6. AUTOIMMUNE DISEASES AND THE PROMISE OF STEM CELL-BASED THERAPIES

One of the more perplexing questions in biomedical research is—why does the body's protective shield against infections, the immune system, attack its own vital cells, organs, and tissues? The answer to this question is central to understanding an array of autoimmune diseases, such as rheumatoid arthritis, type 1 diabetes, systemic lupus erythematosus, and Sjogren's syndrome. When some of the body's cellular proteins are recognized as "foreign" by immune cells called T lymphocytes, a destructive cascade of inflammation is set in place. Current therapies to combat these cases of cellular mistaken identity dampen the body's immune response and leave patients vulnerable to life-threatening infections. Research on stem cells is now providing new approaches to strategically remove the misguided immune cells and restore normal immune cells to the body. Presented here are some of the basic research investigations that are being guided by adult and embryonic stem cell discoveries.

INTRODUCTION

The body's main line of defense against invasion by infectious organisms is the immune system. To succeed, an immune system must distinguish the many cellular components of its own body (self) from the cells or components of invading organisms (nonself). "Nonself" should be attacked while "self" should not. Therefore, two general types of errors can be made by the immune system. If the immune system fails to quickly detect and destroy an invading organism, an infection will result. However, if the immune system fails to recognize self cells or components and mistakenly attacks them, the result is known as an autoimmune disease. Common autoimmune diseases include rheumatoid arthritis, systemic lupus erythematosis (lupus), type 1 diabetes, multiple sclerosis, Sjogren's syndrome and inflammatory bowel disease. Although each of these diseases has different symptoms, they share the unfortunate reality that, for some reason, the body's immune system has turned against itself (see Box 6.1. Immune System Components: Common Terms and Definitions).

HOW DOES THE IMMUNE SYSTEM NORMALLY KEEP US HEALTHY?

The "soldiers" of the immune system are white blood cells, including T and B lymphocytes, which originate in the bone marrow from hematopoietic stem cells. Every day the body comes into contact with many organisms such as bacteria, viruses, and parasites. Unopposed, these organisms have the potential to cause serious infections, such as pneumonia or AIDS. When a healthy individual is infected, the body responds by activating a variety of immune cells. Initially, invading bacteria or viruses are engulfed by an antigen presenting cell (APC), and their component proteins (antigens) are cut into pieces and displayed on the cell's surface. Pieces of the foreign protein (antigen) bind to the major histocompatibility complex (MHC) proteins, also known as human leukocyte antigen (HLA) molecules, on the surface of the APCs (see Figure 6.1 Immune Response to Self or Foreign Antigens). This complex, formed by a foreign protein and an MHC protein, then binds to a T cell receptor on the surface of another type of immune cell, the CD4 helper T cell. They are so named because they "help" immune responses proceed and have a protein called CD4 on their surface. This complex enables these T cells to focus the immune response to a specific invading organism. The antigen-specific CD4 helper T cells divide and multiply while secreting substances called cytokines, which cause inflammation and help activate other immune cells. The particular cytokines secreted by the CD4 helper T cells act on cells known as the CD8 "cytotoxic" T cells (because they can kill the cells that are infected by the invading organism and have the CD8

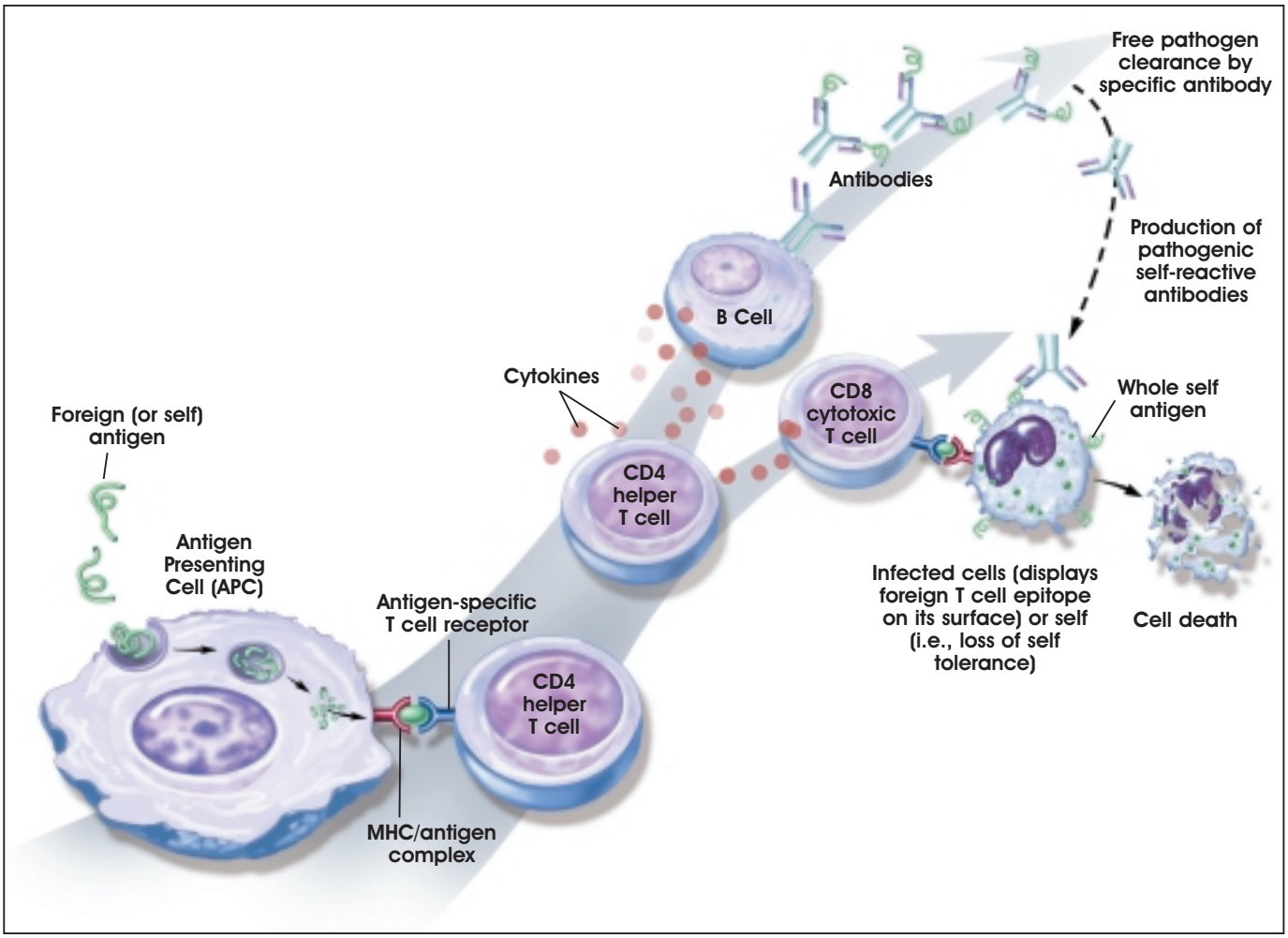

Figure 6.1. Immune Response to Self or Foreign Antigens.

protein on their surface). The helper T cells can also activate antigen-specific B cells to produce antibodies, which can neutralize and help eliminate bacteria and viruses from the body. Some of the antigen-specific T and B cells that are activated to rid the body of infectious organisms become long-lived "memory" cells. Memory cells have the capacity to act quickly when confronted with the same infectious organism at later times. It is the memory cells that cause us to become "immune" from later reinfections with the same organism.

HOW DO THE IMMUNE CELLS OF THE BODY KNOW WHAT TO ATTACK AND WHAT NOT TO?

All immune and blood cells develop from multipotent hematopoietic stem cells that originate in the bone marrow. Upon their departure from the bone marrow, immature T cells undergo a final maturation process in the thymus, a small organ located in the upper chest, before being dispersed to the body with the rest of the immune cells (e.g., B cells). Within the thymus, T cells undergo an important process that "educates" them to distinguish between self (the proteins of their own body) and nonself (the invading organism's) antigens. Here, the T cells are selected for their ability to bind to the particular MHC proteins expressed by the individual. The particular array of MHCs varies slightly between individuals, and this variation is the basis of the immune response when a transplanted organ is rejected. MHCs and other less easily characterized molecules called minor histocompatibility antigens are genetically determined and this is the reason why donor organs from relatives of the recipient are preferred over unrelated donors.

Box 6.1

Immune System Components: Common Terms and Definitions

Antibody — A Y-shaped protein secreted by B cells in response to an antigen. An antibody binds specifically to the antigen that induced its production. Antibodies directed against antigens on the surface of infectious organisms help eliminate those organisms from the body.

Antigen — A substance (often a protein) that induces the formation of an antibody. Antigens are commonly found on the surface of infectious organisms, transfused blood cells, and organ transplants.

Antigen presenting cells (APC) — One of a variety of cells within the body that can process antigens and display them on their surface in a form recognizable by T cells.

Autoantibody — An antibody that reacts with antigens found on the cells and tissues of an individual's own body. Autoantibodies can cause autoimmune diseases.

Autoimmune disease — A condition that results from the formation of antibodies that attack the cells or tissues of an individual's own body.

B cells — Also known as B lymphocytes. Each B cell is capable of making one specific antibody. When stimulated by antigen and helper T cells, B cells mature into plasma cells that secrete large amounts of their specific antibody

Bone marrow — The soft, living tissue that fills most bone cavities and contains hematopoietic stem cells, from which all red and white blood cells evolve. The bone marrow also contains mesenchymal stem cells that a number of cell types come from, including chondrocytes, which produce cartilage.

Cytokines — A generic term for a large variety of regulatory proteins produced and secreted by cells and used to communicate with other cells. One class of cytokines is the interleukins, which act as intercellular mediators during the generation of an immune response.

Immune system cells — White blood cells or leukocytes that originate from the bone marrow. They include antigen presenting cells, such as dendritic cells, T and B lymphocytes, and neutrophils, among many others.

Lymphatic system — A network of lymph vessels and nodes that drain and filter antigens from tissue fluids before returning lymphocytes to the blood.

Memory cells — A subset of antigen-specific T or B cells that "recall" prior exposure to an antigen and respond quickly without the need to be activated again by CD4 helper T cells.

Major histocompatibility complex (MHC) — A group of genes that code for cell-surface histocompatibility antigens. These antigens are the primary reason why organ and tissue transplants from incompatible donors fail.

T cells — Also known as T lymphocytes. There are two primary subsets of T cells. CD4 helper T cells (identified by the presence of the CD4 protein on their surfaces) are instrumental in initiating an immune response by supplying special cytokines. CD8 cytotoxic (killer) T cells (identified by the presence of the CD8 protein on their surfaces), after being activated by the CD4 helper cells, are capable of killing infected cells in the body. CD4 helper T cells are destroyed by the HIV virus in AIDS patients, resulting in an ineffective immune system.

Thymus — A lymphoid organ located in the upper chest cavity. Maturing T cells leave the bone marrow and go directly to the thymus, where they are educated to discriminate between self and nonself proteins. (See tolerance.)

Tolerance — A state of specific immunologic unresponsiveness. Individuals should normally be tolerant of the cells and tissues that make up our own bodies. Should tolerance fail, an autoimmune disease may result.

In the bone marrow, a highly diverse and random array of T cells is produced. Collectively, these T cells are capable of recognizing an almost unlimited number of antigens. Because the process of generating a T cell's antigen specificity is a random one, many immature T cells have the potential to react with the body's own (self) proteins. To avoid this potential disaster, the thymus provides an environment where T cells that recognize self-antigens (autoreactive or self-reactive T cells) are deleted or inactivated in a process called tolerance induction.

Tolerance usually ensures that T cells do not attack the "autoantigens" (self-proteins) of the body. Given the importance of this task, it is not surprising that there are multiple checkpoints for destroying or inactivating T cells that might react to auto-antigens.

Autoimmune diseases arise when this intricate system for the induction and maintenance of immune tolerance fails. These diseases result in cell and tissue destruction by antigen-specific CD8 cytotoxic T cells or autoantibodies (antibodies to self-proteins) and the

accompanying inflammatory process. These mechanisms can lead to the destruction of the joints in rheumatoid arthritis, the destruction of the insulin-producing beta cells of the pancreas in type 1 diabetes, or damage to the kidneys in lupus. The reasons for the failure to induce or maintain tolerance are enigmatic. However, genetic factors, along with environmental and hormonal influences and certain infections, may contribute to tolerance and the development of autoimmune disease [4, 7].

HEMATOPOIETIC STEM CELL THERAPY FOR AUTOIMMUNE DISEASES

The current treatments for many autoimmune diseases include the systemic use of anti-inflammatory drugs and potent immunosuppressive and immunomodulatory agents (i.e., steroids and inhibitor proteins that block the action of inflammatory cytokines). However, despite their profound effect on immune responses, these therapies are unable to induce clinically significant remissions in certain patients. In recent years, researchers have contemplated the use of stem cells to treat autoimmune disorders. Discussed here is some of the rationale for this approach, with a focus on experimental stem cell therapies for lupus, rheumatoid arthritis, and type 1 diabetes.

The immune-mediated injury in autoimmune diseases can be organ-specific, such as type 1 diabetes which is the consequence of the destruction of the pancreatic beta islet cells or multiple sclerosis which results from the breakdown of the myelin covering of nerves. These autoimmune diseases are amenable to treatments involving the repair or replacement of damaged or destroyed cells or tissue (see Chapter 7. Stem Cells and Diabetes and Chapter 11. Use of Genetically Modified Stem Cells in Experimental Gene Therapies). In contrast, non-organ-specific autoimmune diseases, such as lupus, are characterized by widespread injury due to immune reactions against many different organs and tissues.

One approach is being evaluated in early clinical trials of patients with poorly responsive, life-threatening lupus. This is a severe disease affecting multiple organs in the body including muscles, skin, joints, and kidneys as well as the brain and nerves. Over 239,000 Americans, of which more than 90 percent are women, suffer from lupus. In addition, lupus disproportionately afflicts African-American and Hispanic women [11]. A major obstacle in the treatment of non-organ-specific autoimmune diseases such as lupus is the lack of a single specific target for the application of therapy.

The objective of hematopoietic stem cell therapy for lupus is to destroy the mature, long-lived, and autoreactive immune cells and to generate a new, properly functioning immune system. In most of these trials, the patient's own stem cells have been used in a procedure known as autologous (from "one's self") hematopoietic stem cell transplantation. First, patients receive injections of a growth factor, which coaxes large numbers of hematopoietic stem cells to be released from the bone marrow into the blood stream. These cells are harvested from the blood, purified away from mature immune cells, and stored. After sufficient quantities of these cells are obtained, the patient undergoes a regimen of cytotoxic (cell-killing) drug and/or radiation therapy, which eliminates the mature immune cells. Then, the hematopoietic stem cells are returned to the patient via a blood transfusion into the circulation where they migrate to the bone marrow and begin to differentiate to become mature immune cells. The body's immune system is then restored. Nonetheless, the recovery phase, until the immune system is reconstituted represents a period of dramatically increased susceptibility to bacterial, fungal, and viral infection, making this a high-risk therapy.

Recent reports suggest that this replacement therapy may fundamentally alter the patient's immune system. Richard Burt and his colleagues [18] conducted a long-term follow-up (one to three years) of seven lupus patients who underwent this procedure and found that they remained free from active lupus and improved continuously after transplantation, without the need for immunosuppressive medications. One of the hallmarks of lupus is that during the natural progression of disease, the normally diverse repertoire of T cells become limited in the number of different antigens they recognize, suggesting that an increasing proportion of the patient's T cells are autoreactive. Burt and colleagues found that following hematopoietic stem cell transplantation, levels of T cell diversity were restored to those of healthy individuals. This finding provides evidence that stem cell replacement may be beneficial in reestablishing tolerance in T cells, thereby decreasing the likelihood of disease reoccurrence.

DEVELOPMENT OF HEMATOPOIETIC STEM CELL LINES FOR TRANSPLANTATION

The ability to generate and propagate unlimited numbers of hematopoietic stem cells outside the body—whether from adult, umbilical cord blood, fetal, or embryonic sources—would have a major impact on the safety, cost, and availability of stem cells for transplantation. The current approach of isolating hematopoietic stem cells from a patient's own peripheral blood places the patient at risk for a flare-up of their autoimmune disease. This is a potential consequence of repeated administration of the stem cell growth factors needed to mobilize hematopoietic stem cells from the bone marrow to the blood stream in numbers sufficient for transplantation. In addition, contamination of the purified hematopoietic stem cells with the patient's mature autoreactive T and B cells could affect the success of the treatment in some patients. Propagation of pure cell lines in the laboratory would avoid these potential drawbacks and increase the numbers of stem cells available to each patient, thus shortening the at-risk interval before full immune reconstitution.

Whether embryonic stem cells will provide advantages over stem cells derived from cord blood or adult bone marrow hematopoietic stem cells remains to be determined. However, hematopoietic stem cells, whether from umbilical cord blood or bone marrow, have a more limited potential for self-renewal than do pluripotent embryonic stem cells. Although new information will be needed to direct the differentiation of embryonic stem cells into hematopoietic stem cells, hematopoietic cells are present in differentiated cultures from human embryonic stem cells [9] and from human fetal-derived embryonic germ stem cells [17].

One potential advantage of using hematopoietic stem cell lines for transplantation in patients with autoimmune diseases is that these cells could be generated from unaffected individuals or, as predisposing genetic factors are defined, from embryonic stem cells lacking these genetic influences. In addition, use of genetically selected or genetically engineered cell types may further limit the possibility of disease progression or reemergence.

One risk of using nonself hematopoietic stem cells is of immune rejection of the transplanted cells. Immune rejection is caused by MHC protein differences between the donor and the patient (recipient). In this scenario, the transplanted hematopoietic stem cells and their progeny are rejected by the patient's own T cells, which are originating from the patient's surviving bone marrow hematopoietic stem cells. In this regard, embryonic stem cell-derived hematopoietic stem cells may offer distinct advantages over cord blood and bone marrow hematopoietic stem cell lines in avoiding rejection of the transplant. Theoretically, banks of embryonic stem cells expressing various combinations of the three most critical MHC proteins could be generated to allow close matching to the recipient's MHC composition.

Additionally, there is evidence that embryonic stem cells are considerably more receptive to genetic manipulation than are hematopoietic stem cells (see Chapter 11. Use of Genetically Modified Stem Cells in Experimental Gene Therapies).

This characteristic means that embryonic stem cells could be useful in strategies that could prevent their recognition by the patient's surviving immune cells. For example, it may be possible to introduce the recipient's MHC proteins into embryonic stem cells through targeted gene transfer. Alternatively, it is theoretically possible to generate a universal donor embryonic stem cell line by genetic alteration or removal of the MHC proteins. Researchers have accomplished this by genetically altering a mouse so that it has little or no surface expression of MHC molecules on any of the cells or tissues. There is no rejection of pancreatic beta islet cells from these genetically altered mice when the cells are transplanted into completely MHC-mismatched mice [13]. Additional research will be needed to determine the feasibility of these alternative strategies for prevention of graft rejection in humans [6].

Jon Odorico and colleagues have shown that expression of MHC proteins on mouse embryonic stem cells and differentiated embryonic stem cell progeny is either absent or greatly decreased compared with MHC expression on adult cells [8]. These preliminary findings raise the intriguing possibility that lines derived from embryonic stem cells may be inherently less susceptible to rejection by the recipient's immune

system than lines derived from adult cells. This could have important implications for the transplantation of cells other than hematopoietic stem cells.

Another potential advantage of using pure populations of donor hematopoietic stem cells achieved through stem cell technologies would be a lower incidence and severity of graft-versus-host disease, a potentially fatal complication of bone marrow transplantation. Graft-versus-host disease results from the immune-mediated injury to recipient tissues that occurs when mature organ-donor T cells remain within the organ at the time of transplant. Such mature donor alloreactive T cells would be absent from pure populations of multipotent hematopoietic stem cells, and under ideal conditions of immune tolerance induction in the recipient's thymus, the donor-derived mature T cell population would be tolerant to the host.

GENE THERAPY AND STEM CELL APPROACHES FOR THE TREATMENT OF AUTOIMMUNE DISEASES

Gene therapy is the genetic modification of cells to produce a therapeutic effect (see Chapter 11. Use of Genetically Modified Stem Cells in Experimental Gene Therapies). In most investigational protocols, DNA containing the therapeutic gene is transferred into cultured cells, and these cells are subsequently administered to the animal or patient. DNA can also be injected directly, entering cells at the site of the injection or in the circulation. Under ideal conditions, cells take up the DNA and produce the therapeutic protein encoded by the gene.

Currently, there is an extensive amount of gene therapy research being conducted in animal models of autoimmune disease. The goal is to modify the aberrant, inflammatory immune response that is characteristic of autoimmune diseases [15, 19]. Researchers most often use one of two general strategies to modulate the immune system. The first strategy is to block the actions of an inflammatory cytokine (secreted by certain activated immune cells and inflamed tissues) by transferring a gene into cells that encodes a "decoy" receptor for that cytokine. Alternatively, a gene is transferred that encodes an anti-inflammatory cytokine, redirecting the auto-inflammatory immune response to a more "tolerant" state. In many animal studies, promising results have been achieved by using these approaches, and the studies have advanced understanding of the disease processes and the particular inflammatory cytokines involved in disease progression [15, 19].

Serious obstacles to the development of effective gene therapies for humans remain, however. Foremost among these are the difficulty of reliably transferring genetic material into adult and slowly dividing cells (including hematopoietic stem cells) and of producing long-lasting expression of the intended protein at levels that can be tightly controlled in response to disease activity. Importantly, embryonic stem cells are substantially more permissive to gene transfer compared with adult cells, and embryonic cells sustain protein expression during extensive self-renewal. Whether adult-derived stem cells, other than hematopoietic stem cells, are similarly amenable to gene transfer has not yet been determined.

Ultimately, stem cell gene therapy should allow the development of novel methods for immune modulation in autoimmune diseases. One example is the genetic modification of hematopoietic stem cells or differentiated tissue cells with a "decoy" receptor for the inflammatory cytokine interferon gamma to treat lupus. For example, in a lupus mouse model, gene transfer of the decoy receptor, via DNA injection, arrested disease progression [12]. Other investigators have used a related but distinct approach in a mouse model of type 1 diabetes. Interleukin-12 (IL-12), an inflammatory cytokine, plays a prominent role in the development of diabetes in these mice. The investigators transferred the gene for a modified form of IL-12, which blocks the activity of the natural IL-12, into pancreatic beta islet cells (the target of autoimmune injury in type 1 diabetes). The islet cell gene therapy prevented the onset of diabetes in these mice [20]. Theoretically, embryonic stem cells or adult stem cells could be genetically modified before or during differentiation into pancreatic beta islet cells to be used for transplantation. The resulting immune-modulating islet cells might diminish the occurrence of ongoing autoimmunity, increase the likelihood of long-term function of the transplanted cells, and eliminate the need for immunosuppressive therapy following transplantation.

Researchers are exploring similar genetic approaches to prevent progressive joint destruction and loss of cartilage and to repair damaged joints in animal

models of rheumatoid arthritis. Rheumatoid arthritis is a debilitating autoimmune disease characterized by acute and chronic inflammation, in which the immune system primarily attacks the joints of the body. In a recent study, investigators genetically transferred an anti-inflammatory cytokine, interleukin-4 (IL-4), into a specialized, highly efficient antigen-presenting cell called a dendritic cell, and then injected these IL-4-secreting cells into mice that can be induced to develop a form of arthritis similar to rheumatoid arthritis in humans. These IL-4-secreting dendritic cells are presumed to act on the CD4 helper T cells to reintroduce tolerance to self-proteins. Treated mice showed complete suppression of their disease and, in addition to its immune-modulatory properties, IL-4 blocked bone resorption (a serious complication of rheumatoid arthritis), making it a particularly attractive cytokine for this therapy [10]. However, one obstacle to this approach is that human dendritic cells are difficult to isolate in large numbers.

Investigators have also directed the differentiation of dendritic cells from mouse embryonic stem cells, indicating that a stem cell-based approach might work in patients with rheumatoid arthritis [5]. Longer-term follow-up and further characterization will be needed in animal models before researchers proceed with the development of such an approach in humans. In similar studies, using other inhibitors of inflammatory cytokines such as a decoy receptor for tumor necrosis factor–α (a prominent inflammatory cytokine in inflamed joints), an inhibitor of nuclear factor–κB (a protein within cells that turns on the production of many inflammatory cytokines), and interleukin-13 (an anti-inflammatory cytokine), researchers have shown promising results in animal models of rheumatoid arthritis [19]. Because of the complexity and redundancy of immune system signaling networks, it is likely that a multifaceted approach involving inhibitors of several different inflammatory cytokines will be successful, whereas approaches targeting single cytokines might fail or produce only short-lived responses. In addition, other cell types may prove to be even better vehicles for the delivery of gene therapy in this disease.

Chondrocytes, cells that build cartilage in joints, may provide another avenue for stem cell-based treatment of rheumatoid arthritis. These cells have been derived from human bone marrow stromal stem cells derived from human bone marrow [14]. Little is known about the intermediate cells that ultimately differentiate into chondrocytes. In addition to adult bone marrow as a source for stromal stem cells, human embryonic stem cells can differentiate into precursor cells believed to lead ultimately to the stromal stem cells [16]. However, extensive research is needed to reliably achieve the directed derivation of the stromal stem cells from embryonic stem cells and, subsequently, the differentiation of chondrocytes from these stromal stem cells.

The ideal cell for optimum cartilage repair may be a more primitive cell than the chondrocyte, such as the stromal cell, or an intermediate cell in the pathway (e.g., a connective tissue precursor) leading to the chondrocyte. Stromal stem cells can generate new chondrocytes and facilitate cartilage repair in a rabbit model [3]. Such cells may also prove to be ideal targets for the delivery of immune-modulatory gene therapy. Like hematopoietic stem cells, stromal stem cells have been used in animal models for delivery of gene therapy [1]. For example, a recent study demonstrated that genetically engineered chondrocytes, expressing a growth factor, can enhance the function of transplanted chondrocytes [2].

Two obstacles to the use of adult stromal stem cells or chondrocytes are the limited numbers of these cells that can be harvested and the difficulties in propagating them in the laboratory. Embryonic stem cells, genetically modified and expanded before directed differentiation to a connective tissue stem cell, may be an attractive alternative.

Collectively, these results illustrate the tremendous potential these cells may offer for the treatment of rheumatoid arthritis and other autoimmune diseases.

CONCLUSION

Stem cell-based therapies offer many exciting possibilities for the development of novel treatments, and perhaps even cures, for autoimmune diseases. A challenging research effort remains to fully realize this potential and to address the many remaining questions, which include how best to direct the differentiation of specific cell types and determine which particular type of stem cell will be optimum for each therapeutic approach. Gene therapy with cytokines or their inhibitors is still in its infancy, but stem cells or

their progeny may provide one of the better avenues for future delivery of immune-based therapies. Ultimately, the potential to alleviate these devastating chronic diseases with the use of stem cell-based technologies is enormous.

REFERENCES

1. Allay, J.A., Dennis, J.E., Haynesworth, S.E., Majumdar, M.K., Clapp, D.W., Shultz, L.D., Caplan, A.I., and Gerson, S.L. (1997). LacZ and interleukin-3 expression in vivo after retroviral transduction of marrow-derived human osteogenic mesenchymal progenitors. Hum. Gene Ther. *8*, 1417-1427.

2. Brower-Toland, B.D., Saxer, R.A., Goodrich, L.R., Mi, Z., Robbins, P.D., Evans, C.H., and Nixon, A.J. (2001). Direct adenovirus-mediated insulin-like growth factor I gene transfer enhances transplant chondrocyte function. Hum. Gene Ther. *12*, 117-129.

3. Caplan, A.I., Elyaderani, M., Mochizuki, Y., Wakitani, S., and Goldberg, V.M. (1997). Principles of cartilage repair and regeneration. Clin. Orthop. *342*, 254-269.

4. Cooper, G.S., Dooley, M.A., Treadwell, E.L., St Clair, E.W., Parks, C.G., and Gilkeson, G.S. (1998). Hormonal, environmental, and infectious risk factors for developing systemic lupus erythematosus. Arthritis Rheum. *41*, 1714-1724.

5. Fairchild, P.J., Brook, F.A., Gardner, R.L., Graca, L., Strong, V., Tone, Y., Tone, M., Nolan, K.F., and Waldmann, H. (2000). Directed differentiation of dendritic cells from mouse embryonic stem cells. Curr. Biol. *10*, 1515-1518.

6. Gearhart, J. (1998). New potential for human embryonic stem cells. Science. *282*, 1061-1062.

7. Grossman, J.M. and Tsao, B.P. (2000). Genetics and systemic lupus erythematosus. Curr. Rheumatol. Rep. *2*, 13-18.

8. Harley, C.B., Gearhart, J., Jaenisch, R., Rossant, J., and Thomson, J. (2001). Keystone Symposia. Pluripotent stem cells: biology and applications. Durango, CO.

9. Itskovitz-Eldor, J., Schuldiner, M., Karsenti, D., Eden, A., Yanuka, O., Amit, M., Soreq, H., and Benvenisty, N. (2000). Differentiation of human embryonic stem cells into embryoid bodies comprising the three embryonic germ layers. Mol. Med. *6*, 88-95.

10. Kim, S.H., Kim, S., Evans, C.H., Ghivizzani, S.C., Oligino, T., and Robbins, P.D. (2001). Effective treatment of established murine collagen-induced arthritis by systemic administration of dendritic cells genetically modified to express IL-4. J. Immunol. *166*, 3499-3505.

11. Lawrence, R.C., Helmick, C.G., Arnett, F.C., Deyo, R.A., Felson, D.T., Giannini, E.H., Heyse, S.P., Hirsch, R., Hochberg, M.C., Hunder, G.G., Liang, M.H., Pillemer, S.R., Steen, V.D., and Wolfe, F. (1998). Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. Arthritis Rheum. *41*, 778-799.

12. Lawson, B.R., Prud'homme, G.J., Chang, Y., Gardner, H.A., Kuan, J., Kono, D.H., and Theofilopoulos, A.N. (2000). Treatment of murine lupus with cDNA encoding IFN-gammaR/Fc. J. Clin. Invest. *106*, 207-215.

13. Osorio, R.W., Ascher, N.L., Jaenisch, R., Freise, C.E., Roberts, J.P., and Stock, P.G. (1993). Major histocompatibility complex class I deficiency prolongs islet allograft survival. Diabetes. *42*, 1520-1527.

14. Pittenger, M.F., Mackay, A.M., Beck, S.C., Jaiswal, R.K., Douglas, R., Mosca, J.D., Moorman, M.A., Simonetti, D.W., Craig, S., and Marshak, D.R. (1999). Multilineage potential of adult human mesenchymal stem cells. Science. *284*, 143-147.

15. Prud'homme, G.J. (2000). Gene therapy of autoimmune diseases with vectors encoding regulatory cytokines or inflammatory cytokine inhibitors. J. Gene. Med. *2*, 222-232.

16. Schuldiner, M., Yanuka, O., Itskovitz-Eldor, J., Melton, D., and Benvenisty, N. (2000). Effects of eight growth factors on the differentiation of cells derived from human embryonic stem cells. Proc. Natl. Acad. Sci. U. S. A. *97*, 11307-11312.

17. Shamblott, M.J., Axelman, J., Littlefield, J.W., Blumenthal, P.D., Huggins, G.R., Cui, Y., Cheng, L., and Gearhart, J.D. (2000). Human embryonic germ cell derivatives express a broad range of develpmentally distinct markers and proliferate extensively *in vitro*. Proc. Natl. Acad. Sci. U. S. A. *98*, 113-118.

18. Traynor, A.E., Schroeder, J., Rosa, R.M., Cheng, D., Stefka, J., Mujais, S., Baker, S., and Burt, R.K. (2000). Treatment of severe systemic lupus erythematosus with high-dose chemotherapy and haemopoietic stem-cell transplantation: a phase I study. Lancet. *356*, 701-707.

19. Tsokos, G.C. and Nepom, G.T. (2000). Gene therapy in the treatment of autoimmune diseases. J. Clin. Invest. *106*, 181-183.

20. Yasuda, H., Nagata, M., Arisawa, K., Yoshida, R., Fujihira, K., Okamoto, N., Moriyama, H., Miki, M., Saito, I., Hamada, H., Yokono, K., and Kasuga, M. (1998). Local expression of immunoregulatory IL-12p40 gene prolonged syngeneic islet graft survival in diabetic NOD mice. J. Clin. Invest. *102*, 1807-1814.