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Immunologic and Inflammatory Reactions to Exogenous Stem Cells

Implications for Experimental Studies and Clinical Trials for Myocardial Repair

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Intense research is under way to determine the optimal stem cell type and regimen for repairing diseased myocardium. Although initial studies in humans focused on the use of homologous stem cells, allogeneic or xenogeneic stem cells have been studied extensively in experimental work. Clinical trials with allogeneic stem cells are now under way, an approach based on the premise that stem cells and precursor cells are characterized as being immunotolerant. However, evidence indicates that stem cells may gain immune potency in vivo, especially when delivered to inflamed tissue, such as acutely infarcted myocardium. Histopathologic studies show the presence of a lymphohistiocytic inflammatory reaction at the sites of delivery of allogeneic stem cells, a response that is exaggerated with the use of xenogeneic stem cells. The immune-mediated inflammatory reaction to allogeneic and xenogeneic stem cells may elicit a spectrum of effects, ranging from beneficial (e.g., increased paracrine activity) to detrimental (e.g., accelerated damage and removal of stem cells). Although the issue of immune-mediated inflammatory responses to non-self stem cells requires further evaluation, non-self stem cells should not be considered as immunologically inert or exclusively immunosuppressive in vivo. (J Am Coll Cardiol 2010;56:1693–700) © 2010 by the American College of Cardiology Foundation

With the advent of regenerative medicine, considerable effort has been directed at overcoming the fundamental biology of the mammalian myocardium to achieve not only myocardial repair, but also myocardial regeneration in response to injury. Results of initial experimental studies indicated the beneficial effects of stem cells on damaged myocardium, and human clinical trials quickly followed after the publication of observations suggesting that exogenously administered stem cells may contribute to myocardial regeneration (1–3).

In initial clinical trials, autologous stem cells were harvested from the bone marrow of the patient undergoing cell therapy, processed in culture, and delivered to the myocardium by various methods (1–6). However, in most of the experimental studies preceding these trials, allogeneic or xenogeneic stem cells were used in animal models of myocardial injury, and immunologic reactions were controlled by using various strategies (i.e., immunosuppressive drugs or immunodeficient animals). Moreover, the underlying assumption was that stem cells exhibit immune tolerance and low immunogenicity. This putative immunotolerance of certain types of stem cells has led to the emergence of new clinical programs involving the administration of proprietary allogeneic human stem cells to patients with heart disease. Given these considerations, the goal of this commentary is to increase awareness of the immunologic and inflammatory reactions to exogenously administered stem cells—a topic that has received only limited attention.

Immunology of Stem Cells

An exogenous cell delivered to a host is expected to encounter some form of host resistance. Recognition of foreign antigen initiates an immune response that involves the activation and proliferation of specific immune cells. At the heart of this response is a set of specific antigens called major histocompatibility complex (MHC) antigens that are expressed on each cell of the body. The MHC antigens were originally recognized for their role in initiating T-cell responses that lead to the rejection of transplanted tissue. By binding to foreign antigens, MHC molecules form complexes that are recognized by specific T cells, thus initiating a cascade of events to identify and eliminate foreign invaders. The MHC class I antigens are traditionally associated with the activation of CD8+ cytotoxic T lymphocytes, whereas MHC class II antigens are recognized by CD4+ T

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Abbreviations and Acronyms

ESC = embryonic stem cell
HSC = hematopoetic stem cell
iPS = induced pluripotent stem
MHC = major histocompatibility complex
MSC = mesenchymal stem cell
SCID = severe combined immunodeficiency

lymphocytes. However, many stem cells express no or only low levels of MHC antigens and have been considered to be immunoprivileged, or lacking in the ability to induce an immune response. In fact, embryonic stem cells (ESCs) and mesenchymal stem cells (MSCs) have been presented as the prototype of the immunoprivileged cell for cell transplantation studies (7,8).

As shown in Figure 1, stem and precursor cells exhibit a spectrum of immunologic properties, and a slowly increasing body of literature has begun to question the immunomodulatory and immunoprivileged status of various stem cells (7,9). Given the importance of the immune status of these cells in cell therapy, we will review the experimental evidence for the immunoprivileged status of ESCs and MSCs and present recent evidence that contradicts this assumption.

ESCs. Undifferentiated ESCs are characterized by the basic traits of self-renewal, clonogenicity, and pluripotency (for differentiation into multiple cell types). Our current understanding of the immunoprivileged status of human ESCs is based on in vitro and in vivo xenogeneic transplantation studies (7). These studies have collectively shown that human ESCs express low levels of MHC class I antigens but do not express MHC class II antigens or costimulatory molecules (10–12). Furthermore, human ESCs have been found to evade recognition by natural killer cells and inhibit T-cell–induced stimulation by antigen-presenting cells. These studies have also shown that human ESCs do not induce an inflammatory response, or presumably, an immune response upon injection into immunocompetent mice and are, thus, candidates for immunomodulation and tolerance induction (7). In regenerative studies, ESCs have been reported to have salutary effects on organ repair, including the heart (13–16).

However, some studies contradict the idea of ESC immunoprivilege and suggest that ESCs are associated with immune rejection and teratogenicity (7–9,17–19). Transplantation of undifferentiated allogeneic murine and human ESCs into mouse hearts has led to the formation of cardiac teratomas in immunodeficient animals (18,19) (Fig. 2). Furthermore, treatment of immunocompetent animals with undifferentiated murine and human ESCs has resulted in immunologic rejection and intense inflammation after several weeks and the up-regulation of class I and class II MHC molecules (18–23) (Fig. 2). In a comparative experiment, mouse ESCs were injected into the injured myocar-

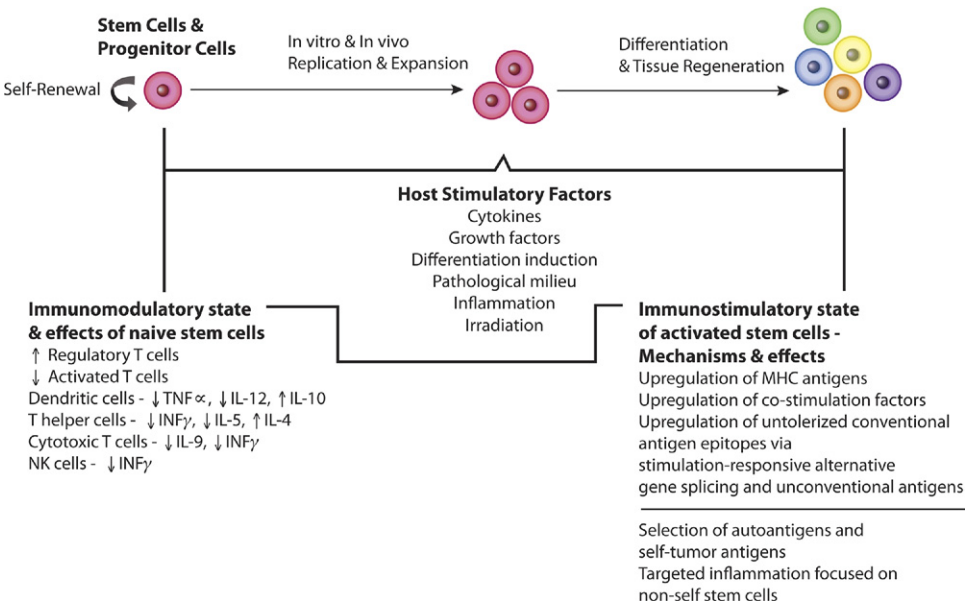

Figure 1 **Immunologic Properties of Stem Cells**

Naive embryonic stem cells and mesenchymal stem cells function to modulate and dampen the native and acquired immune responses of the host through cytokine-mediated interactions with various populations of lymphocytes (B cells, T helper cells, cytotoxic T cells, and natural killer [NK] cells) and dendritic cells and macrophages. Stem cells also respond to perturbations of the host by proliferation and differentiation, which may result in tissue repair and regeneration. Concomitantly, stem cells are exposed to several host factors leading to their activation. In the process, the phenotype and functional properties of the stem cells are changed, as mediated and manifested by up-regulaton of major histocompatibility complex (MHC) class I and II molecules, costimulation factors, and untolerized conventional antigen epitopes through stimulation-responsive alternative gene splicing and unconventional antigens. One consequence is that the stem cells undergo stimulation-responsive splicing for the selection of autoantigens and self-tumor antigens. However, if the stem cells are non-self (allogeneic or xenogeneic), they also become subject to targeted immune and inflammatory responses by the host. Adapted from Yang (8). IL = interleukin; INF = interferon; TNF = tumor necrosis factor.

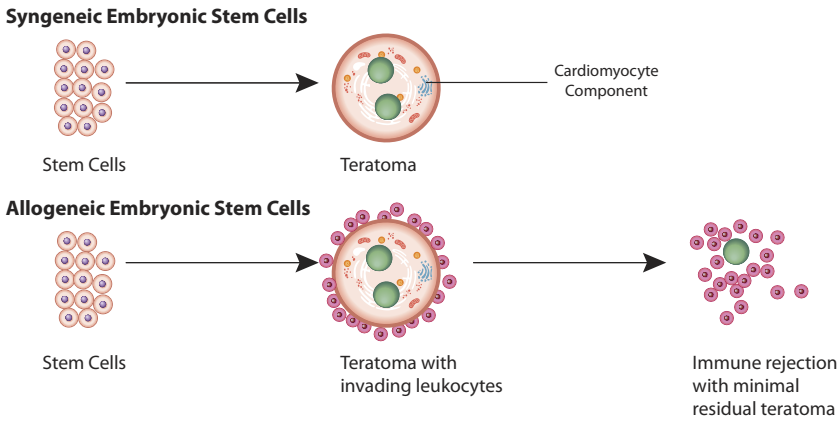

Figure 2 **Undifferentiated ESCs Implanted Into the Myocardium**

Host reactions to undifferentiated embryonic stem cells (ESCs) implanted into the myocardium of syngeneic and allogeneic recipients. In syngeneic recipients, the undifferentiated stem cells proliferate and form teratomas. In allogeneic recipients, the ESCs initially proliferate to form teratomas, but at the same time, an immune reaction occurs, leading to an intense, targeted inflammatory reaction resulting in destruction of the teratomas and rejection of the ESCs.

dium of syngeneic, allogeneic, and severe combined immunodeficient (SCID) mouse recipients (22). In allogeneic mice, ESCs triggered an intense cellular inflammatory response consisting of T lymphocytes (CD3+) and dendritic cells (CD11c), as well as a humoral response. However, fewer CD3+ T cells were elicited in the syngeneic group, and no CD3+ T cells were elicited in the SCID mice. Immunologic reactions and lymphocytic infiltration have also been observed after the transplantation of neural stem cells derived from ESCs (24). These observations indicate that up-regulation of MHC expression occurs when ESCs are used to treat conditions associated with ongoing inflammation (8). This becomes of particular consideration in the cardiac field as inflammation and immune cells play a prominent role in the early phases of myocardial infarction (see section "Stem Cells, Immunogenicity, and the Infarcted Heart").

MSCs. MSCs have been described as having a range of immunologic traits from immunoprivilege and immunotolerance to immunosuppression, all supported by appropriate in vitro studies (25–35). Adult MSCs derived from the bone marrow and other sources have been reported to express fewer antigens and are considered to be immunotolerant when delivered to allorecipients (29,31–34). Unlike most allogeneic cells, MSCs generally do not generate a T-cell proliferative response in an in-vitro mixed lymphocyte reaction. In addition, cocultivation of cells with MSCs reduces the reaction to other cell types and immune-stimulating molecules, leading to speculation that MSCs may have a local anti-inflammatory effect in vivo (29,32). MSCs have also been reported as capable of bimodal anti-inflammatory and immune-enhancing functions, conferring immune plasticity in these cells (25,33,34).

The ability of MSCs to suppress immune responses, however, has not always held true in in-vivo studies (36–42). In a mouse model, Badillo et al. (40) showed that the introduction of allogeneic MSCs into an immunologically competent mouse elicits both a cellular and humoral host immune response and does not induce tolerance. In a similar study, Poncelet et al. (42) found that allogeneic MSCs, when injected subcutaneously or into ischemic myocardium in pigs, induced both a cellular and humoral response in vivo, despite demonstrating that these cells did not elicit a proliferative T-cell response in vitro. Zangi et al. (39) used direct imaging to examine the survival of luciferase-labeled MSCs transplanted into allogeneic mouse hearts. Although MSCs survived longer than did fibroblasts, MSC survival was significantly shorter in allogeneic recipients than in syngeneic mice, immune-deficient Balb-nude mice, or nonobese diabetic-SCID mice. By using T-cell antigen receptor transgenic mouse recipients, Zangi et al. (39) demonstrated that MSCs may induce memory against allogeneic MHC class I and MHC class II molecules in CD8+ and CD4+ T cells. Similarly, Nauta et al. (36) showed that the injection of allogeneic donor MSCs in naïve mice induced a memory T-cell response. Others have reported that allogeneic MSCs were not effective against graft-versus-host disease because of loss of their immunosuppressive effects in vivo (37,38).

Stem Cells, Immunogenicity, and the Infarcted Heart

Although often overlooked, innate and adaptive immune mechanisms contribute in an important way to the natural history of acute myocardial infarction (2,43). Myocardial infarction leads to an intense inflammatory cell influx within the myocardium and the activation of local and systemic immune signaling pathways. Thus, stem cells are delivered into an immunologically activated milieu when applied after

myocardial infarction, which some investigators consider as a trigger for the up-regulation of stem cell MHC expression (44). Moreover, stem cells themselves can produce various immunomodulatory signaling factors, including both proinflammatory and anti-inflammatory molecules (33,34).

The effects of exogenously administered stem cells and precursor cells on the evolution of myocardial infarction have been studied in mouse and rat models (1,2,13,19,22,45–48). In most of these studies, stem cells were delivered to syngeneic mice or rats, or xenogeneic stem cells (including those from humans) were used in severely immunosuppressed mice or rats, such as nude or SCID mice. Both approaches have been designed to minimize immunologic and inflammatory reactions against stem cells. The benefits of stem cell therapy have varied widely, ranging from minimal to substantial effects on myocardial regeneration and repair. Of note, the inflammatory response to exogenously delivered stem cells has been reported in several rodent models (18–23,36–39,47–49); in 1 study, prominent pathologic calcification was reported (45).

In cell therapy studies in dogs and swine, autologous bone-marrow–derived precursor cells have been shown to improve cardiac function, with minimal effects on the extent of myocardial scarring and no evidence of either transdifferentiation or development of an immune response (50–53). In experimental settings, allogeneic (in dogs [54], swine [55–57], and sheep [58–60]) or xenogeneic (in various animal models [61–66]) cells have been used more frequently than autologous stem cells. In these models, the outcome on myocardial infarction and repair has been similar to that seen with autologous stem cells. Specifically, cell therapy has had beneficial effects on cardiac function, sometimes in association with increased vascularity, but with little evidence of myocardial regeneration (2).

In most reports, little emphasis has been placed on potential inflammatory or immunologic aspects of these models, and detailed histopathologic studies are often lacking. However, critical review of the literature indicates that lymphohistiocytic inflammatory infiltrates are directed at foci of non-self stem cells. In a dog model of myocardial infarction, we observed lymphohistiocytic infiltrates in relationship to administered allogeneic dog stem cells (54). Similar findings have been described in swine and sheep models (56,58). Immunocytochemical studies in dogs have shown that the cells infiltrating at cell injection sites stained positively for T cells (CD3+) and macrophages (54). The residual MSCs showed no evidence of transdifferentiation or proliferation (Ki 67). These observations support the concept that allogeneic stem cells develop increased immunogenicity in vivo (Fig. 3). In a xenogeneic model reported by Kim et al. (65), CD3+ T cells and macrophages were found at the sites of stem cell delivery. In our experience with a similar xenogeneic model, sites of stem cell implantation showed evidence of a very florid lymphohistiocytic infiltrate with a granulomatous component, characterized by the presence of multinucleated giant cells (66) (Fig. 3). This response developed in spite of immunosuppressive therapy with cyclosporine.

The findings described in the preceding text may affect the retention of stem cells delivered into the myocardium. In a rat model, Yasuda et al. (67) examined the retention of male smooth muscle cells in the female normal and infarcted myocardium and found that only 50% of the transplanted cells remained in the area after 1 h, with a progressive decrease in cell survival thereafter. At 1 and 4 weeks after cell transplantation, about half the number of live transplanted cells was retained in infarcted myocardium compared with normal myocardium; the mean number of live cells in the infarcted myocardium was 9% of the original dose at 4 weeks. Although the decrease in cell retention in the normal myocardium points to a baseline wash-out effect, these findings are also consistent with other observations regarding the negative effect of the inflammatory milieu of the infarcted myocardium on the fate of injected stem cells, an effect that may be accelerated by an immune reaction (2). In a mouse myocardial infarction model, administration of either bone marrow cell extract or bone marrow cells resulted in a similar modest improvement in ejection fraction (68). Although bone marrow cells were identified in the myocardium by a prominent green fluorescence protein signal at 1 day, the fluorescent signal in the myocardium was minimal by 25 days. The investigators (68) postulated that intact stem cells may not be necessary for a functional effect and that death of implanted cells may initiate a major component of the benefit. The low retention rates described in these studies may result from a combination of stem cell death and wash-out of viable cells. In vivo imaging techniques hold promise for providing dynamic information regarding the fate of exogenously administered stem cells (69).

Approaches to Circumvent the Immune Response

Several strategies have been identified to control the immune and inflammatory responses targeted to non-self, activated stem cells. However, given the complexity of the system, experimental approaches are still under way. Several strategies have been previously reviewed in detail (8,9,29). Administration of naive, nonactivated MSCs has been beneficial in controlling graft versus host disease. However, studies described in this review (36–42) indicate that chronic exposure of non-self MSCs to an inflamed tissue is likely to lead to their activation and elicitation of a targeted immune response.

The use of immunosuppressants such as cyclosporine and mycophenolate acid seems to achieve only a suboptimal effect in dampening the immune response in the setting of allogeneic stem cell delivery, particularly in large-animal models. In a recent study, Poncelet et al. (70) showed that the addition of a short course of tacrolimus may overcome the immune response to intracardiac-transplanted allogeneic MSCs, while also preserving cell viability.

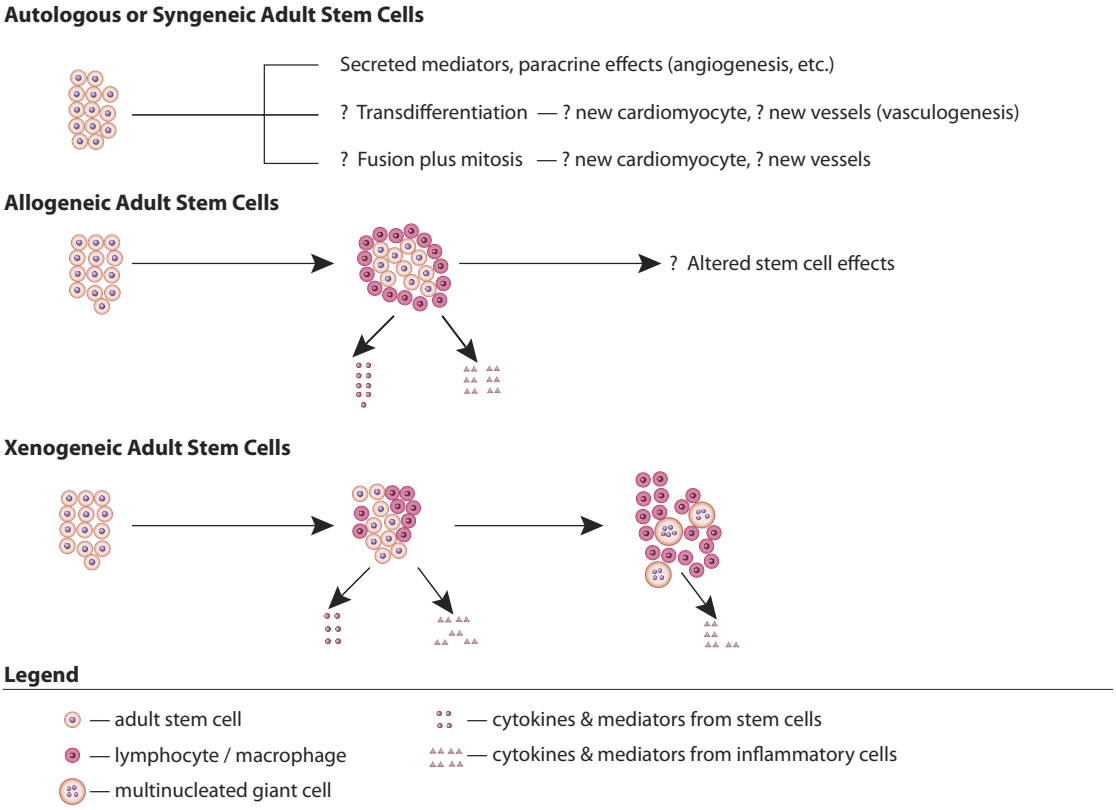

Figure 3 **Host Reactions to Adult Stem Cells Implanted Into the Myocardium Under Autologous, Syngeneic, Allogeneic, and Xenogeneic Conditions**

Adult stem cells implanted into myocardium of autologous or syngeneic recipients may undergo some proliferation and secrete chemical mediators that contribute to paracrine effects, leading to the proliferation of native blood vessels (angiogenesis) and effects on cardiomyocyte function. Transplanted stem cells may result in limited myocardial regeneration through transdifferentiation into new cardiomyocytes and new blood vessels (vasculogenesis) and/or by fusion with existing myocardial cells and subsequent mitosis that produces new cells. However, major remuscularization of the myocardium by implanted stem cells has yet to be consistently demonstrated. Furthermore, stem cells injected into allogeneic recipients induce a targeted lymphohistiocytic inflammatory reaction, which likely leads to significant and accelerated destruction of the stem cells. This reaction is exaggerated in the xenogeneic setting in which stem cells of 1 species are implanted into the myocardium of a different species.

As discussed in the preceding text, undifferentiated human ESCs produce teratomas in immunodeficient animals and trigger an intense inflammatory and immunologic reaction in immunocompetent animals. In a new approach designed to overcome these limitations, ESCs have been driven toward cardiomyocyte differentiation in vitro and then delivered as myocytic precursor cells in animal models of cardiac injury (71). Thus, these committed cells most likely develop an intermediate level of immunogenicity (71). However, long-term studies showed that the effects of these committed cells on myocardial recovery were not sustained (72).

A new technology—induced pluripotent stem (iPS) cells—is under intense investigation and may provide an innovative way to help circumvent the immune response. This approach involves genetically reprogramming adult somatic cells from various body sites to resemble ESCs (73). These dedifferentiated cells share similar morphology, gene expression profiles, and differentiation potential with ESCs. The forced differentiation of iPS cells to a cardiomyocyte lineage has been demonstrated (74). Using patient-derived iPS cells has the obvious ethical advantage over ESCs and the theoretical benefit of avoiding the immune response generated with an allogeneic approach. However, other issues such as cell longevity and cancer development may be of concern with the use of iPS cells.

Clinical Implications

The full range of implications and effects of the host response to allogeneic and xenogeneic stem cells requires further examination. Although definitive conclusions cannot be reached, some evidence-based speculation can be made. It is possible that inflammatory cells contribute chemical mediators to the paracrine effects triggered by the stem cells, or the inflammatory and immunologic reactions may lead to the destruction of stem cells and the blunting of their potential effects.

In considering the implications of experimental studies for clinical application, species variability should be considered. Human MSCs and ESCs may have lower immunogenicity in vivo than cells from other species. Clinical programs involving the administration of proprietary allogeneic human MSCs to patients with heart disease are under way (75,76). The launching of such programs has been based almost exclusively on the initial presumption of the immunotolerance of allogeneic stem cells. To date, adverse effects or safety issues associated with the use of allogeneic MSCs have not been reported in clinical trials (32,75–77), although cellular and humoral responses in the myocardium have not been well studied.

Because of untoward consequences of human gene therapy trials, concerns have been raised regarding clinical trials with stem cells, particularly human ESCs, given that information derived from experimental work is not comprehensive (78,79). The important considerations include oncogenicity and immunogenicity. The latter issues relate to the possibility that donor cells/tissue may eventually express human leukocyte antigens that would activate immunologic and inflammatory responses, leading to uncertain outcomes.

Conclusions

Considerable interest has developed in the clinical applications of ESCs and MSCs, mostly due to their immunoprivileged status. This protected status is now being brought into question. Naïve stem cells appear to display immunomodulatory properties in vitro; however, the effect of transplantation itself, as well as delivery to a hostile environment, can trigger increased immunogenicity in these cells.

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