiency delays lupus nephritis in MRL-Faslpr mice: the IL-6 pathway as a new therapeutic target in treatment of autoimmune kidney disease in systemic lupus erythematosus. J Rheumatol 37:60–70
84. Tang B, Matsuda T, Akira S, Nagata N, Ikehara S, Hirano T, Kishimoto T (1991) Age-associated increase in interleukin 6 in MRL/lpr mice. Int Immunol 3:273–278
85. Boswell JM, Yui MA, Burt DW, Kelley VE (1988) Increased tumor necrosis factor and IL-1 beta gene expression in the kidneys of mice with lupus nephritis. J Immunol 141:3050–3054
86. Lemay S, Mao C, Singh AK (1996) Cytokine gene expression in the MRL/lpr model of lupus nephritis. Kidney Int 50:85–93
87. Esfandiari E, McInnes IB, Lindop G, Huang FP, Field M, Komai-Koma M, Wei X, Liew FY (2001) A proinflammatory role of IL-18 in the development of spontaneous autoimmune disease. J Immunol 167:5338–5347
88. Favilli F, Anzilotti C, Martinelli L, Quattroni P, De Martino S, Pratesi F, Neumann D, Beermann S, Novick D, Dinarello CA, Boraschi D, Migliorini P (2009) IL-18 activity in systemic lupus erythematosus. Ann N Y Acad Sci 1173:301–309
89. Yin Z, Bahtiyar G, Zhang N, Liu L, Zhu P, Robert ME, McNiff J, Madaio MP, Craft J (2002) IL-10 regulates murine lupus. J Immunol 169:2148–2155
90. Sugiyama N, Nakashima H, Yoshimura T, Sadanaga A, Shimizu S, Masutani K, Igawa T, Akahoshi M, Miyake K, Takeda A, Yoshimura A, Hamano S, Yoshida H (2008) Amelioration of human lupus-like phenotypes in MRL/lpr mice by overexpression of interleukin 27 receptor alpha (WSX-1). Ann Rheum Dis 67:1461–1467
91. Hron JD, Peng SL (2004) Type I IFN protects against murine lupus. J Immunol 173:2134–2142
92. Schwarting A, Paul K, Tschirner S, Menke J, Hansen T, Brenner W, Kelley VR, Relle M, Galle PR (2005) Interferon-β: a therapeutic for autoimmune lupus in MRL-Faslpr mice. J Am Soc Nephrol 16:3264–3272
93. Herber D, Brown TP, Liang S, Young DA, Collins M, Dunussi-Joannopoulos K (2007) IL-21 has a pathogenic role in a lupus-prone mouse model and its blockade with IL-21R.Fc reduces disease progression. J Immunol 178:3822–3830
94. Murphy ED, Roths JB (1979) A Y chromosome associated factor in strain BXSB producing accelerated autoimmunity and lymphoproliferation. Arthritis Rheum 22:1188–1194
95. Maibaum MA, Haywood ME, Walport MJ, Morley BJ (2000) Lupus susceptibility loci map within regions of BXSB derived from the SB/Le parental strain. Immunogenetics 51:370–372
96. Hudgins CC, Steinberg RT, Klinman DM, Reeves MJ, Steinberg AD (1985) Studies of consomic mice bearing the Y chromosome of the BXSB mouse. J Immunol 134:3849–3854
97. Merino R, Shibata T, De Kossodo S, Izui S (1989) Differential effect of the autoimmune Yaa and lpr genes on the acceleration of lupus-like syndrome in MRL/MpJ mice. Eur J Immunol 19:2131–2137
98. Bolland S, Ravetch JV (2000) Spontaneous autoimmune disease in Fc(gamma)RIIB-deficient mice results from strain-specific epistasis. Immunity 13:277–285
99. Bolland S, Yim YS, Tus K, Wakeland EK, Ravetch JV (2002) Genetic modifiers of systemic lupus erythematosus in FcgammaRIIB(-/-) mice. J Exp Med 195:1167–1174
100. Izui S, Higaki M, Morrow D, Merino R (1988) The Y chromosome from autoimmune BXSB/MpJ mice induces a lupus-like syndrome in (NZW x C57BL/6)F1 male mice, but not in C57BL/6 male mice. Eur J Immunol 18:911–915
101. Pisitkun P, Deane JA, Difilippantonio MJ, Tarasenko T, Satterthwaite AB, Bolland S (2006) Autoreactive B cell responses to RNA-related antigens due to TLR7 gene duplication. Science 312:1669–1672
102. Deane JA, Pisitkun P, Barrett RS, Feigenbaum L, Town T, Ward JM, Flavell RA, Bolland S (2007) Control of toll-like receptor 7 expression is essential to restrict autoimmunity and dendritic cell proliferation. Immunity 27:801–810

103. Mizutani H, Engelman RW, Kinjoh K, Kurata Y, Ikehara S, Matsuzawa Y, Good RA (1994) Calorie restriction prevents the occlusive coronary vascular disease of autoimmune (NZW x BXSB)F1 mice. Proc Natl Acad Sci U S A 91:4402–4406
104. Mizutani H, Engelman RW, Kurata Y, Ikehara S, Good RA (1994) Energy restriction prevents and reverses immune thrombocytopenic purpura (ITP) and increases life span of ITP-prone (NZW x BXSB) F1 mice. J Nutr 124:2016–2023
105. Liu K, Mohan C (2006) What do mouse models teach us about human SLE? Clin Immunol 119:123–130
106. Gu Z, Akiyama K, Ma X, Zhang H, Feng X, Yao G, Hou Y, Lu L, Gilkeson GS, Silver RM, Zeng X, Shi S, Sun L (2010) Transplantation of umbilical cord mesenchymal stem cells alleviates lupus nephritis in MRL/lpr mice. Lupus 19:1502–1514
107. Ji S, Guo Q, Han Y, Tan G, Luo Y, Zeng F (2012) Mesenchymal stem cell transplantation inhibits abnormal activation of Akt/GSK3beta signaling pathway in T cells from systemic lupus erythematosus mice. Cell Physiol Biochem 29:705–712
108. Choi EW, Shin IS, Song JW, Yun TW, Yang J, Choi KS, Seong JK (2015) Transplantation of adipose tissue-derived mesenchymal stem cells prevents the development of lupus dermatitis. Stem Cells Dev 24:2041–2051
109. Liu RH, Li YQ, Zhou WJ, Shi YJ, Ni L, Liu GX (2014) Supplementing mesenchymal stem cells improves the therapeutic effect of hematopoietic stem cell transplantation in the treatment of murine systemic lupus erythematosus. Transplant Proc 46:1621–1627
110. Youd M, Blickarz C, Woodworth L, Touzjian T, Edling A, Tedstone J, Ruzek M, Tubo R, Kaplan J, Lodie T (2010) Allogeneic mesenchymal stem cells do not protect NZBxNZW F1 mice from developing lupus disease. Clin Exp Immunol 161:176–186
111. Kidd S, Spaeth E, Dembinski JL, Dietrich M, Watson K, Klopp A, Vl B, Wei M, Andreeff M, Marini FC (2009) Direct evidence of mesenchymal stem cell tropism for tumor and wounding microenvironments using in vivo bioluminescent imaging. Stem Cells 27:2614–2623
112. Passweg J, Tyndall A (2007) Autologous stem cell transplantation in autoimmune diseases. Semin Hematol 44:278–285
113. Jang E, Jeong M, Kim S, Jang K, Kang BK, Lee DY, Bae SC, Kim KS, Youn J (2015) Infusion of human bone marrow-derived mesenchymal stem cells alleviates autoimmune nephritis in a lupus model by suppressing follicular helper T cell development. Cell Transplant. doi:10.3727/096368915X688173
114. Gu F, Molano I, Ruiz P, Sun L, Gilkeson GS (2012) Differential effect of allogeneic versus syngeneic mesenchymal stem cell transplantation in MRL/lpr and (NZB/NZW)F1 mice. Clin Immunol 145:142–152
115. Choi YH, Kurtz A, Stamm C (2011) Mesenchymal stem cells for cardiac cell therapy. Hum Gene Ther 22:3–17
116. Li Y, Raman I, Du Y, Yan M, Min S, Yang J, Fang X, Li W, Lu J, Zhou XJ, Mohan C, Li QZ (2013) Kallikrein transduced mesenchymal stem cells protect against anti-GBM disease and lupus nephritis by ameliorating inflammation and oxidative stress. PLoS One 8:e67790
117. Lu S, Zeumer L, Sorensen H, Yang H, Ng Y, Yu F, Riva A, Croker B, Wallet S, Morel L (2015) The murine Pbx1-d lupus susceptibility allele accelerates mesenchymal stem cell differentiation and impairs their immunosuppressive function. J Immunol 194:43–55
118. Che N, Li X, Zhang L, Liu R, Chen H, Gao X, Shi S, Chen W, Sun L (2014) Impaired B cell inhibition by lupus bone marrow mesenchymal stem cells is caused by reduced CCL2 expression. J Immunol 193:5306–5314
119. Schena F, Gambini C, Gregorio A, Mosconi M, Reverberi D, Gattorno M, Casazza S, Uccelli A, Moretta L, Martini A, Traggiai E (2010) Interferon-γ-dependent inhibition of B cell activation by bone marrow-derived mesenchymal stem cells in a murine model of systemic lupus erythematosus. Arthritis Rheum 62:2776–2786
120. Chang JW, Hung SP, Wu HH, Wu WM, Yang AH, Tsai HL, Yang Y, Lee OK (2011) Therapeutic effects of umbilical cord blood-derived mesenchymal stem cell transplantation in experimental lupus nephritis. Cell Transplant 20:245–257

Anti-inflammatory Effects of Adipose-Derived Stem Cells (ASCs)

A.C. Bowles, R.M. Wise, and B.A. Bunnell

Abbreviations

ASCs	Adipose-derived stem cells
IL-1Ra	Interleukin-1 receptor antagonist
IL-6	Interleukin-6
IL-10	Interleukin-10
TGF-β	Transforming growth factor-β
TNF-α	Tumor necrosis factor-α
IFN-γ	Interferon-γ

A.C. Bowles
Center for Stem Cell Research and Regenerative Medicine, Tulane University School of Medicine, New Orleans, LA 70112, USA

Department of Cell and Molecular Biology, Tulane University School of Science and Engineering, New Orleans, LA 70118, USA

R.M. Wise
Center for Stem Cell Research and Regenerative Medicine, Tulane University School of Medicine, New Orleans, LA 70112, USA

Neuroscience Program, Brain Institute, Tulane University School of Science and Engineering, New Orleans, LA

B.A. Bunnell, Ph.D. (✉)
Center for Stem Cell Research and Regenerative Medicine, Tulane University School of Medicine, New Orleans, LA 70112, USA

Department of Pharmacology, Tulane University School of Medicine, New Orleans, LA 70112, USA
e-mail: bbunnell@tulane.edu

C.J. Malemud, E. Alsberg (eds.), *Mesenchymal Stem Cells and Immunomodulation*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-3-319-46733-7_4

Th	Helper T
Tregs	Regulatory T cells
BALF	Bronchoalveolar lavage fluid
COPD	Chronic obstructive pulmonary disease
MIP-2	Macrophage inflammatory protein-2
G-CSF	Granulocyte-colony stimulating factor
GM-CSF	Granulocyte-macrophage-colony stimulating factor
PD	Parkinson's disease
AD	Alzheimer's disease
MCP-1	Monocyte chemoattractant protein-1
BDNF	Brain-derived neurotrophic factor

Adipose-Derived Stem Cells

What was once considered merely an energy reservoir or structural cushion is now known as a complex endocrine organ mediating energy homeostasis, feeding, and immune surveillance. Even more interesting, adipose tissue, or fat, is emerging as an abundant source of cells with regenerative capabilities [1, 2]. The potential for regeneration is attributed to the heterogeneous composition of cells including the adipocytes, or fat cells, and the cells of the stromal vascular fraction (SVF). Once the harvested fat is digested, the composite of released cells is known as the SVF. The SVF contains several cell types, namely, adipose stromal cells (ASCs; 15–30 %), endothelial cells (10–20 %), pericytes (3–5 %), and immune cells (25–45 %) [3, 4]. The ASCs have been the focus of much attention due to their multilineage differentiation potential, immune privileged status, regenerative properties, and immunomodulatory effects. ASCs efficiently differentiate into mesodermal lineage cells. They have demonstrated the ability to differentiate along adipogenic, osteogenic, chondrogenic, and myogenic lineages. ASCs appear to be immune privileged due to their lack of expression of MHC class II molecules and costimulatory molecules [5, 6].

ASCs have highly comparable properties and therapeutic effects to bone marrow-derived mesenchymal stem cells (BMSCs); however, ASCs offer distinct advantages [7, 8]. Compared to the procedures to obtain BMSCs, the ease of harvesting fat by liposuction of subcutaneous adipose tissue can be in large volumes with minimal risk. Moreover, fat contains a higher frequency of ASCs, yielding 100–500 times more cells per tissue volume [2, 9]. Similar to BMSCs, ASCs can be culture expanded once isolated from associated tissue utilizing the characteristic plastic adherence capability. ASCs have a comparable spindle-like morphology, are multipotent, can self-renew for clonal expansion, and express a unique cell surface profile indicative of mesenchymal stem cells. ASCs have demonstrated potent immunomodulatory, angiogenic, and anti-inflammatory qualities which make them attractive candidates for therapeutic use [1–3, 5].

Adipose-Derived Stem Cells Mediate Anti-inflammatory Effects

The therapeutic properties of ASCs are multifaceted which makes them ideal candidates for comprehensively treating various disease states. Of these properties, ASCs have a pronounced ability to efficiently quiet disease-associated inflammation. By production of anti-inflammatory factors or modulation of immune frequency and activity, ASCs squelch inflammation and promote an environment that reestablishes homeostasis.

Production of Anti-inflammatory Factor by ASCs

When introduced into an inflammatory environment, ASCs secrete an array of soluble factors including cytokines, chemokines, enzymes, and other small molecules that modulate the surrounding inflammatory milieu [10]. Typically, these factors mediate anti-inflammatory effects by attenuating production of pro-inflammatory mediators, promoting anti-inflammatory phenotypes of cells, upregulating the production of regulatory cell types, inhibiting further recruitment of pro-inflammatory cells, or suppressing the activation and/or expansion of pro-inflammatory cells in the affected area [3, 4, 6, 8]. Several of these anti-inflammatory effects mediated by ASCs have been demonstrated in vitro and in vivo and are described in the following chapter.

Cytokines and Chemokines

The cytokine profiles of ASCs have been elucidated in vitro to evaluate possible mechanistic effects that may be correlative in vivo. When stimulated, ASCs secrete high levels of anti-inflammatory cytokines including interleukin-1 receptor antagonist, interleukin-6, and interleukin-10 which can be collected, measured, or even therapeutically administered in what is known as conditioned media [11]. It is important to note that IL-6 can function in both a pro- and anti-inflammatory manner [11, 12].

Interleukin-1 Receptor Antagonist

Interleukin-1 receptor antagonist (IL-1Ra) acts as an anti-inflammatory cytokine by way of opposing the activities of IL-1, a potent mediator of inflammation and tissue damage. Evidence from studies of rheumatoid arthritis and experimental animal models of arthritis indicates a direct role for IL-1 in inflammation-induced pathology [13]. Treatment with anti-inflammatory cytokine IL-4 induced gene

expression of IL-1Ra which resulted in amelioration of disease in animal models of arthritis [14]. Therapeutic administration of IL-1Ra reduced initial joint swelling and inhibited joint swelling after reactivation of arthritis [15].

Interleukin-6

Interleukin-6 (IL-6) is a pleiotropic cytokine which produces either anti- or pro-inflammatory effects depending on the surrounding environment [16]. Here the anti-inflammatory effects of IL-6 are discussed with respect to the administration of ASCs. IL-6 secreted by mesenchymal stem cells is associated with the induction of regulatory cells that promote immune tolerance, polarization of cells to a more anti-inflammatory phenotype, and/or enhanced expression of other anti-inflammatory molecules in vitro [16–20]. Attenuation of inflammatory pathologies by administration of ASCs is associated with elevated IL-6 and IL-10 levels in the affected tissue [1, 21].

Interleukin-10

The cytokine interleukin-10 (IL-10) produced by ASCs has been demonstrated to be potently anti-inflammatory in several inflammatory diseases [18, 21, 22]. Many studies have shown increased IL-10 levels in the locally affected area following administration of ASCs. ASCs are capable of directly producing IL-10 as well as inducing regulatory cell types which can also produce IL-10 [21–24]. IL-10 production leads to immunosuppressive activities against T cell and macrophage populations [25]. IL-10 is also a regulatory cytokine that is involved in the induction of regulatory T cells (Tregs) that are capable of countering autoimmunity and in the promotion of an M2-like phenotype of macrophages that are potently anti-inflammatory [26–28].

Transforming Growth Factor-β

Transforming growth factor-β (TGF-β) is a potent anti-inflammatory cytokine, and is thus a common marker measured after treatment with ASCs. The levels of TGF-β were markedly increased after administration of ASCs, which was also accompanied by an increase in the Treg population during inflammation [26]. The enhanced levels of TGF-β were detected in the local pathologic area as well as the associated draining lymph nodes [23, 26]. To correlate the direct effects of TGF-β to ASCs, researchers manipulated ASCs to hinder their ability to produce TGF-β. After administration of these ASCs, the immunomodulatory effects necessary to counter inflammation in vivo were abrogated [29]. In vitro assays of mononuclear cells further demonstrated the upregulation of TGF-β that was directly associated with cocultures containing ASCs [26].

Other Anti-inflammatory Molecules

Prostaglandin E-2

Prostaglandin E-2 (PGE-2) is a small molecule that functions as a ubiquitous homeostatic factor. By modulating chemokines and chemotaxis of pro-inflammatory cells, PGE-2 is described as a key mediator of immunopathology. All cells are capable of producing PGE-2; however, most of the local production of PGE-2 is generated by myeloid and stromal cells. Dendritic cells respond to PGE-2 by suppressing the recruitment of naïve, memory, and effector T cells. The selective suppression of effector activities of macrophages and neutrophils can also be mediated by PGE-2 [30]. During inflammatory responses, PGE-2 can function as an anti-inflammatory mediator by thwarting T cell activation [19, 31–33]. PGE-2 can modulate the local environment by promoting Th2, Th17, and Tregs responses [30]. The administration of ASCs increases the expression of PGE-2, which is accompanied by the induction of Tregs in the local milieu. The connection between PGE-2 and Tregs has been demonstrated in several animal models of pathologic conditions including allergic inflammation, asthma, and rheumatoid arthritis [23, 26, 32, 34, 35].

Indoleamine 2, 3-Dioxygenase

Tryptophan-metabolizing enzyme indoleamine 2, 3-dioxygenase (IDO) plays a critical role in immune tolerance, autoimmunity, and infection by inhibiting T cell responses [36, 37]. Tryptophan catabolism occurs primarily at local sites of inflammation, and it is believed that the expression of IDO modulates the inflammatory cascade to attenuate tissue damage [38]. For example, IDO-mediated tryptophan catabolism has been shown to induce apoptosis of T cells [39]. Additionally, IDO exerts immunomodulatory effects by inhibiting proliferation of activated T cells in vivo and in vitro [36, 40]. As a result of administering ASCs, high levels of IDO in locally affected areas were accompanied by induction of Tregs [23].

Modulation of Immune Cell Recruitment, Activity, and Repertoire

Reduction in Levels of Infiltrating Immune Cells

Pathology can arise from many causative agents including the mobilization of pathogenic cells to afflicted areas where damage ensues. The pathogenesis of many inflammatory and neurodegenerative diseases arises from this infiltration of damaging cells. ASCs have the ability to thwart this process by suppressing the infiltration

of cells or mediating the removal of infiltrated cells from local areas that are responsible for pathology. Following the administration of ASCs, evidence from in vivo studies showed a dramatic reduction in the levels of infiltrating cells, especially those promoting neuroinflammation. More specifically, a marked reduction in infiltrating macrophages was seen in neurodegenerative disease models treated with ASCs [21, 41, 42].

The immunomodulatory effects of ASCs are attributed to the suppression of T cell recognition. In a rat kidney transplantation study, prolonged survival of the tissue graft resulted from attenuated acute cellular rejection. The significant reduction in T cell frequency was attributed to treatment with ASCs [43].

Animals modeling autoimmune diseases are ideal candidates for therapies using ASCs. A dramatic reduction in the levels of lymphocyte infiltration was observed in the thyroid glands of animals affected by autoimmune thyroiditis subsequent to infusion with ASCs. Similarly, central nervous system tissue showed a significant decrease in the extent of cellular infiltrates including macrophages and lymphocytes in neurodegenerative and autoimmune diseases [41, 44].

T Cell Immunomodulation

The quality of ASCs to be immune privileged, thus evading T cell recognition in vivo, is believed to be due to their low immunogenicity, potent immunosuppressive effects, or a combination of both. Much of this evidence results from the direct effects of ASCs on T cells in vitro [45, 46]. These qualities are necessary to demonstrate the safety of therapeutic applications using ASCs.

A well-determined mechanism of action of mesenchymal stem cell administration is the modulation of T cells, especially of the helper T (Th) cell subtypes. The associated factors that these cells express achieve a phenotype beneficial to reestablish homeostasis. These mechanisms are integral to maintaining the balance between autoimmune disease and immune tolerance. To investigate the direct effects, in vitro analysis of ASCs cultured with spleen mononuclear cells determined that a significant reduction in factors IL-4, IFN-γ, and IL-17 occurred, which correlated with reduced Th1 cells, Th2 cells, and Th17 cells, respectively [28].

Moreover, the inflammatory cytokine IL-1β is associated with autoimmune diseases and is a known inducer of pathogenic Th17 cells [28, 47]. The ability of ASCs to enhance the expression of anti-inflammatory mediators, and their earlier described effects on T cell subsets, demonstrates robust immunomodulatory effects that show promise for treating inflammatory and autoreactive disease milieus [28].

For many inflammatory airway diseases, a Th2 cell-driven immune response perpetuates lung inflammation. Investigations of treatments with ASCs have demonstrated attenuation of Th2-mediated pathologies in these disease models. It is suggested that ASCs have the ability to modulate the Th1/Th2 balance to counter the pathologic effects of Th2 by reducing IL-4, IL-5, and TGF-β. This effect was accompanied by a decrease in Th2 cells and a concomitant increase in Th1 cells [48].

Induction of Tregs

Once known as suppressor T cells, Tregs are critical regulators of immune responses. By direct cell contact or secretion of cytokines, Tregs target pathogenic T cells, B cells, or other antigen-presenting cells to suppress autoimmunity. Tregs are also activated by dendritic cells and cytokines [49]. IL-6 secreted by ASCs is linked to induction of Tregs and a subsequent increase in the anti-inflammatory factor IL-10 expression [17, 28]. High levels of IDO, TGF-β, and PGE-2 were associated with an increase in Tregs [26]. TGF-β, IL-10, and PGE-2, all of which are secreted by ASCs, result in the expression and maintenance of Foxp3, a transcription factor expressed specifically by Tregs [28].

Shifting Macrophages Toward an Anti-inflammatory Phenotype

Macrophages are vital phagocytic cells which reside in all tissues. Depending on the conditions and their niche, macrophages present with two phenotypically distinct subtypes which are conceptually comparable to the Th1/Th2 paradigm [19]. The identity of the distinct phenotype can be determined by their metabolic activity and expression of specific factors [20, 27]. Several studies have demonstrated that the switch from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype occurs with the administration of ASCs. With the switch to the M2-like phenotype, macrophages will begin to express elevated levels of arginase 1 and reduced levels of inducible nitric oxide synthase. Additionally, the phenotypic changes result in changes to transcription factors that regulate inflammatory and autoimmune responses such as NF-κB and STAT3/STAT6 [27].

Direct and Indirect Effects on B Cells

B cells are major contributors to several autoimmune disorders by functioning as antigen-presenting cells, producing cytokines, and by differentiating into plasma cells that produce antigen-specific immunoglobulins or antibodies. B cells directly interact with T cells to perpetuate immune responses making them targets for immunotherapy [24].

In vitro assays of human ASCs and human tonsil-derived B cells demonstrated several mechanisms that ASCs influence B cells. The results suggested that the presence of ASCs inhibited differentiation and proliferation of B cells induced by activated T cells. However, without T cell-mediated stimulation, ASCs have a direct effect on the inhibition of B cell differentiation but not on proliferation. More importantly, ASCs induced a regulatory phenotype of B cells. Similar to

Tregs, the infusion of ASCs induced expression of IL-10 in B cells. This study determined that ASCs indirectly modulated T cell-mediated proliferation of B cells and differentiation into antibody-producing plasma cells, and directly induced a regulatory phenotype of B cells [24, 48, 49].

Preclinical and Clinical Applications

Much evidence of the anti-inflammatory impact of ASCs is generated from studies in inflammatory and autoimmune disease models. Following therapeutic administration of ASCs, systemic and local inflammation can be attenuated or resolved. Demonstrations of the various clinical applications for the robust anti-inflammatory and immunomodulatory effects of ASCs are provided by numerous studies modeling tissue repair, autoimmune diseases, and neurodegenerative disorders.

Wound Healing

The dynamic properties of ASCs are not aimed specifically at tissue niches or types of insults, yet comprehensively benefit various forms of cellular/tissue damage. An in vitro system was created to investigate the direct effects of macrophages on tendon fibroblasts. Macrophages cocultured with ASCs switched their phenotype from pro- to anti-inflammatory. The evidence of this switch was demonstrated when the macrophages were cocultured with tendon fibroblasts, which diminished the production of pro-inflammatory cytokines IL-1β and TNFα by the tendon fibroblasts [50]. Treatment with ASCs demonstrated anti-inflammatory effects when administered to pressure ulcers of both young and old mice by reducing the infiltration of mononuclear cells that promote inflammation. Mechanistically, this anti-inflammatory effect induced by ASCs was concomitant with the activation of reparative genes which were suggested to optimally prepare the local milieu for tissue repair [51].

Ischemia–Reperfusion/Infarction

With cardiovascular disease being one of the leading causes of death worldwide, the employment of therapies to resolve ischemic heart disease is critical. The inadequate levels of oxygen (hypoxia) to the heart tissue leads to myocardial infarction which in turn results in a loss of cardiomyocytes, an irreversible event. A study using a rodent model of myocardial infarction showed that the delivery of ASCs not only resulted in engraftment of donor ASCs into heart tissue under hypoxic

conditions, but also showed a significant improvement in cardiac function and reduced infarction [52]. These results were attributed to a significant reduction in pro-inflammatory levels of TNF-α and an increase in anti-inflammatory IL-10 levels in ventricular tissue of ASCs-treated animals [49, 52].

Autoimmune Diseases

Pro-inflammatory T cells play a critical role in many autoimmune diseases. The Th1/Th2 cell imbalance along with the induction of Th17-mediated responses have been associated with autoimmune diseases. Effector T cells perpetuate antigen-specific responses that drive the cascade of pathologic events promoting widespread inflammation [8, 53].

Multiple Sclerosis

Multiple sclerosis (MS) is an autoimmune disease against integral components of the central nervous system (CNS) that leads to neurodegeneration and inflammation. Using the murine experimental autoimmune encephalomyelitis model of MS, several studies have identified many anti-inflammatory effects attributable to ASCs. Splenocytes harvested from mice treated with ASCs were assessed for proliferation in vitro after stimulation with the antigen used to induce the autoimmune reaction in this MS model. Upon stimulation, the proliferation of splenocytes harvested from mice treated with ASCs was suppressed compared to the splenocytes from untreated mice [46]. Furthermore, serum levels revealed a significant reduction in IL-12 and IFN-γ following treatment with ASCs [54]. These anti-inflammatory and immunomodulatory effects collectively resulted in a reduction in tissue damage, cellular infiltrates, and preservation of myelin in the CNS which ameliorated symptoms of this disease [46, 54].

Rheumatoid Arthritis

In a murine model for rheumatoid arthritis (RA), the production of granulocyte-macrophage-colony stimulating factor (GM-CSF) by effector T cells led to several pathologic processes. Administration of ASCs provided anti-inflammatory effects by reducing the frequency of pathogenic T cells and associated production of GM-CSF in the spleen and peripheral blood. Moreover, an increase in the Treg population within the lymph nodes of treated mice was also reported. Increased numbers of T cells expressing IL-10 were also detected in the lymph nodes after treatment with ASCs. The modulation of these cells and associated

factors correlated with a significant decrease in the severity of arthritis. The data also suggested reestablishment of immune tolerance in the peripheral lymphoid tissues [55]. Other studies of RA also noticed an induction of IL-10 production and generation of antigen-specific Tregs with ASCs treatment [8, 35]. These studies further demonstrated that ASCs promoted inhibition of inflammatory mediators and reduced antigen-specific Th1/Th17 cell expansion. Thus, anti-inflammatory and immunomodulatory effects attenuated the incidence and severity of experimental arthritis [8, 35, 55].

Inflammatory Diseases/Disorders

Under inflammatory conditions, ASCs respond by modulating immune cell activities, producing anti-inflammatory proteins, or promoting the differentiation of anti-inflammatory or regulatory cell types. Thus, ASCs promote an anti-inflammatory environment to counter pathologic processes induced by inflammation.

Acute Respiratory Distress Syndromes

As a result of conditions such as sepsis, trauma, gastric aspiration, and pneumonia, acute lung injury can develop which can progress into acute respiratory distress syndromes. Symptoms of these conditions are a result of pulmonary inflammation and the infiltration of immune cells that largely contribute to lung tissue damage. A study treating murine models of acute lung injury demonstrated that the anti-inflammatory effects from the administration of human and mouse ASCs were able to significantly attenuate lung damage and inflammation. The therapeutic effects were attributed to an ASC-induced increase in the expression of anti-inflammatory IL-6 and IL-10 detected only in treated lungs [9, 56]. Furthermore, a reduction in the gene expression levels of pro-inflammatory cytokines macrophage inflammatory protein-2 (MIP-2), IL-1α, IL-1β, and TNF-α in the lungs was reported. Quantitative comparison of proteins in the lungs revealed a significant increase of the anti-inflammatory cytokine IL-10 and a reduction in the pro-inflammatory proteins, MIP-2, IL-1α, IL-1β, IFN-γ, and GM-CSF [9].

Asthma

ASCs have shown multiple mechanisms by which they produce anti-inflammatory effects that can significantly ameliorate symptoms manifesting from allergic airway inflammation. The administration of ASCs resulted in a marked increase in the levels of anti-inflammatory factors, IDO, TGF-β, and PGE-2, which was accompanied

by induction of Tregs in the lungs of animals afflicted with asthma [23]. It was determined that ASCs were able to attenuate asthma-associated inflammation by several mechanisms including reduction of infiltration of eosinophils, suppression of local goblet cell hyperplasia, and systemically reducing the levels of total immunoglobulins and allergen-specific IgE and IgG1 [26]. Another study reported that ASCs were capable of rapidly resolving inflammation, tissue remodeling, and bronchial hyper-responsiveness. Lung tissue treated with ASCs revealed suppression of neutrophilic inflammation and total IgE production, preservation of alveolar architecture by inhibition of lymphoplasmacytic infiltrates, and negligible smooth muscle hyperplasia/hypertrophy in peribronchiolar areas, the characteristic features of occupational asthma [57]. Another study using a murine model of allergic asthma determined the anti-inflammatory effects from ASCs administration by measuring inhibition of Th2-driven responses. Airway hyper-responsiveness, eosinophilia, and mucus production were markedly reduced after administration of ASCs [48].

Chronic Obstructive Pulmonary Disease

Repetitive exposure to cigarette smoke induces a progressive lung disease called chronic obstructive pulmonary disease (COPD) which is one of the six leading causes of death in the United States [58]. Cigarette smoke-induced COPD is a result of infiltration of neutrophils and eosinophils to the lungs and the bronchoalveolar lavage fluid (BALF). Immune cell infiltration leads to damage from inflammatory responses and oxidative stress to the lungs, as well as a decrease in tracheal hyper-responsiveness. Treatment with ASCs in a guinea pig model of COPD revealed a significant decrease in the percentages of eosinophils, neutrophils, and lymphocytes in the BALF. Moreover, ASCs administration was able to restore tracheal hyper-responsiveness [59].

Inflammatory Bowel Disease/Crohn's Disease

Inflammatory bowel disease (IBD) is an inflammatory immune system disorder characterized by chronic inflammation of the intestines that closely resembles Crohn's disease [60]. ASC-induced macrophage polarization toward an M2-like phenotype promotes the further production of anti-inflammatory factors necessary to alleviate the progression of chronic colitis and to prevent disease recurrence in animal models of experimental colitis and sepsis [25]. Furthermore, administration of ASCs to a murine model of IBD revealed a substantial reduction in pro-inflammatory factors IL-12, IFN-γ, and TNF-α. Changes to these cytokine levels were correlative with a significant reduction in the degree of inflammation as determined by histology [60]. Another study focused on the modulation of Th1 cells following treatment with ASCs. Treatment with ASCs demonstrated impairment of Th1 cell

activation, induction of Tregs, and enhanced IL-10 production in colonic mucosa and draining lymph nodes. These changes led to the amelioration of severe sepsis by the reduction of inflammatory infiltrates in various affected organs and down-regulation of several inflammatory mediators responsible for pathologic processes underlying acute and chronic colitis [53, 61].

Obesity

Obesity is believed to be a low-grade systemic inflammatory disease characterized by high serum levels of inflammatory proteins including C-reactive protein, TNF-α, and leptin [62]. In a study delivering ASCs to obese mice demonstrated a marked shift in the macrophages from pro-inflammatory M1 toward anti-inflammatory M2 phenotype. This phenomenon was also demonstrated in vitro using a transwell coculture system with ASCs and macrophages as well as by culturing macrophages with conditioned media from ASCs to examine the effects of paracrine factors. This shift was associated with an attenuation of adipocyte hypertrophy, inflammation of white adipose tissue, and TNF-α production [27].

Osteoarthritis

Often patients with osteoarthritis develop synovitis, inflammation of the synovial membrane. This occurs as a result of high levels of pro-inflammatory macrophages and associated inflammatory factors. Using a collagenase-induced experimental mouse model of osteoarthritis, serum levels of known protein products of synovitis called alarmins S100A8/A9 were used as a measure of the degree of synovial inflammation. This study demonstrated that 6 h after local administration of ASCs there was a decrease in the expression levels of pro-inflammatory mediators S100A8/A9, IL-1β, and KC (mouse IL-8) in the synovium. These data suggested that ASCs provided a protective action by secreting anti-inflammatory factors when administered during high levels of synovial inflammation [47].

Neurodegenerative Diseases

The anti-inflammatory effects produced by ASCs make them therapeutic candidates for neurodegenerative diseases where secondary neuroinflammation exacerbates the pathogenic features and disease. The anti-inflammatory and trophic factors produced by ASCs make them ideal for sustaining the survival of critical brain cells. Several reports investigating the therapeutic benefit of ASCs in preclinical animal models have been published.

Alzheimer's Disease

As the most prevalent neurodegenerative disease in the United States, Alzheimer's disease (AD) is characterized by the progressive loss of cognitive functions, especially learning and memory. These debilities are caused by the accumulation of neuropathologic features known as amyloid plaques and neurofibrillary tangles [63].

The administration of human ASCs in a mouse model of AD was shown to attenuate learning and memory impairment and to reduce the number of amyloid plaques and associated precursor proteins in the brain. Alterations in the levels of IL-10 were attributed to the benefits of ASC treatment. The anti-inflammatory effects of IL-10 production and neuroprotective benefits from trophic factors were observed following local and systemic delivery of ASCs. Evidence showed a reduction of pro-inflammatory cytokines and an increase in amyloid-β-degrading enzymes in the brains of AD mice treated with ASCs. Moreover, marked upregulation of several neurotrophic factors including vascular endothelial growth factor, glial-derived neurotrophic factor, neurotrophin-3, and NeuroD1 was observed [63, 64]. The effects from the administration of ASCs were reported to persist for more than 4 months [63].

Huntington's Disease

The genetic mutations in the *huntingtin* gene and loss of striatal neurons are features of Huntington's disease that result in progressive cognitive impairment, neuropsychiatric symptoms, and loss of voluntary motor control [63, 65].

Human ASCs administered to a mouse model of Huntington's disease led to attenuation of induced striatal lesions, delays in the phenotypic progression of motor impairments, and reduced neurodegeneration by attenuating brain atrophy, neuronal loss, and formation of pathologic aggregates in the brains of affected animals. These therapeutic effects demonstrated that ASCs provide neuroprotective effects in addition to their known anti-inflammatory effects [64].

Krabbe's Disease

Krabbe's disease is one of over 40 lysosomal storage diseases that are characterized by rapid neurodegeneration and CNS inflammation that leads to early death. The genetic deficiency of an essential lysosomal enzyme galactocerebrosidase results in the accumulation of a toxic substrate and subsequent demyelination within the central and peripheral nervous system [41, 42].

The administration of murine ASCs in the twitcher mouse model of Krabbe's disease resulted in significant changes to the inflammatory profiles present in the

serum of treated animals. Following treatment with ASCs, marked decreases in the expression levels of pro-inflammatory IL-1α, IL-1β, IL-6, TNF-α, granulocyte-colony stimulating factor (G-CSF), and MCP-1 were measured. Correlatively, protein levels of G-CSF, IL-1 α, and MCP-1 were significantly reduced with ASC treatment. In the CNS tissues of treated mice, a reduction in macrophage infiltration and microglial activation was reported. Together, treatment with ASCs led to a significant increase in lifespan of animals which implicates treatment with ASCs as a promising therapy for the other lysosomal storage disorders [41].

Parkinson's Disease

Parkinson's disease (PD) is the second most common progressive neurodegenerative disorder in which crucial neurons degenerate and the development of Lewy bodies within the cells causes deleterious effects to normal motor function [63, 66]. Pathologic markers of disease in both human and murine PD include high levels of TGF-β and monocyte chemoattractant protein-1 (MCP-1) and low levels of brain-derived neurotrophic factor (BDNF), brain dopamine, and brain tyrosine hydroxylase which are typically present in the serum [66].

The syngeneic administration of ASCs in a rat model of PD resulted in marked changes to key factors driving pathogenesis. The levels of TGF-β and MCP-1 in the serum were significantly decreased. Additionally, the expression of BDNF, brain dopamine, and brain tyrosine kinase was all significantly increased in serum. The results of this study demonstrated that the improvements associated with the disease were the result of the anti-inflammatory, immunomodulatory, and neurotrophic effects attributed to the ASCs [66]. Another study investigated the non-motor symptoms of PD after treatment with human ASCs as a disease-modifying therapy. Transplantation of ASCs directly into the brain provided anti-inflammatory and trophic effects that enhanced dopamine levels and upregulated the expression of anti-inflammatory cytokines in the periphery. These ASC-mediated effects promoted neurogenesis in hippocampal and subventricular regions and boosted memory functions in the PD rodents [67].

Conclusion

With the vast evidence demonstrating the potency of ASCs to modulate various pathologic states that occur in numerous diseases, the field of regeneration flourishes with the potential. The growing advances that are elucidated at the bench top demonstrate the unique capabilities of ASCs. The potency of ASCs is attributed to their ability to respond to endogenous environments and comprehensively promote homeostasis. The support from preclinical applications is promising to one day achieve bedside practices with ASCs.

References

1. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH (2001) Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 7:211–228
2. D'Andrea F, De Francesco F, Ferraro GA, Desiderio V, Tirino V, De Rosa A, Papaccio G (2008) Large-scale production of human adipose tissue from stem cells: a new tool for regenerative medicine and tissue banking. Tissue Eng Part C Methods 14:233–242
3. Bourin P, Bunnell BA, Casteilla L, Dominici M, Katz AJ, March KL, Redl H, Rubin JP, Yoshimura K, Gimble JM (2013) Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). Cytotherapy 15:641–648
4. Lee MJ, Wu Y, Fried SK (2013) Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity complications. Mol Aspects Med 34:1–11
5. Gimble JM, Katz AJ, Bunnell BA (2007) Adipose-derived stem cells for regenerative medicine. Circ Res 100:1249–1260
6. DelaRosa O, Sanchez-Correa B, Morgado S, Ramirez C, del Rio B, Menta R, Lombardo E, Tarazona R, Casado JG (2012) Human adipose-derived stem cells impair natural killer cell function and exhibit low susceptibility to natural killer-mediated lysis. Stem Cells Dev 21:1333–1343
7. Yi H, Kang KY, Kim Y, Jung H, Rim YA, Park N, Kim J, Jung SM, Park SH, Ju JH (2015) Human adipose-derived mesenchymal stem cells attenuate collagen antibody-induced autoimmune arthritis by inducing expression of FCGIIB receptors. BMC Musculoskelet Disord 16:170
8. Zhou B, Yuan J, Zhou Y, Ghawji M Jr, Deng YP, Lee AJ, Lee AJ, Nair U, Kang AH, Brand DD, Yoo TJ (2011) Administering human adipose-derived mesenchymal stem cells to prevent and treat experimental arthritis. Clin Immunol 141:328–337
9. Zhang S, Danchuk SD, Imhof KM, Semon JA, Scruggs BA, Bonvillain RW, Strong AL, Gimble JM, Betancourt AM, Sullivan DE, Bunnell BA (2013) Comparison of the therapeutic effects of human and mouse adipose-derived stem cells in a murine model of lipopolysaccharide-induced acute lung injury. Stem Cell Res Ther 4:13
10. Hattori H, Ishihara M (2015) Altered protein secretions during interactions between adipose tissue- or bone marrow-derived stromal cells and inflammatory cells. Stem Cell Res Ther 6:70
11. Blaber SP, Webster RA, Hill CJ, Breen EJ, Kuah D, Vesey G, Herbert BR (2012) Analysis of *in vitro* secretion profiles from adipose-derived cell populations. J Transl Med 10:172
12. Kilroy GE, Foster SJ, Wu X, Ruiz J, Sherwood S, Heifetz A, Ludlow JW, Stricker DM, Potiny S, Green P, Halvorsen YD, Cheatham B, Storms RW, Gimble JM (2007) Cytokine profile of human adipose-derived stem cells: expression of angiogenic, hematopoietic, and pro-inflammatory factors. J Cell Physiol 212:702–709
13. Arend WP (2002) The balance between IL-1 and IL-1Ra in disease. Cytokine Growth Factor Rev 13:323–340
14. Allen JB, Wong HL, Costa GL, Bienkowski MJ, Wahl SM (1993) Suppression of monocyte function and differential regulation of IL-1 and IL-1ra by IL-4 contribute to resolution of experimental arthritis. J Immunol 151:4344–4351
15. Schwab JH, Anderle SK, Brown RR, Dalldorf FG, Thompson RC (1991) Pro- and anti-inflammatory roles of interleukin-1 in recurrence of bacterial cell wall-induced arthritis in rats. Infect Immun 59:4436–4442
16. Kishimoto T, Hibi M, Murakami M, Narazaki M, Saito M, Taga T (1992) The molecular biology of interleukin 6 and its receptor. Ciba Found Symp 167:5–16, discussion, 16–23
17. Ivanova-Todorova E, Bochev I, Dimitrov R, Belemezova K, Mourdjeva M, Kyurkchiev S, Kinov P, Altankova I, Kyurkchiev D (2012) Conditioned medium from adipose tissue-derived mesenchymal stem cells induces CD4+FOXP3+ cells and increases IL-10 secretion. J Biomed Biotechnol 2012:295167

18. Adutler-Lieber S, Ben-Mordechai T, Naftali-Shani N, Asher E, Loberman D, Raanani E, Leor J (2013) Human macrophage regulation via interaction with cardiac adipose tissue-derived mesenchymal stromal cells. J Cardiovasc Pharmacol Ther 18:78–86
19. Waterman RS, Tomchuck SL, Henkle SL, Betancourt AM (2010) A new mesenchymal stem cell (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an Immunosuppressive MSC2 phenotype. PLoS One 5:e10088
20. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM (2000) M-1/M-2 macrophages and the Th1/Th2 paradigm. J Immunol 164:6166–6173
21. Semon JA, Maness C, Zhang X, Sharkey SA, Beuttler MM, Shah FS, Pandey AC, Gimble JM, Zhang S, Scruggs BA, Strong AL, Strong TA, Bunnell BA (2014) Comparison of human adult stem cells from adipose tissue and bone marrow in the treatment of experimental autoimmune encephalomyelitis. Stem Cell Res Ther 5:2
22. Park KH, Mun CH, Kang MI, Lee SW, Lee SK, Park YB (2016) Treatment of collagen-induced arthritis using immune modulatory properties of human mesenchymal stem cells. Cell Transplant 25:1057–1072
23. Cho KS, Park MK, Kang SA, Park HY, Hong SL, Park HK, Yu HS, Roh HJ (2014) Adipose-derived stem cells ameliorate allergic airway inflammation by inducing regulatory T cells in a mouse model of asthma. Mediators Inflamm 2014:436476
24. Franquesa M, Mensah FK, Huizinga R, Strini T, Boon L, Lombardo E, DelaRosa O, Laman JD, Grinyo JM, Weimar W, Betjes MG, Baan CC, Hoogduijn MJ (2015) Human adipose tissue-derived mesenchymal stem cells abrogate plasmablast formation and induce regulatory B cells independently of T helper cells. Stem Cells 33:880–891
25. Anderson RJ (2012) Acute kidney injury with sepsis: is nitric oxide a friend, foe, both, or neither? Crit Care Med 40:2511–2512
26. Cho KS, Lee JH, Park MK, Park HK, Yu HS, Roh HJ (2015) Prostaglandin E2 and transforming growth factor-beta play a critical role in suppression of allergic airway inflammation by adipose-derived stem cells. PLoS One 10:e0131813
27. Shang Q, Bai Y, Wang G, Song Q, Guo C, Zhang L, Wang Q (2015) Delivery of adipose-derived stem cells attenuates adipose tissue inflammation and insulin resistance in obese mice through remodeling macrophage phenotypes. Stem Cells Dev 24:2052–2064
28. Mohammadzadeh A, Pourfathollah AA, Shahrokhi S, Hashemi SM, Moradi SL, Soleimani M (2014) Immunomodulatory effects of adipose-derived mesenchymal stem cells on the gene expression of major transcription factors of T cell subsets. Int Immunopharmacol 20:316–321
29. Yanez R, Lamana ML, Garcia-Castro J, Colmenero I, Ramirez M, Bueren JA (2006) Adipose tissue-derived mesenchymal stem cells have in vivo immunosuppressive properties applicable for the control of the graft-versus-host disease. Stem Cells 24:2582–2591
30. Kalinski P (2012) Regulation of immune responses by prostaglandin E_2. J Immunol 188:21–28
31. Wiemer AJ, Hegde S, Gumperz JE, Huttenlocher A (2011) A live imaging cell motility screen identifies prostaglandin E_2 as a T cell stop signal antagonist. J Immunol 187:3663–3670
32. Carrade Holt DD, Wood JA, Granick JL, Walker NJ, Clark KC, Borjesson DL (2014) Equine mesenchymal stem cells inhibit T cell proliferation through different mechanisms depending on tissue source. Stem Cells Dev 23:1258–1265
33. Yanez R, Oviedo A, Aldea M, Bueren JA, Lamana ML (2010) Prostaglandin E2 plays a key role in the immunosuppressive properties of adipose and bone marrow tissue-derived mesenchymal stromal cells. Exp Cell Res 316:3109–3123
34. Carceller MC, Guillen MI, Ferrandiz ML, Alcaraz MJ (2015) Paracrine *in vivo* inhibitory effects of adipose tissue-derived mesenchymal stromal cells in the early stages of the acute inflammatory response. Cytotherapy 17:1230–1239
35. Gonzalez MA, Gonzalez-Rey E, Rico L, Buscher D, Delgado M (2009) Treatment of experimental arthritis by inducing immune tolerance with human adipose-derived mesenchymal stem cells. Arthritis Rheum 60:1006–1019
36. Curti A, Trabanelli S, Salvestrini V, Baccarani M, Lemoli RM (2009) The role of indoleamine 2,3-dioxygenase in the induction of immune tolerance: focus on hematology. Blood 113:2394–2401

37. Wolf AM, Wolf D, Rumpold H, Moschen AR, Kaser A, Obrist P, Fuchs D, Brandacher G, Winkler C, Geboes K, Rutgeerts P, Tilg H (2004) Overexpression of indoleamine 2,3-dioxygenase in human inflammatory bowel disease. Clin Immunol 113:47–55
38. Varga J, Yufit T, Hitraya E, Brown RR (1996) Control of extracellular matrix degradation by interferon-gamma. The tryptophan connection. Adv Exp Med Biol 398:143–148
39. Fallarino F, Grohmann U, Vacca C, Bianchi R, Orabona C, Spreca A, Fioretti MC, Puccetti P (2002) T cell apoptosis by tryptophan catabolism. Cell Death Differ 9:1069–1077
40. Mellor AL, Keskin DB, Johnson T, Chandler P, Munn DH (2002) Cells expressing indoleamine 2,3-dioxygenase inhibit T cell responses. J Immunol 168:3771–3776
41. Ripoll CB, Flaat M, Klopf-Eiermann J, Fisher-Perkins JM, Trygg CB, Scruggs BA, McCants ML, Leonard HP, Lin AF, Zhang S, Eagle ME, Alvarez X, Li YT, Li SC, Gimble JM, Bunnell BA (2011) Mesenchymal lineage stem cells have pronounced anti-inflammatory effects in the twitcher mouse model of Krabbe's disease. Stem Cells 29:67–77
42. Scruggs BA, Zhang X, Bowles AC, Gold PA, Semon JA, Fisher-Perkins JM, Zhang S, Bonvillain RW, Myers L, Li SC, Kalueff AV, Bunnell BA (2013) Multipotent stromal cells alleviate inflammation, neuropathology, and symptoms associated with globoid cell leukodystrophy in the twitcher mouse. Stem Cells 31:1523–1534
43. Kato T, Okumi M, Tanemura M, Yazawa K, Kakuta Y, Yamanaka K, Tsutahara K, Doki Y, Mori M, Takahara S, Nonomura N (2014) Adipose tissue-derived stem cells suppress acute cellular rejection by TSG-6 and CD44 interaction in rat kidney transplantation. Transplantation 98:277–284
44. Yousefi F, Ebtekar M, Soleimani M, Soudi S, Hashemi SM (2013) Comparison of *in vivo* immunomodulatory effects of intravenous and intraperitoneal administration of adipose-tissue mesenchymal stem cells in experimental autoimmune encephalomyelitis (EAE). Int Immunopharmacol 17:608–616
45. McIntosh K, Zvonic S, Garrett S, Mitchell JB, Floyd ZE, Hammill L, Kloster A, Di Halvorsen Y, Ting JP, Storms RW, Goh B, Kilroy G, Wu X, Gimble JM (2006) The immunogenicity of human adipose-derived cells: temporal changes *in vitro*. Stem Cells 24:1246–1253
46. Scruggs BA, Semon JA, Zhang X, Zhang S, Bowles AC, Pandey AC, Imhof KM, Kalueff AV, Gimble JM, Bunnell BA (2013) Age of the donor reduces the ability of human adipose-derived stem cells to alleviate symptoms in the experimental autoimmune encephalomyelitis mouse model. Stem Cells Trans Med 2:797–807
47. Schelbergen RF, van Dalen S, ter Huurne M, Roth J, Vogl T, Noel D, Jorgensen C, van den Berg WB, van de Loo FA, Blom AB, van Lent PL (2014) Treatment efficacy of adipose-derived stem cells in experimental osteoarthritis is driven by high synovial activation and reflected by S100A8/A9 serum levels. Osteoarthritis Cartilage 22:1158–1166
48. Park MJ, Kwok SK, Lee SH, Kim EK, Park SH, Cho ML (2015) Adipose tissue-derived mesenchymal stem cells induce expansion of interleukin-10-producing regulatory B cells and ameliorate autoimmunity in a murine model of systemic lupus erythematosus. Cell Transplant 24:2367–2377
49. Beissert S, Schwarz A, Schwarz T (2006) Regulatory T cells. J Invest Dermatol 126:15–24
50. Manning CN, Martel C, Sakiyama-Elbert SE, Silva MJ, Shah S, Gelberman RH, Thomopoulos S (2015) Adipose-derived mesenchymal stromal cells modulate tendon fibroblast responses to macrophage-induced inflammation *in vitro*. Stem Cell Res Ther 6:74
51. Strong AL, Bowles AC, MacCrimmon CP, Frazier TP, Lee SJ, Wu X, Katz AJ, Gawronska-Kozak B, Bunnell BA, Gimble JM (2015) Adipose stromal cells repair pressure ulcers in both young and elderly mice: potential role of adipogenesis in skin repair. Stem Cells Trans Med 4:632–642
52. Paul A, Srivastava S, Chen G, Shum-Tim D, Prakash S (2013) Functional assessment of adipose stem cells for xenotransplantation using myocardial infarction immunocompetent models: comparison with bone marrow stem cells. Cell Biochem Biophys 67:263–273
53. Gonzalez MA, Gonzalez-Rey E, Rico L, Buscher D, Delgado M (2009) Adipose-derived mesenchymal stem cells alleviate experimental colitis by inhibiting inflammatory and autoimmune responses. Gastroenterology 136:978–989

54. Semon JA, Zhang X, Pandey AC, Alandete SM, Maness C, Zhang S, Scruggs BA, Strong AL, Sharkey SA, Beuttler MM, Gimble JM, Bunnell BA (2013) Administration of murine stromal vascular fraction ameliorates chronic experimental autoimmune encephalomyelitis. Stem Cells Trans Med 2:789–796
55. Lopez-Santalla M, Mancheno-Corvo P, Menta R, Lopez-Belmonte J, DelaRosa O, Bueren JA, Dalemans W, Lombardo E, Garin MI (2015) Human adipose-derived mesenchymal stem cells modulate experimental autoimmune arthritis by modifying early adaptive T cell responses. Stem Cells 33:3493–3503
56. Zhang S, Danchuk SD, Bonvillain RW, Xu B, Scruggs BA, Strong AL, Semon JA, Gimble JM, Betancourt AM, Sullivan DE, Bunnell BA (2014) Interleukin 6 mediates the therapeutic effects of adipose-derived stromal/stem cells in lipopolysaccharide-induced acute lung injury. Stem Cells 32:1616–1628
57. Martinez-Gonzalez I, Roca O, Masclans JR, Moreno R, Salcedo MT, Baekelandt V, Cruz MJ, Rello J, Aran JM (2013) Human mesenchymal stem cells overexpressing the IL-33 antagonist soluble IL-1 receptor-like-1 attenuate endotoxin-induced acute lung injury. Am J Respir Cell Mol Biol 49:552–562
58. Jemal A, Ward E, Hao Y, Thun M (2005) Trends in the leading causes of death in the United States, 1970-2002. JAMA 294:1255–1259
59. Feizpour A, Boskabady MH, Ghorbani A (2014) Adipose-derived stromal cell therapy affects lung inflammation and tracheal responsiveness in guinea pig model of COPD. PLoS One 9:e108974
60. Jung WY, Kang JH, Kim KG, Kim HS, Jang BI, Park YH, Song IH (2015) Human adipose-derived stem cells attenuate inflammatory bowel disease in IL-10 knockout mice. Tissue Cell 47:86–93
61. Gonzalez-Rey E, Anderson P, Gonzalez MA, Rico L, Buscher D, Delgado M (2009) Human adult stem cells derived from adipose tissue protect against experimental colitis and sepsis. Gut 58:929–939
62. Das UN (2001) Is obesity an inflammatory condition? Nutrition 17:953–966
63. Chang KA, Lee JH, Suh YH (2014) Therapeutic potential of human adipose-derived stem cells in neurological disorders. J Pharmacol Sci 126:293–301
64. Kim S, Chang KA, Kim J, Park HG, Ra JC, Kim HS, Suh YH (2012) The preventive and therapeutic effects of intravenous human adipose-derived stem cells in Alzheimer's disease mice. PLoS One 7:e45757
65. Lee ST, Chu K, Jung KH, Im WS, Park JE, Lim HC, Won CH, Shin SH, Lee SK, Kim M, Roh JK (2009) Slowed progression in models of Huntington disease by adipose stem cell transplantation. Ann Neurol 66:671–681
66. Ahmed H, Salem A, Atta H, Ghazy M, Aglan H (2014) Do adipose tissue-derived mesenchymal stem cells ameliorate Parkinson's disease in rat model? Hum Exp Toxicol 33:1217–1231
67. Schwerk A, Altschuler J, Roch M, Gossen M, Winter C, Berg J, Kurtz A, Akyuz L, Steiner B (2015) Adipose-derived human mesenchymal stem cells induce long-term neurogenic and anti-inflammatory effects and improve cognitive but not motor performance in a rat model of Parkinson's disease. Regen Med 10:431–446

Similarities and Differences in Stem Cells Between Cancer, Normal, and Injured Brain

Lei Huang and Peng Huang

Introduction

Mesenchymal stem cells (MSCs) have recently garnered tremendous interest within the field of neuroscience because MSCs communicate and interact with the nervous system during brain development, injuries, and even tumor formation. Also, MSCs are easily isolated, cultured, and manipulated. Furthermore, MSCs have several unique characteristics, like immunomodulation, homing to sites of injury and secreting trophic factors. All of these make MSCs as a promising candidate to treat neurological diseases. In this chapter, we are trying to answer several questions involving the relationship between MSC, brain development, and pathology based on an increasing amount of experimental evidences. For example, is MSC-initiated neuronal transdifferentiation possible? Where are MSCs located in the brain? How and why can MSCs be successfully used to treat brain injuries? What are the relationships between MSCs and brain tumors?

L. Huang, Ph.D. (✉)
Research Imaging Institute, University of Texas Health Science Center at San Antonio, 8403 Floyd Curl Drive, San Antonio, TX 78229, USA
e-mail: huangl@uthscsa.edu

P. Huang, M.D., Ph.D.
Department of Laboratory Medicine and Pathology, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL 32224, USA
e-mail: Huang.Peng@Mayo.edu

C.J. Malemud, E. Alsberg (eds.), *Mesenchymal Stem Cells and Immunomodulation*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-3-319-46733-7_5

MSC and the Brain

MSC Transdifferentiate into Neural Cells

Transdifferentiation is a process where different stem cells are capable of crossing the germ layer boundary to form cell types of alternative layers. The transdifferentiation concept has changed the notion that multipotent stem cells are restricted in their potency to form the cell types of a derived germ layer. For example, numerous studies show that mesodermal MSCs could transdifferentiate into ectodermal cells like neurons and astrocytes in vivo and in vitro [1–9, 20, 21]. It is a promising concept as this will make MSCs a good candidate for treatment of neurodegenerative disease, aiming to replace damaged or lost cells. However, to fully prove the possibility is still challenging.

Last decade, Woodbury et al. [1] and Sanchez-Ramos et al. [2] demonstrated for the first time the concept of MSCs participating in vitro in neuronal transdifferentiation. Their studies reported that neurons can be obtained from MSCs treated with chemicals or a cocktail of trophic factors [1, 2]. However, subsequent studies challenged both methods [3, 4] and raised the question as to whether MSCs neuronal differentiation was an artifact.

Until now, four major approaches have been proposed in order to transdifferentiate MSCs into neurons or glial cells in vitro:

1. Chemical induction (chemical compounds): For example, Woodbury et al. [1] previously treated MSC with beta-mercaptoethanol, followed by dimethyl sulfoxide (DMSO) and butylated hydroxyanisole (BHA); Deng et al. [5] used dibutyryl cyclic AMP(dbcAMP) and isobutylmethylxanthine (IBMX) for 3 days to induce MSC transdifferentiation; Francesco et al. [6] modified a neuronal induction medium by adding forskolin and valproic acid, but left out BHA. After induction, some of the cells had neuronal-like morphology and expressed neural markers such as neuron-specific nuclear protein (NeuN) and neuron-specific enolase (NSE). However, some follow-up studies questioned the conclusion derived from these protocols. Studies showed the formation of neuronal morphologies did not only take place in MSCs but also in human embryonic kidney (HEK)-293 cells and pheochromocytoma cell (PC)-12 cells after chemical induction [3, 7], which was probably due to the consequences of cell shrinkage and cytoskeleton alterations. Also, some neural proteins were spontaneously expressed on MSCs under standard culture conditions [4]. More importantly, these studies lacked functional electrophysiological evidence that shows excitatory properties of typical neuron.
2. Trophic factors: Brain-derived neurotrophic factor (BDNF) [2] or basic fibroblast growth factor (bFGF) [8, 9] combined with/without retinoic acid (RA) has been shown to induce neural differentiation. After induction, cells showed neuronal morphology and expressed neural marker-NeuN, microtubule-associated protein 2 (MAP2), or glial marker–glial fibrillary acidic protein (GFAP). An electrophysiological study demonstrated K^+ current and K^+ channels on the MSCs exposed to trophic factors FGF and EGF [10]. Furthermore, Cho et al. [11] confirmed that

MSCs treated with RA had spontaneous electrical activity and postsynaptic current, which is a unique characteristic of neuronal cells. Although this trophic factor induction appears to be promising, the function of neural-like MSC-derived cells still needs to be tested before translating this method to clinical usage, especially function on synaptic transmission and neurotransmitter regulation. Also, the host microenvironment may affect the characteristic of neural-like MSC after transplantation, and maintenance of the neuronal property after trophic factor induction needs to be further evaluated.

3. Genetic manipulation: A study showed that upregulating BDNF gene induced neuronal transdifferentiation of MSC following RA induction, which also increased the survival rate of MSCs compared to trophic factor induction alone [12]. A high ratio of neurons also can be obtained by Notch intracellular domain (NICD) transfection of MSCs followed with treatment with three trophic factors, bFGF, forskolin, and ciliary neurotrophic factor [13, 14]. Na^+, K^+ current and action potentials, as well as expression of a neural marker, were found on these cells. Meanwhile, another study has confirmed that MSCs formed neurospheres and successfully differentiated into neurons, also by NICD transfection [13]. Most importantly, these cells improved functional recovery of "stroke" rats after transplantation and showed extended long neurites [13]. Upregulating expression of neurogenin1 (Ngn1) was also sufficient to induce MSCs differentiate into neurons [15], with expression of neuron-specific proteins and voltage-gated Ca^{2+} and Na^+ channels. Not only upregulating proneural gene expression could achieve neuronal transdifferentiation of MSCs, but also knocking down neuronal-related gene has shown the possibility of inducing MSCs into neurons which involved downregulating gene RE-1 silencing factor (REST) using siRNA [16]. Taken together the results of these studies indicated gene manipulation plus trophic factor induction as a better strategy for MSC transdifferentiation with long-term maintenance of neuronal characteristics and better electrophysiological function compared to trophic factor alone. However, more risk exists with viral gene transfection for clinical usage. Therefore, the long-term effects of gene manipulation of such cells need to be further evaluated in vivo and in vitro.
4. Coculture of MSCs with neural cell types: A few studies showed that coculture with several neuronal types of cells, like cerebellar granule neuron [17], or astrocyte [18], can induce MSCs to differentiate into neurons with morphologic and molecular evidence. However, the effect may be due to trophic factors secreted by cocultured cells thus making it hard to tell the role of direct cell–cell interaction in this process.

Although tremendous progress has been made in MSCs neural transdifferentiation studies in vitro, to completely fulfill the 'neuron' definitions on single neural-like MSC is still challenging, like whether synaptic transmission of neuron induced from MSC can be regulated by neurotransmitters.

Evidence from in vitro studies indicated that the neuronal microenvironment could be important factors for MSCs neural induction. Indeed, this MSCs neural transdifferentiation phenomenon has been demonstrated from in vivo studies. Pioneer studies from Azizi et al. [19] and Kopen et al. [20] transplanted human

MSCs into adult brain or lateral ventricle of neonatal mice in which the engrafted MSCs survived and migrated throughout the brain. Some MSCs expressed neural or glial marker (GFAP). Furthermore, rat MSCs were infused into embryonic rat brain to evaluate their survival and phenotypic expression [21]. After infusion, engrafted MSCs migrated along the radial glial process and expressed neural marker NeuN. Although these results were exciting, unfortunately, in vivo studies cannot totally avoid the concern that neural transdifferentiation of MSC may be caused by spontaneous cell fusion, even though it happens at an extremely low frequency, as it has been shown that MSCs can fuse with neural cell types spontaneously [22]. So in vivo studies need to better separate engrafted MSCs and host cells using various methods and demonstrate the function of neuron-like MSCs in the future.

Brain Pericytes

MSCs were initially isolated from the bone marrow of an adult organism. However, subsequent studies demonstrated MSCs can also be obtained from nonmarrow tissues, such as adult muscle [23], adipose tissue [24], even brain [25]. Using the same culture method for bone marrow-derived MSCs, MSCs were successfully isolated from mouse brain with expression of mouse MSCs marker as well as their ability to undergo mesodermal differentiation [26]. Similarly, a group of cells, isolated from human brain ventricular wall and neocortex, expressed MSCs marker and have true multilineage potential toward a mesodermal and neuroectodermal phenotype [27].

Although MSCs can be isolated retrospectively from different tissues, the native distribution of MSCs has long been a mystery. Two landmark studies [28, 29] published in 2008 partly unveiled the reason for this mystery. Thus, Crisan et al. [28] identified a subset of pericytes from multiple adult tissues, which expressed CD146, neural/glial antigen 2 (NG2), and platelet-derived growth factor (PDGF)-Rβ, and exhibited the same osteogenic, chondrogenic, adipogenic, and myogenic potential as MSCs. This indicated that the pericyte may be integral to the origin of the elusive MSCs [28]. Since then, the characteristics and function of pericyte have been reexplored and recognized, as this type of cells was first found 140 years ago [30]. Pericytes are perivascular cells, which form an incomplete layer on the abluminal surface of capillary endothelial cells. In addition, the known functions of pericytes include vascular support, participating in angiogenesis, matrix protein synthesis, vessel stabilization, and regulation of vascular tone [31]. Most importantly, recent studies showed that pericytes have been regarded as a potential reservoir of stem cells for adult tissue repair.

In the central nervous system, the pericyte is an important part of the neurovascular unit (NVU), which consists of neural cells and vascular cells. The pericyte is involved in the regulation of angiogenesis, vascular tone, and blood–brain barrier function. They are mainly distributed around cerebral capillaries and cover more than 30 % surface of capillaries [30]. Paul et al. [27] indicated that adult brain pericytes have all the features of MSCs, such as expressing MSCs immunological markers, $CD105^+$, $CD90^+$, $CD73^+$, as well as mesenchymal differentiation potential [27].

MSCs and Brain Injury

Therapeutic Roles of MSC for Brain Injury

MSC transplantation in human patients began in 1995, aimed at promoting the survival of engrafted hematopoietic stem cell. Based on the safety of MSC transplantation and multiple potentials of MSCs, subsequent studies have been performed to investigate the therapeutic role in numerous diseases and disorders, including brain injury.

Stroke and traumatic brain injury (TBI) are the leading causes of adult disability worldwide, arising from the loss of neurons and impairment of neurological function. Unfortunately, there is limited treatment for these diseases. Preclinical studies, using MSCs transplantation to treat stroke and TBI, began early this century. Li et al. [32] transplanted bone marrow-derived MSCs into "stroke" mouse brain. They found that the engrafted MSCs survived and improved functional recovery. From that, numerous follow-up studies tried to figure out the optimized source of MSCs, delivery routes, time window, and dosage for MSCs transplantation for stroke and TBI.

Delivery Routes

Three major routes have been investigated for stroke treatment: intracerebral [33–36], intracarotid [37, 38], and intravenous [39–41]. A growing number of studies showed MSCs administration decreased infarct size and improved neurological outcome in "stroke" animals through all three routes. However, it remains unclear which route is more efficient based on existing experimental evidence, as these studies lacked a direct comparison with different delivery routes of MSCs. One meta-analysis, based on preclinical studies of MSCs for ischemic stroke, showed that the effect size of intracerebral administration was larger than with the intravenous one [42]. This indicated that direct transplant of MSCs into brain may be more efficient, but it is invasive and needs complex neurosurgery. Furthermore, intracerebral [43, 44] and intravenous [43] MSCs transplantation have also been evaluated for TBI treatment. Both routes of MSCs administration improved functional recovery after TBI. However, which route is ideal remains unclear.

Cell Resources

MSCs, derived from various resources, have been investigated for stroke and TBI treatment, including bone marrow [45, 46], placenta [47], peripheral blood [48], adipose [45, 46], and umbilical cord blood [49, 50]. All of these cells have been shown beneficial impact on neural injury after transplantation. However, few studies have compared the efficacy of different MSCs. There was one study that indicated adipose-derived MSCs maybe a preferable source than bone marrow-derived MSCs for stroke therapy because of higher proliferative activity, more vascular endothelial growth factor (VEGF) secretion, and easier access [45].

Timing

Time of MSCs delivery after stroke varied from 1 h to 1 month [42]. Several studies published recently indicated 24 h after stroke is optimized for MSCs intraarterial or intravenous transplantation with improved behavior and more cell migration to infarcts [51–53]. Also 24 h is clinically reasonable, when patients tend to be stabilized. For TBI, 24 h following TBI were typically used for MSC transplantation, based on known study results [54]. However, the optimized time (i.e., window) for transplantation remains unclear, since it is hard to decide based on current available information.

Dosage

The MSCs dosage used for stroke preclinical studies ranges from 4×10^5 to 1.2×10^8 cells/kg [54]. Chen et al. [41] evaluated the relationship between cell dose and efficacy. High dosage (3×10^6) was more efficient than low dosage (1×10^6) for MSCs intravenous transplantation on the cerebral ischemic rat with better behavioral recovery. Also MSCs transplantation dose dependently restored cerebral blood flow (CBF) and blood–brain barrier (BBB) function [55]. However, various quantity and presentation of cell dosage make it harder to compare the efficacy among different preclinical studies and to directly guide clinical studies. Thus, dosage used for TBI studies varied from 6×10^6 to 3.2×10^8 cells/kg depending on the administration route [54]. However, optimized dosage for stroke and TBI therapy still needs to be explored.

Mechanisms of MSCs Cell Therapy on Brain Injury

Immunomodulation

MSCs undergo crosstalk with the innate and adaptive immune system. Their immunomodulatory functions depend on the microenvironment, through cell contact and independent mechanisms (reviewed by Blanc et al. [56]). Stroke and TBI induce a strong inflammatory response that leads to subsequent recruitment of leukocytes to the infarct zone. MSCs transplantation significantly reduced inflammation and subsequent cell death. Ohtaki et al. [57] used microarray to detect gene changes after MSCs transplantation on global cerebral ischemic mice. Over 10 % of proinflammation genes were downregulated after human bone marrow-derived MSCs transplantation and three neuroprotective genes were upregulated [57]. Similarly, engrafted MSCs reduced brain inflammation and suppressed microglia and macrophage activity after TBI [44, 58]. The resolution of the postinjury inflammatory milieu will also ameliorate brain self-repair, as evidence has showed that MSCs reduced glial scar formation after stroke or TBI [59].

More interestingly, engrafted MSC-induced immunomodulation is not limited to injured brain and it affects peripheral immune organs as well. Thus, a recent study showed a dramatic spleen distribution of MSCs after intravenous administration to rat after induction of stroke [60]. Engrafted MSCs not only had a remote anti-inflammation role on brain but also reduced TNF-α expression in spleen [60].

Trophic Factors

Although numerous studies have confirmed the neural transdifferentiation potential of MSCs in vitro, solid evidences that indicate a therapeutic role for MSCs on stroke and TBI is due to cell replacement is still lacking. On the other hand, bystander effects of MSCs transplantation play a more important role in brain recovery, especially involving secreted trophic factors by engrafts. In vitro studies showed cocultured with stroke and TBI brain extracts upregulated MSCs synthesis and expression of trophic factors, BDNF, NGF, VEGF, and HGF in vitro [61]. Meanwhile, MSCs transplantation increased trophic factors expression not only in engrafted cells but also in host brain tissue after stroke [62]. Also, the expression of host NGF and BDNF genes was significantly increased after intravenous administration of MSCs for TBI [63]. Furthermore, compared to MSC alone, BDNF gene-modified human MSCs resulted in increased BDNF expression and enhanced the therapeutic effect of cell therapy on stroke [64]. As Li and Chopp et al. [65] described, transplanted MSCs work as 'small molecular factories' by continually secreting trophic factors for brain repair. Maybe that's why cell therapy is more efficient than single molecular therapy.

Angiogenesis

Angiogenesis is an important event related to the long-term repair and restoration process of the brain after brain injury. Cultured MSCs continually secrete angiogenic cytokines including, VEGF, bFGF, and placental growth factor (PLGF) [66–69]. Thus, MSC transplantation promoted VEGF secretion, VEGF receptor 2 (VEGFR2) expression, and angiogenesis in the ischemic boundary zone (IBZ) after stroke [70, 71]. A recent study also indicated that only exosomes derived from cultured MSCs were able to enhance angiogenesis in animals following stroke [72]. Furthermore, effect of brain angiogenesis after stroke was greater after transplantation of PLGF gene-modified MSCs, compared to nonmodified MSCs [73].

In addition to secreting angiogenic factors, MSCs also have the potential to differentiate into an endothelial lineage [74]. This unique property could be beneficial for vascular repair after brain injury. Indeed, Liao et al. [50] observed a subset of engrafted cells that differentiated into endothelial cells after intracerebral transplanted human umbilical-derived MSCs (UC-MSCs) in a rat model of stroke. Also, the UC-MSCs treatment increased vascular density and VEGF expression in ipsilateral hemisphere [50].

MSC and Cancer

Cancer Stem Cells (CSCs)

A tumor or cancer can be viewed as an aberrant organ initiated by a tumorigenic cancer cell that acquired the capacity for indefinite proliferation through accumulated mutation [75]. Two hypothetical models, stochastic and hierarchal, have been proposed to explain tumor initiation and development [76]. Cancer-stem-cell theory derived from the hierarchal model asserts that only a rare subset of cells within the tumor have the ability to generate new tumors [75]. In 1997, the confirmatory experimental evidence for this theory was demonstrated by Bonnet and Dick [77]. Since then, numerous studies have verified the existence of cancer stem cells in various kinds of cancer, for example, breast cancer [78], brain tumor [79, 80]. Compared to normal stem cells, cancer stem cells have similar properties of self-renewal and differentiation, but cancer stem cells usually have genomic or karyotypic mutation and aberrant differentiation [80]. The concept of cancer stem cell has propelled researchers in a direction to better understand the oncogenesis and to rethink the strategy for cancer therapy.

MSC and Brain Tumor

Glioblastoma multiforme is an aggressive and invasive neoplasm characterized by extensive neovascularization [76]. Several groups demonstrated tropism of MSC to gliomas by implanting MSCs into gliomas of animals and tracking the migration of MSCs [81, 82]. This tumor-specific migratory pattern makes MSCs a promising cellular vehicle for delivery of therapeutic agents, although whether tumor cells recruit endogenous MSCs remain to be clarified. Meanwhile, glioblastoma stem cells (GSC) are able to transdifferentiate into pericytes or MSC-like cells [83], contributing to the maintenance of microvasculature itself [84, 85]. In addition, the selective elimination of GSC-derived pericytes disrupted the neovasculature and potently inhibited tumor growth [84].

The effect of native MSCs on tumor growth is still controversial. On the one hand, MSCs have been shown to suppress tumor growth of glioma [81, 86], through suppressed tumor angiogenesis [86]. On the other hand, others have reported MSC implantation can promote tumor growth [87], partly by supporting tumor vasculature [82, 88], and by reducing tumor cell apoptosis [87].

Even though the relationship between brain tumor/CSCs and MSCs is controversial, several studies indicated MSCs could be regarded as vector to deliver therapeutic molecules [89, 90], based on the homing property of MSCs to tumor. Sasportas et al. [89] gene-modified MSC to secrete cytokine tumor necrosis factor apoptosis ligand (TRAIL). In vitro and in vivo studies showed TRAIL-MSCs successfully inhibited growth of glioma through inducing tumor cell apoptosis [89]. Similar

results have been verified by another group [90]. Moreover, the results of this study demonstrated that antitumor effect of TRAIL-MSC was better than TRAIL alone using adenovirus-mediated delivery [90]. Other antitumor molecules, such as HSV-tk [91], IL-17 [92], and IFN-α [93], have also been investigated and have shown reduced tumor development. All of these studies indicated using MSCs as a vehicle to target tumor is a promising strategy for future tumor therapy.

References

1. Woodbury D, Schwarz EJ, Prockop DJ, Black IB (2000) Adult rat and human bone marrow stromal cells differentiate into neurons. J Neurosci Res 61:364–370
2. Sanchez-Ramos J, Song S, Cardozo-Pelaez F, Hazzi C, Stedeford T, Willing A, Freeman TB, Saporta S, Janssen W, Patel N, Cooper DR, Sanberg PR (2000) Adult bone marrow stromal cells differentiate into neural cells *in vitro*. Exp Neurol 164:247–256
3. Lu P, Blesch A, Tuszynski MH (2004) Induction of bone marrow stromal cells to neurons: differentiation, transdifferentiation, or artifact? J Neurosci Res 77:174–191
4. Deng J, Petersen BE, Steindler DA, Jorgensen ML, Laywell ED (2006) Mesenchymal stem cells spontaneously express neural proteins in culture and are neurogenic after transplantation. Stem Cells 24:1054–1064
5. Deng W, Obrocka M, Fischer I, Prockop DJ (2001) In vitro differentiation of human marrow stromal cells into early progenitors of neural cells by conditions that increase intracellular cyclic AMP. Biochem Biophys Res Commun 282:148–152
6. Jori FP, Napolitano MA, Melone MA, Cipollaro M, Cascino A, Altucci L, Peluso G, Giordano A, Galderisi U (2005) Molecular pathways involved in neural *in vitro* differentiation of marrow stromal stem cells. J Cell Biochem 94:645–655
7. Neuhuber B, Gallo G, Howard L, Kostura L, Mackay A, Fischer I (2004) Reevaluation of in vitro differentiation protocols for bone marrow stromal cells: disruption of actin cytoskeleton induces rapid morphological changes and mimics neuronal phenotype. J Neurosci Res 77:192–204
8. Trzaska KA, Kuzhikandathil EV, Rameshwar P (2007) Specification of a dopaminergic phenotype from adult human mesenchymal stem cells. Stem Cells 25:2797–2808
9. Barzilay R, Kan I, Ben-Zur T, Bulvik S, Melamed E, Offen D (2008) Induction of human mesenchymal stem cells into dopamine-producing cells with different differentiation protocols. Stem Cells Dev 17:547–554
10. Mareschi K, Novara M, Rustichelli D, Ferrero I, Guido D, Carbone E, Medico E, Madon E, Vercelli A, Fagioli F (2006) Neural differentiation of human mesenchymal stem cells: evidence for expression of neural markers and eag K+ channel types. Exp Hematol 34:1563–1572
11. Cho KJ, Trzaska KA, Greco SJ, McArdle J, Wang FS, Ye JH, Rameshwar P (2005) Neurons derived from human mesenchymal stem cells show synaptic transmission and can be induced to produce the neurotransmitter substance P by interleukin-1 alpha. Stem Cells 23:383–391
12. Zhao LX, Zhang J, Cao F, Meng L, Wang DM, Li YH, Nan X, Jiao WC, Zheng M, Xu XH, Pei XT (2004) Modification of the brain-derived neurotrophic factor gene: a portal to transform mesenchymal stem cells into advantageous engineering cells for neuroregeneration and neuroprotection. Exp Neurol 190:396–406
13. Hayase M, Kitada M, Wakao S, Itokazu Y, Nozaki K, Hashimoto N, Takagi Y, Dezawa M (2009) Committed neural progenitor cells derived from genetically modified bone marrow stromal cells ameliorate deficits in a rat model of stroke. J Cereb Blood Flow Metab 29:1409–1420
14. Dezawa M, Kanno H, Hoshino M, Cho H, Matsumoto N, Itokazu Y, Tajima N, Yamada H, Sawada H, Ishikawa H, Mimura T, Kitada M, Suzuki Y, Ide C (2004) Specific induction of neuronal cells from bone marrow stromal cells and application for autologous transplantation. J Clin Invest 113:1701–1710

15. Kim SS, Yoo SW, Park TS, Ahn SC, Jeong HS, Kim JW, Chang DY, Cho KG, Kim SU, Huh Y, Lee JE, Lee SY, Lee YD, Suh-Kim H (2008) Neural induction with neurogenin1 increases the therapeutic effects of mesenchymal stem cells in the ischemic brain. Stem Cells 26:2217–2228
16. Trzaska KA, Reddy BY, Munoz JL, Li KY, Ye JH, Rameshwar P (2008) Loss of RE-1 silencing factor in mesenchymal stem cell-derived dopamine progenitors induces functional maturity. Mol Cell Neurosci 39:285–290
17. Wislet-Gendebien S, Hans G, Leprince P, Rigo JM, Moonen G, Rogister B (2005) Plasticity of cultured mesenchymal stem cells: switch from nestin-positive to excitable neuron-like phenotype. Stem Cells 23:392–402
18. Bossolasco P, Cova L, Calzarossa C, Rimoldi SG, Borsotti C, Deliliers GL, Silani V, Soligo D, Polli E (2005) Neuro-glial differentiation of human bone marrow stem cells in vitro. Exp Neurol 193:312–325
19. Azizi SA, Stokes D, Augelli BJ, DiGirolamo C, Prockop DJ (1998) Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats--similarities to astrocyte grafts. Proc Natl Acad Sci U S A 95:3908–3913
20. Kopen GC, Prockop DJ, Phinney DG (1999) Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc Natl Acad Sci U S A 96:10711–10716
21. Munoz-Elias G, Marcus AJ, Coyne TM, Woodbury D, Black IB (2004) Adult bone marrow stromal cells in the embryonic brain: engraftment, migration, differentiation, and long-term survival. J Neurosci 24:4585–4595
22. Weimann JM, Charlton CA, Brazelton TR, Hackman RC, Blau HM (2003) Contribution of transplanted bone marrow cells to Purkinje neurons in human adult brains. Proc Natl Acad Sci U S A 100:2088–2093
23. Jiang Y, Vaessen B, Lenvik T, Blackstad M, Reyes M, Verfaillie CM (2002) Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. Exp Hematol 30:896–904
24. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH (2002) Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 13:4279–4295
25. da Silva Meirelles L, Chagastelles PC, Nardi NB (2006) Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci 119:2204–2213
26. Kang SG, Shinojima N, Hossain A, Gumin J, Yong RL, Colman H, Marini F, Andreeff M, Lang FF (2010) Isolation and perivascular localization of mesenchymal stem cells from mouse brain. Neurosurgery 67:711–720
27. Paul G, Ozen I, Christophersen NS, Reinbothe T, Bengzon J, Visse E, Jansson K, Dannaeus K, Henriques-Oliveira C, Roybon L, Anisimov SV, Renstrom E, Svensson M, Haegerstrand A, Brundin P (2012) The adult human brain harbors multipotent perivascular mesenchymal stem cells. PLoS One 7:e35577
28. Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, Andriolo G, Sun B, Zheng B, Zhang L, Norotte C, Teng PN, Traas J, Schugar R, Deasy BM, Badylak S, Buhring HJ, Giacobino JP, Lazzari L, Huard J, Peault B (2008) A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 3:301–313
29. Covas DT, Panepucci RA, Fontes AM, Silva WA Jr, Orellana MD, Freitas MC, Neder L, Santos AR, Peres LC, Jamur MC, Zago MA (2008) Multipotent mesenchymal stromal cells obtained from diverse human tissues share functional properties and gene-expression profile with CD146+ perivascular cells and fibroblasts. Exp Hematol 36:642–654
30. Lange S, Trost A, Tempfer H, Bauer HC, Bauer H, Rohde E, Reitsamer HA, Franklin RJ, Aigner L, Rivera FJ (2013) Brain pericyte plasticity as a potential drug target in CNS repair. Drug Discov Today 18:456–463
31. Winkler EA, Bell RD, Zlokovic BV (2011) Central nervous system pericytes in health and disease. Nat Neurosci 14:1398–1405

32. Li Y, Chopp M, Chen J, Wang L, Gautam SC, Xu YX, Zhang Z (2000) Intrastriatal transplantation of bone marrow nonhematopoietic cells improves functional recovery after stroke in adult mice. J Cereb Blood Flow Metab 20:1311–1319
33. Shichinohe H, Kuroda S, Yano S, Ohnishi T, Tamagami H, Hida K, Iwasaki Y (2006) Improved expression of gamma-aminobutyric acid receptor in mice with cerebral infarct and transplanted bone marrow stromal cells: an autoradiographic and histologic analysis. J Nucl Med 47:486–491
34. Ikeda N, Nonoguchi N, Zhao MZ, Watanabe T, Kajimoto Y, Furutama D, Kimura F, Dezawa M, Coffin RS, Otsuki Y, Kuroiwa T, Miyatake S (2005) Bone marrow stromal cells that enhanced fibroblast growth factor-2 secretion by herpes simplex virus vector improve neurological outcome after transient focal cerebral ischemia in rats. Stroke 36:2725–2730
35. Chen J, Li Y, Wang L, Lu M, Zhang X, Chopp M (2001) Therapeutic benefit of intracerebral transplantation of bone marrow stromal cells after cerebral ischemia in rats. J Neurol Sci 189:49–57
36. Zhao MZ, Nonoguchi N, Ikeda N, Watanabe T, Furutama D, Miyazawa D, Funakoshi H, Kajimoto Y, Nakamura T, Dezawa M, Shibata MA, Otsuki Y, Coffin RS, Liu WD, Kuroiwa T, Miyatake S (2006) Novel therapeutic strategy for stroke in rats by bone marrow stromal cells and ex vivo HGF gene transfer with HSV-1 vector. J Cereb Blood Flow Metab 26:1176–1188
37. Shen LH, Li Y, Chen J, Zhang J, Vanguri P, Borneman J, Chopp M (2006) Intracarotid transplantation of bone marrow stromal cells increases axon-myelin remodeling after stroke. Neuroscience 137:393–399
38. Li Y, Chen J, Wang L, Lu M, Chopp M (2001) Treatment of stroke in rat with intracarotid administration of marrow stromal cells. Neurology 56:1666–1672
39. Cui X, Chen J, Zacharek A, Li Y, Roberts C, Kapke A, Savant-Bhonsale S, Chopp M (2007) Nitric oxide donor upregulation of stromal cell-derived factor-1/chemokine (CXC motif) receptor 4 enhances bone marrow stromal cell migration into ischemic brain after stroke. Stem Cells 25:2777–2785
40. Chen J, Li Y, Katakowski M, Chen X, Wang L, Lu D, Lu M, Gautam SC, Chopp M (2003) Intravenous bone marrow stromal cell therapy reduces apoptosis and promotes endogenous cell proliferation after stroke in female rat. J Neurosci Res 73:778–786
41. Chen J, Li Y, Wang L, Zhang Z, Lu D, Lu M, Chopp M (2001) Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. Stroke 32:1005–1011
42. Vu Q, Xie K, Eckert M, Zhao W, Cramer SC (2014) Meta-analysis of preclinical studies of mesenchymal stromal cells for ischemic stroke. Neurology 82:1277–1286
43. Mahmood A, Lu D, Chopp M (2004) Marrow stromal cell transplantation after traumatic brain injury promotes cellular proliferation within the brain. Neurosurgery 55:1185–1193
44. Zanier ER, Montinaro M, Vigano M, Villa P, Fumagalli S, Pischiutta F, Longhi L, Leoni ML, Rebulla P, Stocchetti N, Lazzari L, De Simoni MG (2011) Human umbilical cord blood mesenchymal stem cells protect mice brain after trauma. Crit Care Med 39:2501–2510
45. Ikegame Y, Yamashita K, Hayashi S, Mizuno H, Tawada M, You F, Yamada K, Tanaka Y, Egashira Y, Nakashima S, Yoshimura S, Iwama T (2011) Comparison of mesenchymal stem cells from adipose tissue and bone marrow for ischemic stroke therapy. Cytotherapy 13:675–685
46. Gutierrez-Fernandez M, Rodriguez-Frutos B, Ramos-Cejudo J, Teresa Vallejo-Cremades M, Fuentes B, Cerdan S, Diez-Tejedor E (2013) Effects of intravenous administration of allogenic bone marrow- and adipose tissue-derived mesenchymal stem cells on functional recovery and brain repair markers in experimental ischemic stroke. Stem Cell Res Ther 4:11
47. Kranz A, Wagner DC, Kamprad M, Scholz M, Schmidt UR, Nitzsche F, Aberman Z, Emmrich F, Riegelsberger UM, Boltze J (2010) Transplantation of placenta-derived mesenchymal stromal cells upon experimental stroke in rats. Brain Res 1315:128–136
48. Ukai R, Honmou O, Harada K, Houkin K, Hamada H, Kocsis JD (2007) Mesenchymal stem cells derived from peripheral blood protects against ischemia. J Neurotrauma 24:508–520

49. Koh SH, Kim KS, Choi MR, Jung KH, Park KS, Chai YG, Roh W, Hwang SJ, Ko HJ, Huh YM, Kim HT, Kim SH (2008) Implantation of human umbilical cord-derived mesenchymal stem cells as a neuroprotective therapy for ischemic stroke in rats. Brain Res 1229:233–248
50. Liao W, Xie J, Zhong J, Liu Y, Du L, Zhou B, Xu J, Liu P, Yang S, Wang J, Han Z, Han ZC (2009) Therapeutic effect of human umbilical cord multipotent mesenchymal stromal cells in a rat model of stroke. Transplantation 87:350–359
51. Lee SH, Jin KS, Bang OY, Kim BJ, Park SJ, Lee NH, Yoo KH, Koo HH, Sung KW (2015) Differential migration of mesenchymal stem cells to ischemic regions after middle cerebral artery occlusion in rats. PLoS One 10:e0134920
52. Yavagal DR, Lin B, Raval AP, Garza PS, Dong C, Zhao W, Rangel EB, McNiece I, Rundek T, Sacco RL, Perez-Pinzon M, Hare JM (2014) Efficacy and dose-dependent safety of intra-arterial delivery of mesenchymal stem cells in a rodent stroke model. PLoS One 9:e93735
53. Toyoshima A, Yasuhara T, Kameda M, Morimoto J, Takeuchi H, Wang F, Sasaki T, Sasada S, Shinko A, Wakamori T, Okazaki M, Kondo A, Agari T, Borlongan CV, Date I (2015) Intra-arterial transplantation of allogeneic mesenchymal stem cells mounts neuroprotective effects in a transient ischemic stroke model in rats: analyses of therapeutic time window and its mechanisms. PLoS One 10:e0127302
54. Monsel A, Zhu YG, Gennai S, Hao Q, Liu J, Lee JW (2014) Cell-based therapy for acute organ injury: preclinical evidence and ongoing clinical trials using mesenchymal stem cells. Anesthesiology 121:1099–1121
55. Borlongan CV, Lind JG, Dillon-Carter O, Yu G, Hadman M, Cheng C, Carroll J, Hess DC (2004) Bone marrow grafts restore cerebral blood flow and blood brain barrier in stroke rats. Brain Res 1010:108–116
56. Le Blanc K, Mougiakakos D (2012) Multipotent mesenchymal stromal cells and the innate immune system. Nat Rev Immunol 12:383–396
57. Ohtaki H, Ylostalo JH, Foraker JE, Robinson AP, Reger RL, Shioda S, Prockop DJ (2008) Stem/progenitor cells from bone marrow decrease neuronal death in global ischemia by modulation of inflammatory/immune responses. Proc Natl Acad Sci U S A 105:14638–14643
58. Zhang R, Liu Y, Yan K, Chen L, Chen XR, Li P, Chen FF, Jiang XD (2013) Anti-inflammatory and immunomodulatory mechanisms of mesenchymal stem cell transplantation in experimental traumatic brain injury. J Neuroinflammation 10:106
59. Li Y, Chen J, Zhang CL, Wang L, Lu D, Katakowski M, Gao Q, Shen LH, Zhang J, Lu M, Chopp M (2005) Gliosis and brain remodeling after treatment of stroke in rats with marrow stromal cells. Glia 49:407–417
60. Acosta SA, Tajiri N, Hoover J, Kaneko Y, Borlongan CV (2015) Intravenous bone marrow stem cell grafts preferentially migrate to spleen and abrogate chronic inflammation in stroke. Stroke 46:2616–2627
61. Chen X, Katakowski M, Li Y, Lu D, Wang L, Zhang L, Chen J, Xu Y, Gautam S, Mahmood A, Chopp M (2002) Human bone marrow stromal cell cultures conditioned by traumatic brain tissue extracts: growth factor production. J Neurosci Res 69:687–691
62. Wakabayashi K, Nagai A, Sheikh AM, Shiota Y, Narantuya D, Watanabe T, Masuda J, Kobayashi S, Kim SU, Yamaguchi S (2010) Transplantation of human mesenchymal stem cells promotes functional improvement and increased expression of neurotrophic factors in a rat focal cerebral ischemia model. J Neurosci Res 88:1017–1025
63. Mahmood A, Lu D, Chopp M (2004) Intravenous administration of marrow stromal cells (MSCs) increases the expression of growth factors in rat brain after traumatic brain injury. J Neurotrauma 21:33–39
64. Kurozumi K, Nakamura K, Tamiya T, Kawano Y, Kobune M, Hirai S, Uchida H, Sasaki K, Ito Y, Kato K, Honmou O, Houkin K, Date I, Hamada H (2004) BDNF gene-modified mesenchymal stem cells promote functional recovery and reduce infarct size in the rat middle cerebral artery occlusion model. Mol Ther 9:189–197
65. Chopp M, Li Y (2002) Treatment of neural injury with marrow stromal cells. Lancet Neurol 1:92–100

66. Schinkothe T, Bloch W, Schmidt A (2008) *In vitro* secreting profile of human mesenchymal stem cells. Stem Cells Dev 17:199–206
67. Kagiwada H, Yashiki T, Ohshima A, Tadokoro M, Nagaya N, Ohgushi H (2008) Human mesenchymal stem cells as a stable source of VEGF-producing cells. J Tissue Eng Regen Med 2:184–189
68. Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchs S, Epstein SE (2004) Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote *in vitro* and *in vivo* arteriogenesis through paracrine mechanisms. Circ Res 94:678–685
69. Eckert MA, Vu Q, Xie K, Yu J, Liao W, Cramer SC, Zhao W (2013) Evidence for high translational potential of mesenchymal stromal cell therapy to improve recovery from ischemic stroke. J Cereb Blood Flow Metab 33:1322–1334
70. Chen J, Zhang ZG, Li Y, Wang L, Xu YX, Gautam SC, Lu M, Zhu Z, Chopp M (2003) Intravenous administration of human bone marrow stromal cells induces angiogenesis in the ischemic boundary zone after stroke in rats. Circ Res 92:692–699
71. Pavlichenko N, Sokolova I, Vijde S, Shvedova E, Alexandrov G, Krouglyakov P, Fedotova O, Gilerovich EG, Polyntsev DG, Otellin VA (2008) Mesenchymal stem cells transplantation could be beneficial for treatment of experimental ischemic stroke in rats. Brain Res 1233:203–213
72. Xin H, Li Y, Cui Y, Yang JJ, Zhang ZG, Chopp M (2013) Systemic administration of exosomes released from mesenchymal stromal cells promote functional recovery and neurovascular plasticity after stroke in rats. J Cereb Blood Flow Metab 33:1711–1715
73. Liu H, Honmou O, Harada K, Nakamura K, Houkin K, Hamada H, Kocsis JD (2006) Neuroprotection by PlGF gene-modified human mesenchymal stem cells after cerebral ischaemia. Brain 129:2734–2745
74. Oswald J, Boxberger S, Jorgensen B, Feldmann S, Ehninger G, Bornhauser M, Werner C (2004) Mesenchymal stem cells can be differentiated into endothelial cells in vitro. Stem Cells 22:377–384
75. Reya T, Morrison SJ, Clarke MF, Weissman IL (2001) Stem cells, cancer, and cancer stem cells. Nature 414:105–111
76. Bexell D, Scheding S, Bengzon J (2010) Toward brain tumor gene therapy using multipotent mesenchymal stromal cell vectors. Mol Ther 18:1067–1075
77. Bonnet D, Dick JE (1997) Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 3:730–737
78. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF (2003) Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 100:3983–3988
79. Ignatova TN, Kukekov VG, Laywell ED, Suslov ON, Vrionis FD, Steindler DA (2002) Human cortical glial tumors contain neural stem-like cells expressing astroglial and neuronal markers in vitro. Glia 39:193–206
80. Vescovi AL, Galli R, Reynolds BA (2006) Brain tumour stem cells. Nat Rev Cancer 6:425–436
81. Nakamura K, Ito Y, Kawano Y, Kurozumi K, Kobune M, Tsuda H, Bizen A, Honmou O, Niitsu Y, Hamada H (2004) Antitumor effect of genetically engineered mesenchymal stem cells in a rat glioma model. Gene Ther 11:1155–1164
82. Bexell D, Gunnarsson S, Tormin A, Darabi A, Gisselsson D, Roybon L, Scheding S, Bengzon J (2009) Bone marrow multipotent mesenchymal stroma cells act as pericyte-like migratory vehicles in experimental gliomas. Mol Ther 17:183–190
83. Tso CL, Shintaku P, Chen J, Liu Q, Liu J, Chen Z, Yoshimoto K, Mischel PS, Cloughesy TF, Liau LM, Nelson SF (2006) Primary glioblastomas express mesenchymal stem-like properties. Mol Cancer Res 4:607–619
84. Cheng L, Huang Z, Zhou W, Wu Q, Donnola S, Liu JK, Fang X, Sloan AE, Mao Y, Lathia JD, Min W, McLendon RE, Rich JN, Bao S (2013) Glioblastoma stem cells generate vascular pericytes to support vessel function and tumor growth. Cell 153:139–152
85. Gilbertson RJ, Rich JN (2007) Making a tumour's bed: glioblastoma stem cells and the vascular niche. Nat Rev Cancer 7:733–736

86. Ho IA, Toh HC, Ng WH, Teo YL, Guo CM, Hui KM, Lam PY (2013) Human bone marrow-derived mesenchymal stem cells suppress human glioma growth through inhibition of angiogenesis. Stem Cells 31:146–155
87. Yu JM, Jun ES, Bae YC, Jung JS (2008) Mesenchymal stem cells derived from human adipose tissues favor tumor cell growth in vivo. Stem Cells Dev 17:463–473
88. Suzuki K, Sun R, Origuchi M, Kanehira M, Takahata T, Itoh J, Umezawa A, Kijima H, Fukuda S, Saijo Y (2011) Mesenchymal stromal cells promote tumor growth through the enhancement of neovascularization. Mol Med 17:579–587
89. Sasportas LS, Kasmieh R, Wakimoto H, Hingtgen S, van de Water JA, Mohapatra G, Figueiredo JL, Martuza RL, Weissleder R, Shah K (2009) Assessment of therapeutic efficacy and fate of engineered human mesenchymal stem cells for cancer therapy. Proc Natl Acad Sci U S A 106:4822–4827
90. Kim SM, Lim JY, Park SI, Jeong CH, Oh JH, Jeong M, Oh W, Park SH, Sung YC, Jeun SS (2008) Gene therapy using TRAIL-secreting human umbilical cord blood-derived mesenchymal stem cells against intracranial glioma. Cancer Res 68:9614–9623
91. Miletic H, Fischer Y, Litwak S, Giroglou T, Waerzeggers Y, Winkeler A, Li H, Himmelreich U, Lange C, Stenzel W, Deckert M, Neumann H, Jacobs AH, von Laer D (2007) Bystander killing of malignant glioma by bone marrow-derived tumor-infiltrating progenitor cells expressing a suicide gene. Mol Ther 15:1373–1381
92. Gunnarsson S, Bexell D, Svensson A, Siesjo P, Darabi A, Bengzon J (2010) Intratumoral IL-7 delivery by mesenchymal stromal cells potentiates IFNgamma-transduced tumor cell immunotherapy of experimental glioma. J Neuroimmunol 218:140–144
93. Sato H, Kuwashima N, Sakaida T, Hatano M, Dusak JE, Fellows-Mayle WK, Papworth GD, Watkins SC, Gambotto A, Pollack IF, Okada H (2005) Epidermal growth factor receptor-transfected bone marrow stromal cells exhibit enhanced migratory response and therapeutic potential against murine brain tumors. Cancer Gene Ther 12:757–768

Controversies in the Use of Mesenchymal Stem Cells for Treating Autoimmune Diseases

Zachary Wolff and Charles J. Malemud

Introduction

Mesenchymal stem cells (MSCs) are currently being employed in clinical trials to test their ultimate use in the treatment of various autoimmune and degenerative diseases. MSCs can be obtained from several sources, most notably, adipose tissue, skeletal muscle, and bone marrow. Owing to the ease in generating and expanding these cells in cell cultures as well as for their known capacity to differentiate into specialized mesenchymal tissue types makes MSCs ideal not only for use in certain forms of tissue repair but also for their immunomodulatory activity. However, even though MSCs have been shown to possess the capacity to induce peripheral tolerance, their immunomodulatory potential as a treatment modality for autoimmune diseases continues to remain quite controversial [1, 2].

Here, we have systematically reviewed the constitutive and regulated expression of molecules produced by MSCs. These encompass the major histocompatibility complex (MHC) class I antigen processing machinery (APM), costimulatory B-7 molecules, and histocompatibility locus antigen (HLA)-G. Furthermore, we have focused attention on the secretion of various factors produced by MSCs such as cytokines, their

Z. Wolff, M.D.
Division of Rheumatic Diseases, Department of Medicine, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA
e-mail: z.wolff73@gmail.com

C.J. Malemud, Ph.D. (✉)
Division of Rheumatic Diseases, Department of Medicine, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA

Division of Rheumatic Diseases, Department of Medicine, University Hospitals Cleveland Medical Center, Foley Medical Building, 2061 Cornell Road, Cleveland, OH 44106-5076, USA
e-mail: cjm4@cwru.edu

C.J. Malemud, E. Alsberg (eds.), *Mesenchymal Stem Cells and Immunomodulation*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-3-319-46733-7_6

functional role in mounting and controlling immune responses directly mediated by different immune cell subpopulations as well as the medical significance of MSCs including those technical obstacles that might limit their clinical application [3, 4].

Multipotential Mesenchymal Stromal Cells: Can They Be Used to Treat Musculoskeletal Diseases?

There is now general agreement that MSCs have a significant application for repairing defects associated with musculoskeletal diseases, such as osteoarthritis [5], osteoporosis [6], long bone fractures [7], and avascular necrosis [8]. For example, in the present context, the interaction between MSCs and immune cells is now considered to be a vital component for inducing bone fracture repair. In this scenario osteoprogenitor MSCs become bone. However, MSCs also induce immune cells to participate in this differentiation process to promote bone repair through the induction of cytokine production [9]. In addition, the activity of the stromal cell-derived factor-1 (SDF-1)-CXCR4 axis was shown to be critical in the recruitment of MSCs, and endothelial progenitor cells, to sites where bone healing occurs [7].

With respect to cartilage defects, although MSC-based cartilage bioengineering retains a long and storied historical belief system the restorative treatment of cartilage defects with MSCs must be weighed against the documented evidence that MSCs while having the ability to migrate to sites of damaged tissue may also differentiate into, as well as potentially interact, with aberrant primary tumors and metastatic cancers [2–4] which could exacerbate the medical condition for which they are being employed. In fact, as pointed out by Bouffi et al. [2], MSCs have actually been associated with tumor growth due to their capacity to migrate to stroma produced by tumor cells. Thus, in the stroma MSCs have been shown to express chemokines involved in carcinoma cell metastasis.

In addition, in the setting of chronic inflammation commonly associated with osteoarticular disease, the capacity for MSCs to differentiate at all may be seriously compromised by the conditions underlying the pathophysiology itself. Therefore, in order to protect against this occurrence the immunomodulatory potential of MSCs may require that antecedent preconditioning occur before using MSCs for therapeutic applications [4]. For example, the activation of proinflammatory cytokines may be required as a precursor for inducing the immunosuppressive activities of MSCs.

Another factor that should be considered here is the compelling evidence from cell cultures studies that human bone marrow-derived MSCs appear to fully differentiate [10–12] in the cell culture expansion phase prior to employing them for future joint reconstruction. Thus, in the context of employing MSCs for articular cartilage repair it will likely be required that we exploit the addition of endogenous biologically active factors which are predicted to dampen or completely inhibit specific gene expressional events that stimulate MSCs to become fully differentiated as would be expected to occur, for example, in growth plate development and maturation [11].

MSCs, Scaffolds, and Growth Factors

It is evident that stromal cells play a major role in the regulation of the immune system and therefore in the subsequent regulation of acute and/or chronic inflammation. In keeping with this contention, another component of evaluating MSCs as immunomodulatory cells must include, an analysis of the role that artificial scaffolds could play in regulating their immune modulating function. In that regard, improvements in the functioning of MSCs were reported when the cells were impregnated into an artificial scaffolding composed of sponges containing [poly (lactic-co-glycolic acid)] [13]. Thus, newly designed scaffolding strategies may also have to include placing MSCs in three-dimensional structural polymer microspheres [14] in the presence or absence of cell survival-inducing factors such as transforming growth factor-β_1 (TGF-β_1), TGF-β3, bone morphogenetic proteins-2 and -7, and bioactive transcription factors, such as the cartilage-specific transcription factor, SOX-9 [13].

Immune Modulation in Animal Models of Arthritis and Osteoarticular Diseases

Experimental studies which employed MSCs in the collagen-induced arthritis model (CIA) in mice have also been revealing with respect to how MSCs might modulate proinflammatory cytokines. Thus, in a focused review of how MSCs influence the course of arthritis in CIA [15], serum levels of tumor necrosis factor-α (TNF-α) and interferon-γ (INF-γ) were modulated by MSCs which were also associated with decreased T_H1-mediated inflammation [15]. The immunomodulatory properties of MSCs have also been reviewed by Klinker and Wei [15] who summarized the disease ameliorating effects in other animal models of autoimmune diseases.

In addition, MSCs appeared to be capable of preventing articular cartilage extracellular matrix protein degradation as a result of stimulating bioactive factors or by reducing matrix metalloproteinase gene expression. The differentiation of MSCs to chondrocytes was also relevant for promoting the repair of articular cartilage defects by reducing the formation of fibrocartilage in damaged articular cartilage. Thus, extending these findings in CIA to human osteoarticular diseases, such as rheumatoid arthritis [16] is likely to be an achievable objective going forward. However, it has been recommended that whatever animal models are chosen to mimic human RA, they should include the three stages of inflammation, the "priming" phase, the joint-specific phase, and the chronic phase of inflammation [16].

Presently, the immunosuppressive activity of MSCs has been principally studied using experimentally induced animal models associated with RA [16]. In that regard, evidence was presented that indicated that MSCs produced high levels of TNF-α when cultured in the presence of Type II collagen [17]. This resulted in reduced T-lymphocyte responses as well as the modulation of other proinflammatory cytokines. However, by far the most impressive result from employing adipose-derived

MSCs in the CIA model was the finding that these MSCs induced the production of antigen-specific $CD4^+CD25^+FoxP3^+$ T-regulatory (T_{reg}) cells with their capacity to suppress self-reactive T effector responses.

By contrast, data has also been published indicating a somewhat paradoxical anti-inflammatory activity of MSC therapy while suggesting that production of TNF-α under these conditions is balanced by the function of other MSC factors. Thus, Tobin et al. [18] reported using an animal model of acute graft-versus-host (GVH) disease that MSCs may actually lose their capacity to "educate" other cells to produce immunosuppressive factors while at the same time also preserving the proinflammatory state which was dependent on the timing of MSC administration. In that regard, protection induced by MSCs in the GVH model was dependent on the timing of MSC administration. Importantly, MSC therapy reduced liver and gut pathology while also increasing animal survival [18]. The results from these studies in this experimentally induced GVH disease model indicated that MSC-treated animals could be provoked to down-regulate the activation of several pathogenic immune mechanisms which contribute to pathology.

Even though the pathogenesis of osteoarthritis (OA) is unlikely to be initiated by immune cell reactivity or by the "classical" components of inflammation it is obvious from the accumulation of persuasive evidence that a form of low-grade "non-classical" form of inflammation constitutes a factor in OA disease progression [19], so much so, that modulating the inflammatory response with MSCs could also reduce the expression of interleukin-1 (IL-1), IL-6, and TNF-α which would predict that the progression of arthritis would be ameliorated. However, it is imperative that we gain a further understanding of the chondrogenic potential of MSCs and as well as further defining the potential adverse side effects of MSCs [20] before they could be routinely employed in a cell-based therapy of osteoarticular diseases.

Are There Clinical Applications for Employing MSCs in Immune-Mediated Disease Processes?

Several experimental studies as well as human Phase I, II, and III clinical trials continue to explore the applicability of MSC-based therapies for treatment of autoimmune-mediated diseases. These include using MSCs to treat progressive neurodegenerative diseases which are at present without any clinically efficacious drug therapy. These would include multiple sclerosis (MS) [21–23] and amyotrophic lateral sclerosis (ALS) [24] which will be discussed in more detail later. To facilitate their ultimate use in human autoimmune diseases, bone marrow-derived MSCs are presently first being tested for their capacity to modulate immune responses in an experimental model of MS [23] and in autoimmune-mediated demyelination disease [25], an animal model for ALS.

Adipose-derived MSCs have also been evaluated for treating steroid-resistant acute graft-versus-host disease (GVHD), poor graft-functioning GVHD, as well as for poor graft-functioning bone marrow [18, 26, 27]. Of note, MSCs appear to be most valuable for treating autoimmune disorders and viral infections, such as

HIV. Thus, MSCS are being evaluated as for their immunomodulatory activity in the treatment of Type I diabetes [28, 29], scleroderma [30], uveitis [31, 32], systemic lupus erythematosus [30, 33–35], and irritable bowel disease [15]. It also appears that umbilical cord-derived MSCs may also have an important role in immune reconstitution in HIV-infected patients [36, 37].

In essence, there are three approved medications for MS, including, interferon-β, glatiramer acetate, and natalizumab [22]. However, MS remains a progressive neurodegenerative disease without any clinically effective medical therapies. Thus, the application of stem cell therapy for the treatment of MS continues to be ongoing. Now that imbalances between T_{reg} cells and T_H17 cells have been noted to play a role in the progression of malaise, urologic dysfunction, and overall MS disease progression [24], MSC-based therapies may take on considerable significance in the future treatment of MS. In addition, the capacity of MSCs to stimulate production of IL-10 by dendritic cells (DC), to promote T_{reg} cell production, as well as stimulating the release of the HLA G isoform appears to be particularly relevant in the context of MS pathology [22, 23].

In a mouse model of MS, where experimental allergic encephalomyelitis (EAE) was induced, mouse immune responses were altered by human bone marrow-derived MSCs [23]. Thus, after treatment with MSCs, activated T_h1 cells producing interferon-γ and T_h17 cells with their associated cytokines were reduced along with a concomitant increase in IL-4-producing T_h2 cells as well as their anti-inflammatory cytokines. Therefore, MSCs may exert an anti-inflammatory effect through their ability to stimulate the production of T_H2 cells. Moreover, in this setting self-tolerance would also be promoted most likely by inhibiting the transformation of DC to "full-time" antigen-presenting-cells. Thus, suppressing DC maturation in combination with effectively decreasing the ability for clonal expansion of autoreactive T-lymphocytes may become a useful future overriding therapy for treating the progressive forms of MS [21].

The production of IL-6 by MSCs also appears to be responsible, in part, for the development of the T_H17 response as well as for inhibiting T_{reg} cell development [22]. Therefore, preventing IL-6 activity by bone marrow-derived MSCs may be a strategically appropriate course for limiting the effectiveness of IL-6 in MS. Another potential candidate as a therapeutic target for MS may be MSC-derived hepatocyte growth factor (HGF) [23]. Thus, Bai et al. [23] demonstrated that conditioned medium from human MSCs (MSC-CM) reduced functional deficits in mouse $MOG_{35–55}$-induced EAE. MSC-CM also promoted the development of oligodendrocytes and neurons. Moreover, functional assays identified HGF and its primary receptor c-Met as critical in MSC-stimulated recovery in EAE, neural cell development, and remyelination [25]. However, MSCs from MS patients down-regulated HGF as well as decreasing IL-10 and TGF-β when compared to a control group.

Amyotrophic Lateral Sclerosis (ALS)

The pathogenesis of ALS has been associated with mutations in copper-containing superoxide dismutase, TAR DNA-binding protein–43, as well as the C9orf72 and TANK-binding kinase-1 genes. However, the heterogeneity among groups of ALS

patients has made it a difficult disease to treat in terms of developing medical therapies. Recently, several stem cell types have been considered for possible use for treating ALS. These have included neural stem cells, MSCs, glial-restricted embryonic stem cells, and induced pluripotent stem (IPS) cells. Recent studies using animal models of ALS have involved the placing of MSCs by peripheral injection or by direct placement into the spinal cord via intraspinal, intracerebral, or intrathecal administration with reported beneficial results [38]. These included a slower loss of motor neurons, improved motor function, and extended survival. Indeed, based primarily on the positive results from preclinical studies, Mazzini et al. [38] have performed the first clinical studies in MS patients to determine the tolerability of transplanted MSCs. Follow-up studies extended 4 years post-MSC placement. Although there was no overall functional improvement post-MSC transplantation, there were no abnormal or adverse effects [38]. These initial findings have been reevaluated in more recent clinical trials which showed that intraspinal, intrathecal, or intracerebral transplants were safely infusible [39].

In the analysis of three recent clinical trials, granulocyte colony-stimulating factor (G-CSF) was used to mobilize endogenous MSCs with no notable increase in major adverse effects. However, due to the effects and inability of G-CSF to cross the intact blood–brain barrier and facilitate peripheral administration, G-CSF has recently been examined more stringently for treating acute and chronic neurodegenerative orders, particularly with regard to CNS spinal regeneration in neurogenic and vasculogenic mechanisms for the treatment of SCI and ALS [40].

Conclusions and Future Perspectives

Ongoing studies now consider MSCs to be a potentially useful cell-based therapy for modifying the pathology of autoimmune diseases. The results of several published studies over the last 5 years have resulted in remarkable advances in extending the positive results from preclinical cell culture and animal models of RA, lupus, and EAE, to name just a few, to an analysis of the extent to which MSCs alter the progression of autoimmune diseases by evaluation of cell-based therapy in human clinical trials. Whereas most of the results from animal models of autoimmune diseases that were treated with MSCs have shown positive results toward reducing inflammation and pathology, a few, exemplified by the results reported by Youd et al. [35] remain controversial. For example, it remains problematic as to whether allogeneic MSCs can reverse pathology in the lupus-prone mouse model of SLE.

As additional data is collected from these analyses it should become evident as to which types of MSCs are most efficient at modulating activated T-cells and B-cells in this and other autoimmune diseases. Importantly, only time will tell if certain neurodegenerative diseases, such as MS and ALS, both of which possess an autoimmune component, but are without any disease-modifying medical therapy, will become amenable to MSC-targeted therapy.

References

1. Ghannam S, Bouffi C, Djouad F, Jorgensen C, Noël D (2010) Immunosuppression by mesenchymal stem cells: mechanisms and clinical applications. Stem Cell Res Ther 1:1
2. Bouffi C, Djouad F, Mathieu M, Noël D, Jorgensen C (2009) Multipotent mesenchymal stromal cells and rheumatoid arthritis: risk or benefit? Rheumatology (Oxford) 48:1185–1189
3. Wang Y, Chen X, Cao W, Shi Y (2014) Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. Nat Immunol 15:1009–1016
4. Ma S, Xie N, Li W, Yuan B, Shi Y, Wang Y (2014) Immunobiology of mesenchymal stem cells. Cell Death Differ 21:216–225
5. Mamidi MK, Das AK, Zakaria Z, Bhonde R (2016) Mesenchymal stromal cells for cartilage repair in osteoarthritis. Osteoarthritis Cartilage 24(8):1307–1316. doi:10.1016/j.joca.2016.03.003, pii: S1063-4584(16)01060-8
6. Gennari L, Rotatori S, Bianciardi S, Gonnelli S, Nuti R, Merlotti D (2015) Appropriate models for novel osteoporosis drug discovery and future perspectives. Expert Opin Drug Discov 10:1201–1216
7. Herrmann M, Verrier S, Alini M (2015) Strategies to stimulate mobilization and homing of endogenous stem and progenitor cells for bone tissue repair. Front Bioeng Biotechnol 3:79
8. Loi F, Córdova LA, Pajarinen J, Lin TH, Yao Z, Goodman SB (2016) Inflammation, fracture and bone repair. Bone 86:119–130
9. Kovach TK, Dighe AS, Lobo PI, Cui Q (2015) Interaction between MSCs and immune cells: implications for bone healing. J Immunol Res 2015:752510
10. Wenger R, Hans MG, Welter JF, Solchaga L, Sheu YR, Malemud CJ (2006) Hydrostatic pressure increased apoptosis in cartilage-constructs produced from human osteoarthritic chondrocytes. Front Biosci 11:1690–1695
11. Malemud CJ (2006) Matrix metalloproteinases: role in skeletal development and growth plate disorders. Front Biosci 11:1702–1715
12. Malemud CJ, Sun Y, Pearlman E, Ginley NM, Awadallah A, Wisler BA, Dennis JE (2012) Monosodium urate and tumor necrosis factor-α increase apoptosis in human chondrocyte cultures. Rheumatolology (Sunnyvale) 2:113
13. Ahmed TA, Hincke MT (2014) Mesenchymal stem cell-based tissue engineering strategies for repair of articular cartilage. Histol Histopathol 29:669–689
14. Solorio LD, Vieregge EL, Dhami CD, Alsberg E (2013) High-density cell systems incorporating polymer microspheres as microenvironmental regulators in engineered cartilage tissues. Tissue Eng Part B Rev 19:209–220
15. Klinker MW, Wei CH (2015) Mesenchymal stem cells in the treatment of inflammatory and autoimmune diseases in experimental animal models. World J Stem Cells 7:556–567
16. Holmdahl R, Malwillmström V, Burkhardt B (2014) Autoimmune priming, tissue attack and chronic inflammation-the three stages of rheumatoid arthritis. Eur J Immunol 44:1593–1599
17. González MA, González-Rey E, Rico L, Büscher D, Delgado M (2009) Treatment of experimental arthritis by inducing immune tolerance with human adipose-derived mesenchymal stem cells. Arthritis Rheum 60:1006–1019
18. Tobin LM, Healy ME, English K, Mahon BP (2013) Human mesenchymal stem cells suppress donor $CD4^+$ T cell proliferation and reduce pathology in a humanized mouse model of acute graft-versus host disease. Clin Exp Immunol 172:333–348
19. Malemud CJ, Schulte ME (2008) Is there a final common pathway for arthritis? Future Rheumatol 3:253–268
20. Maumus M, Guérit D, Toupet K, Jorgensen C, Noël D (2011) Mesenchymal stem cell-based therapies in regenerative medicine: applications in rheumatology. Stem Cell Res Ther 2:14
21. Xiao J, Yang R, Biswas S, Qin X, Zhang M, Deng W (2015) Mesenchymal stem cells and induced pluripotent stem cells as therapies for multiple sclerosis. Int J Mol Sci 16:9283–9302
22. Gharibi T, Ahmad M, Seyfizadeh N, Jadidi-Niaragh F, Yousefi M (2015) Immunomodulatory characteristics of mesenchymal stem cells and their role in the treatment of multiple sclerosis. Cell Immunol 293:113–121

23. Bai L, Lennon DP, Eaton V, Maier K, Caplan AI, Miller SD, Miller RH (2009) Human bone marrow-derived mesenchymal stem cells induce Th2-polarized immune response and promote endogenous repair in animal models of multiple sclerosis. Glia 57:1192–1203
24. Mazzini L, Gelati M, Profico DC, Sgaravizzi G, Projetti Pensi M, Muzi G, Ricciolini C, Rota Nodari L, Carletti C, Giorgi C, Spera C, Domenico F, Bersano E, Petruzzelli F, Cisari C, Maglione A, Sarnelli MF, Stecco A, Querin G, Masiero S, Cantello R, Ferrari D, Zalfa C, Binda E, Visioli A, Trombetta D, Novelli A, Torres B, Bernardini L, Carriero A, Prandi P, Servo S, Cerino A, Cima V, Gaiani A, Nasuelli N, Massara M, Glass J, Sorarù G, Boulis NM, Vescovi AL (2015) Human neural stem cell transplantation in ALS: initial results from a phase I trial. J Transl Med 13:17
25. Payne NL, Dantanarayana A, Sun G, Moussa L, Caine S, McDonald C, Herszfeld D, Bernard CC, Siatskas C (2009) Early intervention with gene-modified mesenchymal stem cells overexpressing interleukin-4 enhances anti-inflammatory responses and functional recovery in experimental autoimmune demyelination. Cell Adh Migr 6:179–189
26. Joo SY, Cho KA, Jung YJ, Kim HS, Park SY, Choi YB, Hong KM, Woo SY, Seoh JY, Cho SJ, Ryu KH (2010) Mesenchymal stromal cells inhibit graft-versus-host disease of mice in a dose-dependent manner. Cytotherapy 12:361–370
27. Christensen ME, Turner BE, Sinfield JJ, Kollar K, Cullup H, Waterhouse NJ, Hart DN, Atkinson K, Rice AM (2010) Mesenchymal stromal cells transiently alter the inflammatory milieu post-transplant to delay graft-versus-host disease. Haematologica 95:2102–2110
28. Fiorina P, Jurewicz M, Augello A, Vergani A, Dada S, La Rosa S, Selig M, Godwin J, Law K, Placidi C, Smith RN, Capella C, Rodig S, Adra CN, Atkinson M, Sayegh MH, Abdi R (2009) Immunomodulatory function of bone marrow-derived mesenchymal stem cells in experimental autoimmune type I diabetes. J Immunol 183:993–1004
29. van Belle TL, Coppieters KT, Herrath MG (2011) Type 1 diabetes: etiology, immunology and therapeutic strategies. Physiol Rev 91:79–118
30. Cras A, Farge D, Carmol T, Lataillade JJ, Wang DD, Sun L (2015) Update on mesenchymal stem cell-based therapy in lupus and scleroderma. Arthritis Res Ther 17:301
31. Tasso R, Ilengo C, Quatro R, Cancedda R, Caspi RR, Pennesi G (2012) Mesenchymal stem cells induce functionally active T-regulatory lymphocytes in a paracrine fashion and ameliorate experimental autoimmune uveitis. Invest Ophthalmol Vis Sci 53:786–793
32. Li G, Yuan L, Ren X, Nian H, Zhang L, Han ZC, Li X, Zhang X (2013) The effect of mesenchymal stem cells on dynamic changes of T cell subsets in experimental autoimmune uveoretinitis. Clin Exp Immunol 173:28–37
33. Sun L, Akiyama K, Zhang H, Yamaza T, Hou Y, Zhao S, Xu T, Le A, Shi S (2009) Mesenchymal stem cell transplantation reverses multiorgan dysfunction in systemic lupus erythematosus mice and humans. Stem Cells 27:1421–1432
34. Gu Z, Akiyama K, Ma X, Zhang H, Feng X, Yao G, Hou Y, Lu L, Gilkeson GS, Silver RM, Zeng X, Shi S, Sun L (2010) Transplantation of umbilical cord mesenchymal stem cells alleviates lupus nephritis in MRL/lpr mice. Lupus 19:1502–1514
35. Youd M, Blickarz C, Woodworth L, Touzjian T, Edling A, Tedstone J, Ruzek M, Tubo R, Kaplan J, Lodie T (2010) Allogeneic mesenchymal stem cells do not protect NZBxNZW F1 mice from developing lupus disease. Clin Exp Immunol 161:176–186
36. Allam O, Samarani S, Ahmad A (2013) Mesenchymal stem cell therapy in HIV-infected HAART-treated nonimmune responders restores immune competence. AIDS 27:1349–1352
37. Zhang Z, Fu J, Xu X, Wang S, Xu R, Zhao M, Nie W, Wang X, Zhang J, Li T, Su L, Wang FS (2013) Safety and immunological responses to human mesenchymal stem cell therapy in difficult-to-treat HIV-1-infected patients. AIDS 27:1283–1293
38. Mazzini L, Vescovi A, Cantello R, Gelati M, Vercelli A (2016) Stem cells therapy for ALS. Expert Opin Biol Ther 16:187–199
39. Mao Z, Zhang S, Chen H (2015) Stem cell therapy for amyotrophic lateral sclerosis. Cell Regen 4:11
40. Wallner S, Peters S, Pitzer C, Resch H, Bogdahn U, Schneider A (2015) The granulocyte-colony stimulating factor has a dual role in neuronal and vascular plasticity. Front Cell Dev Biol 3:48

Index

C.J. Malemud, E. Alsberg (eds.), *Mesenchymal Stem Cells and Immunomodulation*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-3-319-46733-7