

# Chapter 2

## The Immunogenicity of ES Cells and Their Differentiated Progeny

Jeremy I. Pearl and Joseph C. Wu

**Abstract** Embryonic stem (ES) cells are an attractive source for tissue regeneration and repair therapies. This is because in contrast to adult stem cells, ES cells possess unlimited self-renewal and pluripotent capacity. However, for the therapeutic application of ES cells to succeed, the transplanted ES cells must engraft successfully and survive long enough to exert a therapeutic effect. An important obstacle facing the in vivo engraftment and function of ES cells is the immunogenic barrier. In this chapter, we will begin by briefly discussing the safety concerns regarding the transplantation of ES cells and the factors that influence the behavior or misbehavior of transplanted ES cells. We will then discuss the in vitro immunogenic properties of ES cells, including the expression of major histocompatibility (MHC) antigens and minor histocompatibility (mH) antigens and how these properties evolve as undifferentiated cells mature towards more differentiated derivatives. We will also highlight the various (and in some instances conflicting) conclusions regarding the immunogenic properties of ES cells which have been drawn from prior in vitro studies and will conclude with a more extensive discussion of the immunogenic properties of ES cells when transplanted across allogeneic as well as xenogeneic immune barriers.

### 2.1 Introduction

Embryonic stem (ES) cells are a promising option to regenerate tissues and organs. The ability to differentiate into different cell types has stimulated research in generating neurons [1–3], cardiomyocytes [4], hepatocytes [5], hematopoietic

---

J. I. Pearl · J. C. Wu (✉)

Stanford University School of Medicine, Lorrey I Lokey Stem Cell Research Building,  
265 Campus Drive, Rm G1120B, Stanford, CA 94305-5454, USA  
e-mail: joewu@stanford.edu

progenitor cells [6], pancreatic beta cells [7], and other cell types for potential clinical applications. This area of research is generating unprecedented interest in the scientific community because of the expectation of a new horizon in clinical medicine, but thus far it has also been plagued by ethical controversies, potentially unrealistic timelines, and practical hurdles to therapy. With respect to the latter, one of the most vexing and underappreciated problems is immune rejection of ES cells after transplantation into the recipient [8]. This occurs because ES cell-derived therapeutic cells are not “self derived” and can therefore result in an aggressive immune response from the recipient. Another potential problem with ES cell therapy is the potential of undifferentiated ES cells to form teratomas after transplantation. In this chapter, we will first review studies that have attempted to define the potential for ES cell-derived teratoma formation. We will then discuss the data characterizing the *in vitro* immunogenic properties of ES cells and the evidence demonstrating *in vivo* immune rejection of ES cells when transplanted across allogeneic and xenogeneic barriers.

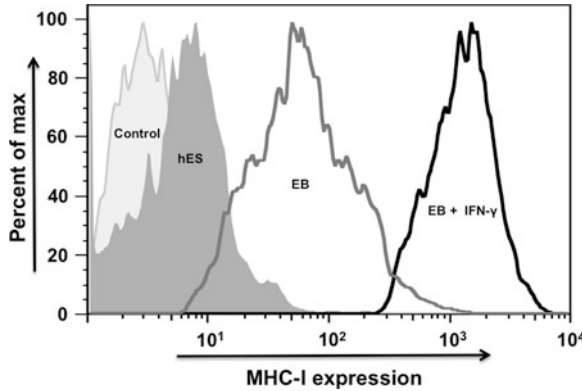
## 2.2 Teratoma Formation

Teratomas are benign germ cell tumors, which in humans occur most often in the gonads, but may occasionally be found in extragonadal sites such as the anterior mediastinum or retroperitoneum [9]. Teratomas differ from most tumors because they are a mixture of many tissue types, whereas most tumors represent a limited diversity of neoplastic cell types. Histological evaluation of a teratoma by definition will demonstrate tissues from all three embryonic germ layers, that are haphazardly arranged throughout the tumor in a way that partly resembles a disorganized embryo [10]. There presently exists no method capable of generating a 100 % pure population of differentiated cells from a pluripotent donor source. Therefore, it is exceedingly difficult to confirm that a preparation of therapeutic cells is not contaminated by residual pluripotent ES cells that have escaped the differentiation process and, consequently, teratoma development is of significant clinical concern [11]. The potential for teratoma formation is influenced by multiple factors, including the immune system [12], transplanted cell number [13], and graft site [14]. The influence of cell number on the potential for teratoma formation is clinically relevant because it establishes a threshold by which to gauge the number of contaminating undifferentiated ES cells that may reliably produce teratoma formation upon transplantation. A previous report investigating the relationship between human ES (hES) cell number and teratoma formation demonstrated that consistent teratoma formation in immunodeficient mice depends both on cell number and transplantation site [13]. Teratoma formation upon transplantation in the myocardium and the skeletal muscle requires  $\sim 1 \times 10^5$  and  $\sim 1 \times 10^4$  hES cells, respectively. This suggests a critical threshold for the number of undifferentiated hES cells to produce teratoma formation. Additionally, the *in vivo* graft site can influence the propensity of

transplanted hES cells to remain in the undifferentiated state. This is exemplified by the observation that when hES cells are intrahepatically versus subcutaneously transplanted into immunodeficient mice, the number of cells that remain undifferentiated and the kinetics of teratoma formation are both enhanced [14]. It is thought that the highly vascular and growth-factor rich environment of the liver may explain why hES cells are less prone to differentiation when transplanted intrahepatically versus subcutaneously [14]. Although these studies involved immunodeficient mice, they illustrate the influence that cell number and transplant location exert on ES cell survival, differentiation, and behavior. As will be discussed in more detail, the literature regarding the immunogenicity of ES cells is rather controversial because there exist numerous reports that have drawn directly conflicting conclusions. When the immunogenicity of ES cells is evaluated based on cell survival in immunocompetent animals, differences in experimental design similar to those mentioned above may help explain the conflicting conclusions.

### 2.3 Cell Surface Expression of Immunogenic Molecules

Numerous groups have attempted to characterize the immunogenic properties of hES cells by assaying their surface expression of potentially “immunogenic” molecules. The first study to do so focused on the expression of major histocompatibility complex (MHC) antigens. MHC antigens are a critical group of antigens that are classically associated with transplant rejection. MHC antigens are divided into class I, which are expressed by most human cells, and class II, which are generally restricted to antigen presenting cells, macrophages, and B-cells [15]. In the undifferentiated state, hES cells express low levels of MHC class I and minimal levels of MHC class II [16]. However, exposing hES cells to IFN- $\gamma$  induces the expression of MHC class I. Similarly, allowing the cells to undergo spontaneous differentiation into embryoid bodies (EB), which are three-dimensional structures composed of an amalgam of hES-derived cell types, stimulates increased MHC class I expression [16] (Fig. 2.1). Interestingly, the expression of MHC class II and co-stimulatory molecules (e.g., CD80, CD84, CD40) by hES cells appears to be very low, and neither incubation with IFN- $\gamma$  nor spontaneous differentiation into EBs induces any substantial increases in the expression of these proteins [17]. The hES cell expression of negative immunoregulatory proteins and cytokines has also been assessed. hES cells do not express the cell surface protein CD95 ligand (Fas ligand), which is a known inducer of apoptosis [18] nor do they secrete the immunosuppressive cytokine interleukin-10 [19]. Thus, in the differentiated state, hES cells possess a comparable cell surface expression pattern to the majority of human cells (e.g., fibroblasts), consisting of MHC class I but not MHC class II, costimulatory molecules, and negative immunoregulatory molecules.



**Fig. 2.1** Florescent activated cell sorting analysis of MHC class I expression by hES cells and their differentiated derivatives. The MHC class I expression increases as hES cells differentiate or are incubated with IFN- $\gamma$ . Control = isotype matched control, hES = undifferentiated hES cells, EB = hES-derived embryoid bodies, EB + IFN- $\gamma$  = EBs incubated with IFN- $\gamma$

## 2.4 In Vitro Immunogenic Properties

To evaluate the in vitro immune response towards hES cells, previous groups have performed mixed leukocyte reactions (MLR), with hES cells serving as the immune “stimulator” cell population. The results from these in vitro assays have produced conflicting results regarding the severity of the immune response elicited by hES cells. One study demonstrated that hES cells do not stimulate proliferation of allogeneic human peripheral blood mononuclear cells (hPBMC), nor do hES cells stimulate proliferation of allogeneic human peripheral blood lymphocytes (hPBL) [17]. Similarly, when the hES cells were differentiated into EBs or incubated with IFN- $\gamma$ , only minimal T cell proliferation was observed. These results seem to indicate that in the undifferentiated state, when they have only marginal MHC class I expression, hES cells induce limited immune stimulation. Surprisingly, when the hES cells differentiate and increase their expression of MHC class I, they still do not provoke immune activation. These results led to the suggestion that hES cells might possess unique immune privileged characteristics [17]. In addition to evidence indicating that hES cells do not themselves activate responder leukocytes, there is evidence suggesting that hES cells actively inhibit the activation of responder leukocytes. Specifically, it has been shown that the inclusion of hES cells in a MLR involving responder hPBL and allogeneic stimulator dendritic cells results in decreased hPBL proliferation compared to allogeneic dendritic cells and hPBMCs alone [17]. This indicates that hES cells actively inhibit the allogeneic T cell response towards third party antigens.

In contrast, other studies using MLRs to characterize the immunogenicity of hES cells have reached opposite conclusions. A MLR using human CD4<sup>+</sup> T cells and dendritic cells from the same donor, mixed with allogeneic hES cells,

demonstrated not only that hES cells lack an inhibitory effect on T cell proliferation, but that hES cells induce T cell proliferation [20]. The level of T cell proliferation stimulated by the hES cells was comparable to that induced by allogeneic human fibroblasts, but it was four-fold less than that induced by allogeneic dendritic cells. This may be because hES cells and fibroblasts express MHC class I, but both lack expression of MHC class II and costimulatory molecules, whereas mature dendritic cells display MHC class I, MHC class II, and costimulatory molecules such as CD80, CD86, and CD40 which confer upon them the potent capacity for T cell activation.

These two studies attempted to define the *in vitro* immunogenic properties of hES cells using MLRs and arrived at contradictory conclusions. The conflicting results likely reflect heterogeneity in experimental design between the two studies. Using a different *in vitro* approach, a third group concluded that hES cells possess a level of immunogenicity that is intermediate to that described by the two studies discussed above. They found that primed cytotoxic T lymphocytes (CTL), have the capacity to recognize and lyse hES cells if the hES cells are rendered sufficiently immunogenic [21]. In this study, CTLs were primed to recognize HLA-A2 antigens because hES cell lines H9 and H13 express HLA-A2. The CTLs were primed by co-culture with irradiated hPBMCs expressing HLA-A2 and loaded with influenza type A peptide (IV/A). When the primed CTLs were mixed with hES cells loaded with IV/A, the CTLs did not lyse the hES cells. However, when the hES cells were infected with influenza virus and MHC class I expression was induced by incubation with IFN- $\gamma$ , efficient CTL mediated lysis was observed. This indicates that with the proper peptide-loading method and sufficient expression of MHC class I, CTLs can recognize and lyse hES cells.

## 2.5 Allogeneic Transplantation of ES Cells

Much of our understanding regarding the immunogenic properties of ES cells has come from the study of mouse ES (mES) cells transplanted into a murine host, because this represents an allogeneic *in vivo* transplantation scenario. Whether the conclusions drawn from the mouse model system can reliably be extrapolated to the human scenario remains to be determined. One of the first reports indicating that ES cells may be immunogenic involved intramyocardial transplantation of mES cells following myocardial infarction [22]. The allogeneic graft site was infiltrated by a significant cellular infiltrate composed of T cells and dendritic cells, and analysis of the host sera demonstrated the presence of alloantibodies. This cellular and humoral immune response was progressive, increasing in intensity from 1 to 4 weeks following transplantation and correlated with the increased expression of MHC class I antigens by mES cells [22].

A similar study involving transplantation of mES cells into ischemic myocardium demonstrated that the allogeneic immune response is of sufficient intensity to prevent the long-term engraftment of mES cells across histocompatibility

barriers [23]. Allogeneic mES cell grafts incited a mild CD4<sup>+</sup> T cell dominated inflammatory infiltrate at 1 week post transplantation, which progressed towards a severe inflammatory infiltrate composed of both CD4<sup>+</sup> and CD8<sup>+</sup> T cells at 4 and 8 weeks after transplantation. In contrast, syngeneic mES cell grafts produced a limited inflammatory infiltrate that was comparable to the sham procedure group at all time points. At 8 weeks after transplantation, mES cells were still detected in syngeneic recipients. By comparison, no evidence of allogeneic mES cell engraftment was observed. The above results were confirmed by a different group that similarly investigated mES cell survival after intramyocardial transplantation into allogeneic and syngeneic recipients [24]. At 3 weeks post transplantation, the inflammatory infiltrate was significantly greater in allogeneic compared to syngeneic grafts. At later time points, the allogeneic grafts were completely rejected, whereas the syngeneic grafts survived indefinitely [24]. In regard to the immunogenicity of mES cells, when mES cells are transplanted across histocompatibility barriers, engraftment will be significantly limited by the host alloimmune response.

If ES cells are recognized as antigenic by the host adaptive immune system, the host will generate immune memory cells with specificity towards these antigens. Upon future exposure to the antigens, these memory cells will orchestrate a more rapid and robust immune response. This was previously demonstrated for mES cells transplanted into MHC-mismatched hosts [12]. When mES cells were intramuscularly transplanted (gastrocnemius muscle) into syngeneic recipients, intramuscular teratoma formation was observed in all recipients by day 28. In contrast, no evidence of mES cell survival was observed in allogeneic recipients at day 28, presumably because of alloantigen specific rejection of the transplanted mES cells [12]. To test if immunologic memory was induced towards mES cells, the kinetics of the secondary immune response were compared to this primary immune response. Upon repeated exposure, mES cells were rejected by day 7, demonstrating an accelerated immune response relative to the 21–28 days required for rejection during the primary exposure [12]. This indicates that immune memory cells are generated as a result of the adaptive immune response against allogeneic mES cells.

The immunogenicity of ES cells may also depend on the differentiation state of the graft. When ES cells differentiate or are exposed to an inflammatory environment (e.g., IFN- $\gamma$ ), MHC class I expression is increased [12, 16, 17]; this may result in a heightened allogeneic immune response to the ES cells. This was addressed by two experiments comparing the survival of undifferentiated and differentiated mES cells transplanted into MHC-mismatched hosts [12]. The first experiment demonstrated that undifferentiated mES cells are immunologically rejected by day 28, but if the cells are allowed to first differentiate *in vivo* and are then isolated and re-transplanted, they are rejected by day 14. The second experiment demonstrated that if a very large number ( $\sim 1 \times 10^7$ ) of undifferentiated mES cells are transplanted into allogeneic recipients, a minority of the grafts ( $\sim 20\%$ ) overcome immune rejection and form teratomas [12]. However, if the mES cells are first allowed to differentiate *in vitro* prior to transplantation, none of the grafts will escape immunological rejection. This accelerated cell death and

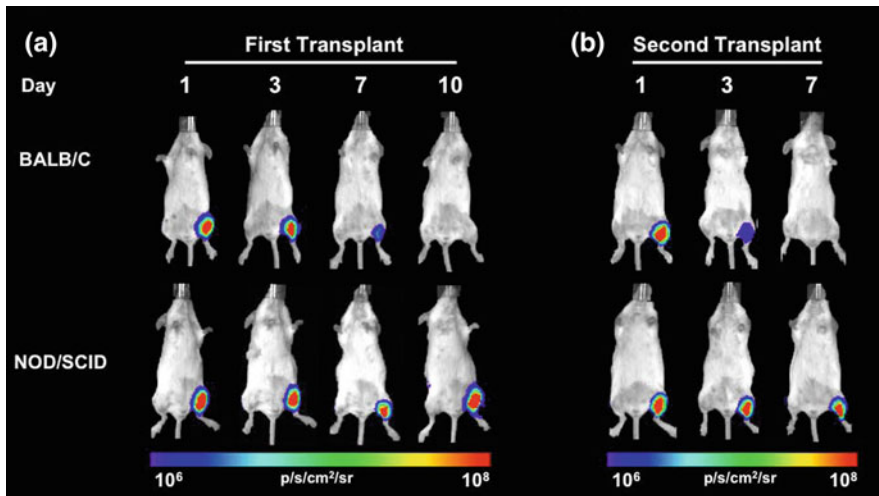
diminished survival may reflect increased immunogenicity of the differentiated mES cells, but is also compatible with the view that when the cells differentiate, their proliferation rate decreases and they become more vulnerable to the immune response than the highly proliferative undifferentiated cells.

The previous examples have demonstrated that transplantation of mES cells across allogeneic MHC barriers can result in immune-mediated rejection. However, in addition to MHC antigens, ES cells express minor histocompatibility (mH) antigens that may contribute to their immunogenicity. The potential impact of mH antigens was investigated by transplanting mES cell-derived EBs into mH antigen mismatched, but MHC matched hosts [25]. EBs which differed only at mH loci were rejected with similar kinetics as both fully MHC-mismatched EBs and MHC-mismatched skin grafts [25]. This finding demonstrates that mES derivatives may be as vulnerable to immune rejection as other types of grafts. Furthermore, it indicates that matching donor and host MHC antigens may not be sufficient to prevent graft rejection, and thus some form of immune intervention will likely be necessary.

## 2.6 Xenogeneic Transplantation of ES Cells

Similar to the mES cell studies, mixed conclusions have been reached regarding the immunogenicity of hES cells. Due to ethical constraints, the *in vivo* immunogenic properties of hES cells have not been studied in a true allogeneic scenario (i.e., human transplantation). Instead the majority of studies have either investigated the immunogenicity of hES cells *in vitro* or in the xenogeneic transplantation setting using rodents as the experimental host.

One of the earlier studies suggesting that hES cells may possess some form of immune privilege involved transplantation of hES cells into the quadriceps muscle of immunocompetent mice. Using histopathological techniques, the investigators were unable to detect an appreciable inflammatory infiltrate at 24 and 48 h after injection. This finding indicates that hES cells may not induce a significant inflammatory infiltrate at the early time points assayed. However, studies which assayed *later* time points demonstrated signs of immune-mediated rejection by 3 days with escalating intensity at 5–7 days post transplantation [20]. The inflammatory cells which infiltrate the hES cell graft are predominantly T and B-cells, indicating the involvement of the adaptive immune system [26]. However, neutrophils and macrophages are also present, likewise suggesting the involvement of the innate immune system [26]. To demonstrate that the immune rejection of xenogeneic hES cells was not unique to a certain mouse strain, a different group of investigators transplanted hES cells into 4 different immunocompetent mouse strains. They found that at 1 month post transplantation, every immunocompetent animal rejected the hES cells, whereas all immunodeficient mice accepted the grafts and demonstrated teratoma formation [21]. The rejection of hES cells appears to be predominantly orchestrated by the CD4<sup>+</sup> T cell subset. When hES cells were transplanted into CD4<sup>-/-</sup> mice, their survival was significantly



**Fig. 2.2** In vivo visualization of hES cell survival. **a** Representative bioluminescent images (BLI) of hES cell transplanted naïve animals (*first transplant*) shows a rapid decrease in BLI signal in immunocompetent (BALB/c) mice, compared to immunodeficient NOD/SCID mice, reaching background levels at day 10 after transplantation. **b** In BALB/c animals that were presensitized with nontransduced hES cells (injection in *right leg*), accelerated loss of the BLI signal was seen upon second transplantation (injection in *left leg*) due to prior pre-sensitization, reaching background intensity by day 7. Color scale bar values are in photons per second per square centimeter per steradian (p/s/cm<sup>2</sup>/sr)

prolonged relative to that observed in CD8<sup>-/-</sup> mice [26]. Interestingly, hES cells were eventually immunologically rejected (albeit with differing kinetics) and failed to engraft in both CD4<sup>-/-</sup> and CD8<sup>-/-</sup> mice, indicating that either T cell subset was sufficient to prevent hES cell engraftment. However, T cell deficient nude mice were unable to reject hES cells, indicating that at least one of the two T cell subsets was necessary for hES cell rejection [26].

The xenogeneic immune response towards hES cells is an adaptive immune response that consists of both a humoral and cellular arm. Murine splenocytes that have been exposed to hES cells in vivo produce significantly increased levels of both IFN- $\gamma$  and IL-4 cytokines compared to splenocytes isolated from naïve mice never exposed to hES cells [26]. IFN- $\gamma$  is produced by T-helper type-1 (Th1) cells which classically induce a cellular immune response. IL-4 is produced by T-helper type-2 (Th2) cells which facilitate the humoral immune response. Indeed, following hES cell transplantation, there are increased quantities of xeno-reactive antibodies in recipient sera relative to control mice. Further proof that hES cells can elicit an adaptive immune response is that, like mES cells, hES cells can stimulate the production of immune memory cells. Primary transplantation of hES cells will result in the complete immunological rejection of xenogeneic grafts by 7–10 days post transplantation. If the same animals are transplanted 14 days after primary challenge with the same number of hES cells, the secondary immune response will accelerate hES cell death and produce complete immunologic

**Table 2.1** Summary of previous studies demonstrating immune rejection of transplanted ES cells

Cell type	Transplant type(s)	Transplant site	Results	Ref
mES cells: undifferentiated	<ul style="list-style-type: none"> <li>• Syngeneic</li> <li>• Fully allogeneic</li> </ul>	Intramyocardial	<ul style="list-style-type: none"> <li>• Increasing infiltration of graft by inflammatory (T and Dendritic) cells from 1–4 weeks</li> <li>• Increasing humoral antibody response from 2–4 weeks</li> <li>• Allogeneic graft incited a Th-1 type immune response consisting of IFN-<math>\gamma</math> and IL-2</li> </ul>	[22]
mES cells: undifferentiated	<ul style="list-style-type: none"> <li>• Syngeneic</li> <li>• Fully allogeneic</li> </ul>	Intramyocardial	<ul style="list-style-type: none"> <li>• At 4 weeks teratoma formation was apparent in both syngeneic and allogeneic hosts</li> <li>• At 8 weeks teratomas persisted in syngeneic hosts, but no cells were detected in allogeneic hosts</li> <li>• Increasing infiltration of allogeneic grafts by inflammatory cells from 1–8 weeks</li> </ul>	[23]
mES cells: undifferentiated	<ul style="list-style-type: none"> <li>• Syngeneic</li> <li>• Fully allogeneic</li> </ul>	Intramyocardial	<ul style="list-style-type: none"> <li>• Teratoma formation in syngeneic hosts at 3 weeks which remained at 5 weeks</li> <li>• Teratoma formation in allogeneic hosts at 3 weeks but 90 % of grafts rejected at 5 weeks</li> <li>• Progressively increasing infiltration of allogeneic grafts by inflammatory cells from 3–5 weeks</li> </ul>	[24]
mES cells: embryoid bodies	<ul style="list-style-type: none"> <li>• Syngeneic</li> <li>• Fully allogeneic</li> <li>• mH antigen mismatch</li> </ul>	Kidney capsule	<ul style="list-style-type: none"> <li>• Syngeneic grafts became well vascularized and increased in size</li> <li>• Fully allogeneic grafts were infiltrated by T cells and macrophages and rejected in all recipients by day 16</li> <li>• Grafts which differ solely at mH antigens were rejected at a rate similar to fully allogeneic grafts</li> </ul>	[25]
mES cells: undifferentiated and differentiated	<ul style="list-style-type: none"> <li>• Syngeneic</li> <li>• Fully allogeneic</li> </ul>	Intramuscular	<ul style="list-style-type: none"> <li>• Syngeneic grafts develop into teratomas by day 28</li> <li>• Allogeneic grafts are fully rejected by day 28 upon primary and by day 7 upon secondary transplantation</li> <li>• Undifferentiated allogeneic cells are rejected by day 28, whereas differentiated grafts are rejected by day 14</li> </ul>	[12]

(continued)

Table 2.1 (continued)

Cell type	Transplant type(s)	Transplant site	Results	Ref
hES cells: undifferentiated	• Xenogeneic	Intramyocardial	• Xenogeneic grafts were infiltrated by T cells and macrophages	[20]
hES cells: undifferentiated	• Xenogeneic	Kidney capsule	• Xenogeneic grafts were rejected by day 4 in immunocompetent mice	
hES cells: undifferentiated and differentiated	• Xenogeneic	Intramuscular	• Xenogeneic grafts were rejected by 1 month in all 4 mouse strains tested	[21]
			• Grafts were infiltrated by T cells (CD4 <sup>+</sup> and CD8 <sup>+</sup> ), B-cells and neutrophils	[26]
			• Xenogeneic grafts elicited a Th-1 type (IFN- $\gamma$ ) and Th-2 type (IL-4) immune response	
			• Donor grafts were undetectable by day 7 upon primary and day 3 upon secondary transplantation	

rejection by day 3 after transplantation [26] (Fig. 2.2). This indicates that hES cells were recognized as antigenic upon primary exposure, leading to the generation of immune memory cells that produced an accelerated adaptive, donor-specific immune response upon secondary immune challenge.

## 2.7 Conclusion

The pluripotent capability of ES cells highlights their potential for future therapeutic applications in regenerative medicine to treat numerous intractable illnesses. However, this pluripotency also underlies the potential risk of teratoma formation if undifferentiated cells are transplanted. Similarly, the immunogenicity of ES cells represents one of the major barriers precluding the successful translation of ES cell based therapies. The immunogenic characteristics of ES cells are dynamic and in constant flux depending on the differentiation state and environment surrounding the ES cells. When ES cells are in the undifferentiated state their high proliferation rate and low expression of potentially immunogenic surface proteins presents an elusive target for the immune system. However, when the cells differentiate and increase their expression of immunogenic cell surface markers, they are placed at increased risk for immunologic rejection. This risk for immune rejection has been demonstrated for mES cells in the allogeneic in vitro and in vivo setting and for hES cells in the allogeneic in vitro and xenogeneic in vivo scenario (Table 2.1). A critical area of investigation for the future success of regenerative medicine will focus on strategies to combat immunological rejection or to induce immunologic tolerance towards ES cells. For the successful development of these approaches, investigators must identify the antigenic components of ES cells that contribute to their immunogenicity, as well as gain a better understanding of the in vivo behaviour of ES cells.

**Acknowledgments** This work was supported by NIH R01 AI085575.

## References

1. Zhang SC, Wernig M, Duncan ID, Brustle O, Thomson JA (2001) In vitro differentiation of transplantable neural precursors from human embryonic stem cells. *Nat Biotechnol* 19:1129–1133
2. Kim JH et al (2002) Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. *Nature* 418:50–56
3. McDonald JW et al (1999) Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. *Nat Med* 5:1410–1412
4. Cao F et al (2008) Transcriptional and functional profiling of human embryonic stem cell-derived cardiomyocytes. *PLoS ONE* 3:e3474
5. Chinzei R et al (2002) Embryoid-body cells derived from a mouse embryonic stem cell line show differentiation into functional hepatocytes. *Hepatology* 36:22–29

6. Chadwick K et al (2003) Cytokines and BMP-4 promote hematopoietic differentiation of human embryonic stem cells. *Blood* 102:906–915
7. Soria B et al (2000) Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. *Diabetes* 49:157–162
8. Bradley JA, Bolton EM, Pedersen RA (2002) Stem cell medicine encounters the immune system. *Nat Rev Immunol* 2:859–871
9. Damjanov I, Solter D (1974) Experimental teratoma. *Curr Top Pathol* 59:69–130
10. Ulbright TM (2005) Germ cell tumors of the gonads: a selective review emphasizing problems in differential diagnosis, newly appreciated, and controversial issues. *Mod Pathol* 18(Suppl 2):S61–S79
11. Li JY, Christophersen NS, Hall V, Soulet D, Brundin P (2008) Critical issues of clinical human embryonic stem cell therapy for brain repair. *Trends Neurosci* 31:146–153
12. Swijnenburg RJ et al (2008) In vivo imaging of embryonic stem cells reveals patterns of survival and immune rejection following transplantation. *Stem Cells Dev* 17:1023–1029
13. Lee AS et al (2009) Effects of cell number on teratoma formation by human embryonic stem cells. *Cell Cycle* 8:2608–2612
14. Cooke MJ, Stojkovic M, Przyborski SA (2006) Growth of teratomas derived from human pluripotent stem cells is influenced by the graft site. *Stem Cells Dev* 15:254–259
15. Janeway CA, Mamula MJ, Rudensky A (1993) Rules for peptide presentation by MHC class II molecules. *Int Rev Immunol* 10:301–311
16. Drukker M et al (2002) Characterization of the expression of MHC proteins in human embryonic stem cells. *Proc Natl Acad Sci U S A* 99:9864–9869
17. Li L et al (2004) Human embryonic stem cells possess immune-privileged properties. *Stem Cells* 22:448–456
18. Grinnem DR, Ferguson TA (2001) The role of Fas ligand in immune privilege. *Nat Rev Mol Cell Biol* 2:917–924
19. Grinnemo KH, Sylven C, Hovatta O, Dellgren G, Corbascio M (2008) Immunogenicity of human embryonic stem cells. *Cell Tissue Res* 331:67–78
20. Grinnemo KH et al (2006) Human embryonic stem cells are immunogenic in allogeneic and xenogeneic settings. *Reprod Biomed Online* 13:712–724
21. Drukker M et al (2006) Human embryonic stem cells and their differentiated derivatives are less susceptible to immune rejection than adult cells. *Stem Cells* 24:221–229
22. Kofidis T et al (2005) They are not stealthy in the heart: embryonic stem cells trigger cell infiltration, humoral and T-lymphocyte-based host immune response. *Eur J Cardiothorac Surg* 28:461–466
23. Swijnenburg RJ et al (2005) Embryonic stem cell immunogenicity increases upon differentiation after transplantation into ischemic myocardium. *Circulation* 112:I166–I172
24. Nussbaum J et al (2007) Transplantation of undifferentiated murine embryonic stem cells in the heart: teratoma formation and immune response. *FASEB J* 21:1345–1357
25. Robertson NJ et al (2007) Embryonic stem cell-derived tissues are immunogenic but their inherent immune privilege promotes the induction of tolerance. *Proc Natl Acad Sci U S A* 104:20920–20925
26. Swijnenburg RJ et al (2008) Immunosuppressive therapy mitigates immunological rejection of human embryonic stem cell xenografts. *Proc Natl Acad Sci U S A* 105:12991–12996



<http://www.springer.com/978-1-4614-5479-3>

The Immunological Barriers to Regenerative Medicine

Fairchild, P.J. (Ed.)

2013, XIV, 334 p., Hardcover

ISBN: 978-1-4614-5479-3

A product of Humana Press