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Neonatal desensitization does not universally prevent xenograft rejection

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To the Editor

Human cells transplanted into immunocompetent animals are generally rejected within 2–4 weeks¹. Severe immune suppression is capable of protecting the grafted cells; however, such treatment is associated with unacceptable side effects². An important advancement in the field was the introduction of immunodeficient animal models that accept xenografts, but these are poor models for human diseases³. A paper published in *Nature Methods* proposed neonatal desensitization without an overall suppression of the immune system⁴ as an alternative. Encouraged by this study, we attempted to use neonatal desensitization to protect grafts of two types of human cells in mice and rats (Fig. 1). The experiments were conducted in two independent laboratories.

The first cell type we tested was immortalized, luciferase-expressing human glial-restricted precursor (hGRP) cells derived from fetal brain (Q Therapeutics). We induced neonatal desensitization by intraperitoneal injection of 1×10^5 hGRP cells into BALB/c mice. The experiment was repeated three times using a total of 37 mice (Supplementary Methods). hGRPs initially produced a strong bioluminescence imaging (BLI) signal; but a dramatic loss of signal was observed within a few days, indicating rapid elimination of transplanted cells in the neonates (Fig. 1a). Once the desensitized animals reached maturity, 3×10^5 hGRP cells were injected into the brain parenchyma; parallel transplantations were conducted in nondesensitized littermates as controls. We monitored the survival of transplanted cells via BLI. Initially, transplanted hGRPs produced a strong BLI signal, which denoted robust survival. However, the signal gradually decreased over time and was undetectable after 2 weeks (Fig. 1b). We observed no difference in the time course of signal loss between desensitized and control animals.

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To validate the BLI results, we performed immunohistochemistry for the human-specific marker HuNu (Fig. 1c) and luciferase (Fig. 1d) on the brain sections of animals that had received human cell transplants. The transplantation site could be easily identified by the area of focal CD45⁺ immune-cell infiltration. The intensity of this infiltration was similar in desensitized (Supplementary Fig. 1a) and control (Supplementary Fig. 1b) animals. Quantification of fluorescence signal for both groups did not show any significant difference (Fig. 1g). hGRP cells transplanted into *rag2*^{-/-} mice (*n* = 5 mice, in parallel with the last cohort of desensitized mice) survived well for several weeks (Fig. 1e).

The second cell type we tested was a nontransformed neural stem cell line derived from human umbilical cord blood (HUCB-NSC). We induced neonatal desensitization by intraperitoneal injection of 1×10^5 HUCB-NSC cells into Wistar rats (five independent experiments with a total of 35 rats). We transplanted 2×10^5 HUCB-NSC cells into the brain of 6-week-old animals (desensitized or controls), which were then killed at different time points (1, 3, 7, 14 and 21 d after transplantation) for histological detection of cell survival using immunohistochemistry against a human-specific marker. We detected many human cells 1 and 3 d after transplantation (Fig. 1h,i), but this number decreased substantially at later time points (7 and 14 d) (Fig. 1j,k). Almost no cells were detectable at day 21 (Fig. 1l). We confirmed good survival of HUCB-NSC cells in *rag2*^{-/-} mice (Fig. 1f). Although some immunodeficient rats are available, *rag2*^{-/-} mice are known to be superior in accepting cellular grafts and were therefore used as the control in our rat experiments. The longest follow-up of grafted HUCB-NSC cells in *rag2*^{-/-} mice was 5 weeks.

In our hands, the neonatal desensitization method failed to induce specific tolerance of the recipient animals to transplanted human cells. Although this method may work for some specific cell types, such as those reported by Kelly *et al.*, our studies show that the method is unfortunately not universal. To date, no other group has confirmed the efficacy of neonatal desensitization to our knowledge. In fact, the induction of host tolerance by exposure to xenogeneic antigens, even within the neonatal period, is counterintuitive. This approach is one of the principles of vaccination at birth (as with the human Bacillus Calmette-Guérin (BCG) vaccine) and leads to stimulation of an immune response rather than tolerance. Indeed, studies on organ transplantation have revealed that neonatal inoculation with allogeneic cells leads to allograft tolerance, whereas neonatal inoculation with xenogeneic cells results in rejection of xenografts⁵. Long-term tolerance to allografts has been shown to correlate well with chimerism, that is, the prolonged survival of the cells used for neonatal desensitization⁶. In our studies, instead of a long-term chimerism, we observed rapid loss of the cells used for desensitization. All together, neonatal desensitization has not proven to be the ‘holy grail’ for preclinical cell transplantation investigations, and the problem of tolerance induction to human xenografts remains unresolved.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Xenograft survival in neonatally desensitized animals. **(a)** *In vivo* bioluminescence of hGRP cells injected intraperitoneally on the day of birth (D₀, *n* = 6). **(b)** *In vivo* bioluminescence of hGRP cells transplanted intracerebrally into adult mice. Open circles, desensitized mice (*n* = 6); filled circles, nondensitized controls (*n* = 6). The shaded area shows the bioluminescence background level. **(c,d)** Immunostaining against HuNu **(c)** and firefly luciferase **(d)** of brain slices 2 weeks after grafting hGRPs in desensitized mice. **(e,f)** Immunostaining against human-specific antigen (HuNu) of hGRPs **(e)** and HUCB-NSC cells **(f)** transplanted into the brains of immunodeficient *rag2*^{-/-} mice 3 weeks after grafting. **(g)** Graph showing quantified signal intensity of CD45 immunofluorescence (in arbitrary units, a.u.) for desensitized (Des; *n* = 6) and control (Con, *n* = 6) mice transplanted with hGRPs. **(h–l)** Detection of HUCB-NSC cells by anti-HuNu staining in desensitized rats killed 1 d **(h)**, 3 d **(i)**, 7 d **(j)**, 14 d **(k)** or 21 d **(l)** after transplantation. Error bars, mean ± s.d. Scale bars: **c–f**, 200 μm; **h–l**, 100 μm.