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Antibody conditioning enables MHC-mismatched hematopoietic stem cell transplants and organ graft tolerance

Benson M. George[1,2], **Kevin S. Kao**[1,2], **Hye-Sook Kwon**[4], **Brenda J. Velasco**[4], **Jessica Poyser**[4], **Angela Chen**[1,3], **Alan C. Le**[4], **Akanksha Chhabra**[4], **Cassandra Burnett**[4], **Devon Cajuste**[1], **Malachia Hoover**[1], **Kyle M. Loh**[1,3], **Judith A. Shizuru**[1,4], **Irving L. Weissman**[1,2,3,5,6,*]

[1]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA

[2]Ludwig Center for Cancer Stem Cell Research and Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA

[3]Department of Developmental Biology and the Stanford-UC Berkeley Stem Cell Institute, Stanford, CA 94305, USA

[4]Department of Blood and Marrow Transplantation, Stanford University School of Medicine, Stanford, CA 94305, USA

[5]Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305, USA

[6]Lead Contact

SUMMARY

Hematopoietic cell transplantation can correct hematological and immunological disorders by replacing a diseased blood system with a healthy one, but currently requires depleting a patient's existing hematopoietic system with toxic and non-specific chemotherapy and/or radiation. Here we report an antibody-based conditioning protocol, with reduced toxicity and enhanced specificity, for robust hematopoietic stem cell (HSC) transplantation and engraftment in recipient mice. Host pre-treatment with six monoclonal antibodies targeting CD47, T cells, NK cells, and HSCs followed by donor HSC transplantation enabled stable hematopoietic system reconstitution in recipients with mismatches at half (haploidentical) or all major histocompatibility complex (MHC)

*Correspondence: irv@stanford.edu.

AUTHOR CONTRIBUTIONS

B.M.G. wrote the manuscript. B.M.G., H.S.K, A.Chh. designed experiments. B.M.G., K.S.K., H.S.K., B.J.V., J.P., M.H., and D.J. performed hematopoietic cell transplants, including conditioning and post-transplant chimerism assessment. B.M.G., A.Che., and K.M.L. performed and analyzed heart graft studies. A.C.L. and C.B. performed data analysis. K.M.L. edited the manuscript. I.L.W. and J.A.S. supervised the project, conceived experiments and edited the manuscript.

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DECLARATIONS OF INTEREST

A.Chh., J.A.S., B.M.G. and I.L.W. are inventors on patents describing antibody-mediated HSC clearance and engraftment (US20180214524A1, US62/041,989, US20100226927A1). I.L.W. is a cofounder, director, and stockholder of Forty Seven, Inc., the company that licensed the above stated patents. B.M.G., J.A.S., K.S.K. and A.Chh. are stockholders of and/or were paid consultants for Forty Seven, Inc. J.A.S. is cofounder and stockholder of Jasper, Inc, which licensed an above stated patent or patents.

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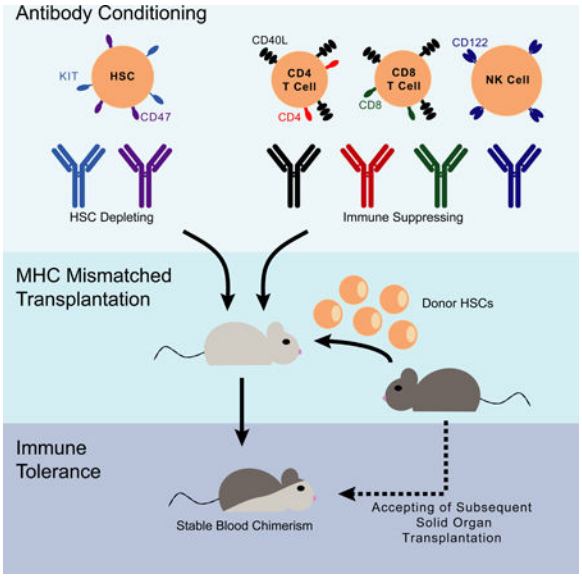
genes. This approach allowed tolerance of heart tissue from HSC donor strains in haploidentical recipients, showing potential applications for solid organ transplantation without immune suppression. Fully mismatched chimeric mice developed antibody responses to nominal antigens, showing preserved functional immunity. These findings suggest approaches for transplanting immunologically-mismatched HSCs and solid organs, with limited toxicity.

Graphical Abstract



eTOC:

Treatment of hematological disorders via donor cell transplantation requires harsh and non-specific conditioning protocols. George et al. report an antibody-based conditioning protocol for HSC engraftment which does not require chemotherapy or irradiation, and allows robust hematopoietic reconstitution even with fully mismatched MHC donor cells.

INTRODUCTION

Hematopoietic stem cells (HSCs) can self-renew and give rise to all blood cell lineages when transplanted into a recipient (Spangrude et al., 1988, Baum et al., 1992; Uchida et al., 1998, Majeti et al., 2007, Müller et al., 2012). For these reasons, hematopoietic cell transplantation (HCT) can be used to replace an individual's diseased blood and immune system. While HCT is most commonly performed to treat malignancies, it can be a curative approach for other disorders, such as thalassemia, sickle cell anemia, inherited immunodeficiencies, autoimmune diseases, and metabolic storage disorders (Lucarelli et al., 1990, Hoogerbrugge et al., 1995, Weissman, 2000, Neven et al., 2009, Bolaños-Meade et al., 2012, Ly et al., 2017). HCT can also induce immunological tolerance wherein tissues from an HSC donor can be transplanted without rejection (Billingham et al., 1953, Weissman, 1967, Weissman, 1973, Gandy and Weissman, 1998). Therefore, HCT can facilitate transplantation of immunologically-mismatched organs without the need for lifelong immune suppression, which is associated with the development of malignancy, disordered

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hematopoiesis, and life-threatening infection (Engels et al., 2011). However, despite the seemingly diverse applicability of HCT, a lack of suitable donors and the toxicities associated with its conventional administration limit its use. Addressing these barriers could allow practitioners to use HCT much more widely in clinical practice and extend its reach into regenerative medicine.

In most transplant situations, donors and recipients are immunologically matched for the major histocompatibility complex (MHC) genes, as they govern rejection of foreign cells (Bix et al., 1991). However, MHC matching of siblings occurs in only 25% of cases, contributing to why many patients do not have a match. Haploidentical transplantation, where donors are matched at half of the *MHC* loci, is becoming more common but is limited by increased rejection, often requiring high-dose immune suppression to sustain donor grafts (Beatty et al., 1985). If it were possible to perform haploidentical transplantation with limited toxicity and consistent engraftment, this would significantly expand the availability of donors, theoretically allowing any individual to receive HCT from their parent, child, or half of their siblings. Beyond this, the ability to form mixed donor-host chimeras (Sachs, Kawai and Sykes, 2014) without MHC matching would enable nearly universal application of HSC transplants and donor specific organ transplant tolerance.

To perform HCT, a recipient's blood system is ablated through a process known as conditioning, which provides both immune suppression and makes HSC niches available for donor cell engraftment. Currently, HCT conditioning requires chemotherapy and/or radiation, which can induce life-threatening side effects, such as a period of profound immune suppression during which the patient is at risk of severe infection, irreversible organ toxicity, veno-occlusive disease, mucositis, and secondary malignancy (Michel et al., 1997, Hartman et al., 1998). Therefore, HCT is used to predominantly treat hematologic malignancies (Passweg et al., 2017), where the benefits of HCT outweigh the associated, potentially fatal, risks. Due to the non-specific nature of conventional conditioning regimens, the safety and risk-benefit ratio of HCT for non-malignant diseases could be considerably improved if more specific agents, such as monoclonal antibodies, could be utilized for conditioning. Various studies and clinical protocols have explored the use of antibodies to condition patients for HCT (Cobbold et al., 1986, Sharabi et al., 1989, Nikolic et al., 2000, Spitzer et al., 2003, Czechowicz et al., 2007, Straathof et al., 2009, Worth et al., 2013, Racine et al., 2014, Chhabra et al., 2016). However, these studies still required the use of chemotherapy/radiation or were limited to MHC matched combinations.

In response to these two major barriers, here we report a strategy to safely engraft MHC-mismatched HSCs without the use of chemotherapy/radiation into immune-competent recipient mice. In our previous work, we showed that antibody-mediated depletion of host HSCs and T cells could facilitate transplantation of HSCs mismatched for *minor* histocompatibility antigens (Chhabra et al., 2016). However, the transplantation of MHC mismatched tissues is much more difficult than in MHC matched situations, partly due to the higher frequency of anti-MHC T cells than *minor* histocompatibility reactive T cells, plus the activity of alloreactive NK cells (Lindhal and Wilson, 1977, Karre et al., 1986). Therefore, to enable *major* histocompatibility-mismatched HSC engraftment, we hypothesized that blocking host T cell activation and depleting NK cells would be necessary.

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We demonstrate that conditioning using six monoclonal antibodies enables mice to receive partially (haploidentical) or fully MHC-mismatched HSCs, thus permitting blood system replacement and induction of tolerance to mismatched donor organs without dependence on chemotherapy or radiation.

RESULTS

We reasoned that expansion of previously demonstrated antibody-conditioning regimens to deplete critical immune subsets would enable more effective HSC transplantation. To deplete HSCs we chose an antibody targeting KIT (anti-CD117), a transmembrane protein expressed on HSCs and hematopoietic progenitor cells, and anti-CD47 which blocks a dominant anti-phagocytic signal. This combination results in macrophage assisted depletion of HSCs (Chhabra et al., 2016). To eliminate host NK cells we used a depleting antibody targeting CD122/Il2Rβ (Tanaka et al. 1993, Seung et al., 2003), which is expressed throughout human and mouse NK cell development (Fathman et al., 2011). To further inhibit T cell mediated rejection we used an antibody that blocks the CD40-CD40L axis; CD40L is a co-stimulatory cell surface molecule expressed by activated T cells whose blockade has been shown to aid in tolerance induction (Lederman et al., 1992, Noelle et al., 1992, Markees et al., 1997, Durham et al., 2000, Wekerle et al., 2000). We tested whether combinations of antibodies to these targets (herein referred to as 4Ab; Fig 1a), would open HSC niches and provide immunosuppression for donor haploidentical or full MHC mismatch HSC engraftment.

Recipient mice were conditioned with 4Ab using a nine-day treatment scheme (Fig 1a). Haploidentical transplantation was performed using AKR x C57BL/6 F_1 mice (hereafter referred to as $AB6F_1$) as donors and BALB/C x C57BL/6 F_1 ($CB6F_1$) mice as recipients (Fig. 1b); these mouse strains are matched at the $H2^b$ haplotype but mismatched for $H2^k$ and $H2^d$. We first transplanted 30×10^6 haploidentical whole bone marrow (WBM) cells into 4Ab-conditioned mice, given that WBM contains T cells and facilitator CD8+ cells that diminish host rejection of the graft (Shizuru et al., 1996; Gandy et al., 1999). Chimerism, defined as the presence of >1% donor cells in peripheral blood lineages, was periodically assessed by measuring expression of donor versus recipient-specific CD45 allelic markers, which are expressed on all nucleated blood cells. While unconditioned mice did not become chimeric, all 4Ab-conditioned mice developed multi-lineage “mixed” chimerism, in that both donor and host blood cells co-existed (Fig S1a). Importantly, chimerism was also observed in the HSC compartment (Fig. S1b), indicating that peripheral donor cells were the product of active hematopoiesis rather than long-lived mature cells circulating after transplantation.

We tested permutations of the 4Ab regimen and found no single antibody alone permitted engraftment (Supplemental Table 1) while 4Ab conditioning induced chimerism in all recipient mice transplanted with haploidentical WBM (Fig. S1c). Anti-KIT and anti-CD47 combined with either anti-CD40L or anti-CD122 alone induced chimerism in a fraction of mice (Fig. S1c), with anti-CD40L proving more effective at increasing chimerism. Since attaining high doses of donor cells can be challenging in the clinical setting (Negrin et al., 2000, Müller et al., 2012), we attempted a 10-fold lower dose of WBM. Since NK cell

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ablation is critical in low-dose HCT (Westerhuis et al., 2005), we attempted engrafting 3×10^6 WBM cells with and without anti-CD122 conditioning (Supplemental Table 1). When anti-CD122 was excluded, the percentage of mice that became chimeric significantly decreased at the lower cell dose (Fig. 1c).

A major limitation of HCT is graft-versus-host disease (GvHD), which is mediated by transplanted donor T cells attacking the host, resulting in potentially lethal organ damage as well as immune deficiency carried out by lymphoid organ homing donor T cells (Gallatin et al 1986, Krensky et al., 1990, Weissman, 2000, Tsao et al., 2009, Müller et al., 2012). GvHD can be avoided by transplanting purified HSCs which lack mature T cells (Shizuru et al., 1996, Uchida et al., 1998). However, in MHC-mismatched transplants, purified HSCs are particularly susceptible to host rejection (Shizuru et al., 1996, Gandy and Weissman, 1998). Therefore, after establishing proof-of-concept in WBM we attempted transplantation of haploidentical Lineage$^-$ Sca1$^+$ KIT$^+$ (LSK) cells, which are highly enriched for HSC and multipotent progenitors (Morrison et al., 1994, Kiel et al., 2005). We transplanted 9×10^3 LSK cells, which is the number of LSK cells present within 3×10^6 WBM cells. However, LSK cells failed to engraft 4Ab-conditioned mice (Fig 1d).

WBM populations engraft more robustly than purified HSCs, as they contain cell populations that may induce immune suppression by actively attacking host immune cells and/or by secreting immunosuppressive factors (Gandy et al., 1999, Grimes et al., 2004, Nilsson et al., 2007). We have previously shown that transplanting purified HSCs can be achieved by additional immune suppression to eliminate T cells (Shizuru et al., 1996; Chhabra et al., 2016); therefore, we added anti-CD4 and anti-CD8 depleting antibodies to the 4Ab regimen. The usage of this six antibody cocktail (anti-KIT, anti-CD47, anti-CD40L, anti-CD122, anti-CD4, and anti-CD8; referred to as 6Ab conditioning hereafter (Fig. 1a)) induced multi-lineage chimerism in recipients transplanted with 9×10^3 haploidentical LSK cells (Fig. 1e). Withholding any single antibody from the 6Ab cocktail resulted in fewer chimeric animals and lower chimerism; removal of both anti-CD40L anti-CD122 antibodies generally prevented any engraftment (Fig. S1d). Both 4Ab and 6Ab regimens specifically depleted desired cell-types, while generally sparing B cells and tissue-resident myeloid cells (Fig. S2a-d), highlighting the targeted nature of these monoclonal antibodies.

To assess whether antibody conditioning could enable fully MHC-mismatched transplants we used DBA/1J (H2^q) mice as donors and CB6F$_1$ (H2$^{b/d}$) as hosts (Fig. 1f). 6Ab conditioning enabled efficient LSK transplantation in all mice while only 40% of 4Ab-conditioned mice receiving WBM achieved chimerism (Fig. 1g). These findings were compared to outcomes in irradiated mice that also received fully MHC-mismatched transplants. Although all irradiated CB6F$_1$ mice transplanted with WBM were chimeric by week 3, 80% died by 9 weeks following transplantation (Fig. S2e). Given that the LSK transplanted radiation-conditioned mice did not succumb and that the WBM transplanted radiation-conditioned mice were initially engrafted, this lethality is possibly due to GvHD in this strain combination. In summary, 6Ab conditioning enabled engraftment of low doses of both haploidentical and fully-MHC mismatched HSCs without recourse to chemotherapy or radiation.

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In chimeric mice, the co-existence of donor and recipient immune systems implied the induction of immune tolerance. However, it was unclear if this was due to central tolerance, where thymic negative selection prevents the formation of donor-reactive host T cells that can induce rejection (Kappler et al., 1987, Guidos et al., 1990). To gauge central tolerance in the transplanted mice, we measured the presence of the Vβ6 chain of the T cell receptor (TCR), which is reactive to the Mtv-7 provirus-encoding super-antigen expressed by the AKR background (Kanagawa et al., 1989; Guidos et al, 1990, Shizuru et al., 2000). The coexistence of AKR x C57BL/6 ($AB6F_1$) cells in $CB6F_1$ mice would likely result from thymic deletion of $V\beta6^+$ T cells by donor HSC-derived thymic medullary dendritic cells and possibly host medullary epithelial cells (Rouse et al., 1979, 1985). Deletion of $V\beta6^+$ T cells was indeed observed in all chimeric animals receiving antibody conditioning for haploidentical WBM or LSK transplants (Fig. 2a-b). Thus, both 4Ab and 6Ab antibody conditioning followed by immune-system replacement can instill central immunological tolerance to the donor mouse strain.

To test whether this central tolerance through antibody conditioning would allow engraftment of donor-matched solid organs, we transplanted neonatal heart grafts (Gandy and Weissman, 1998) from HSC-donor ($AB6F_1$, which are $H2^{k/b}$) or third-party (DBA/1J strain, which are homozygous for $H2^q$) newborn mice pups into the ear pinna of both naïve and 6Ab-conditioned haploidentical transplant chimeras (Fig. 2c). Graft survival was measured by visualization of the beating heart graft. In naïve, unconditioned and untransplanted mice, both $AB6F_1$ and DBA/1J hearts failed to beat at 14 days (Fig. 2d). In 6Ab-conditioned chimeric mice, $AB6F_1$ hearts began beating at 14 days and persisted for at least 181 days while DBA/1J hearts were rejected. At 34 days, $AB6F_1$ hearts were still visible in the pinna while DBA/1J hearts were no longer visible (Fig. 2e). H&E and immunofluorescence (IF) analysis indicated $troponin^+$ cardiac tissue lacking immune cell infiltrates in the $AB6F_1$-engrafted pinna; $troponin^+$ tissue within the pinna containing DBA/1J hearts was not detectable (Fig. 2e). These results indicate that following antibody-conditioning and haploidentical HSC engraftment, tolerance to matched heart grafts is feasible while transplantation immunity against completely foreign tissues is preserved.

Given that 6Ab-conditioned chimeras either share half or no MHC molecules and adaptive immunity is mediated through MHC recognition, it is important to understand whether T cells of donor origin were re-educated to respond to antigens presented by host MHC. To test functional immunity, which requires interactions between T cells, B cells, and antigen presenting cells following immunization, we challenged mice following a fully-MHC mismatched HSC transplant with keyhole limpet hemocyanin (KLH). Animals with intact T cell dependent antibody responses will generate IgG against KLH. Six weeks after transplant, antibody conditioned mice were able to generate anti-KLH antibodies (Fig. 2f); however, their response lagged behind that of unconditioned, untransplanted mice. Importantly, upon second challenge two weeks later, antibody conditioned mice were able to generate a secondary IgG antibody responses comparable to control mice (Fig. 2f).

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DISCUSSION

In sum, here we have developed a method to transplant half (haploidentical) and fully-MHC mismatched purified HSCs into immune-competent animals without the use of chemotherapy and/or radiation. Importantly, the use of purified HSCs dramatically reduces the risk of GvHD (Weissman, 2000). If translatable to human patients, this antibody-based conditioning strategy to overcome immunological barriers could both expand the HSC donor pool to enable most recipients to find a HCT match and decrease the often prohibitive risks of conditioning that prevent widespread use of HCT.

While we have demonstrated the efficacy of antibody conditioning in mice, realizing the potential of such conditioning in humans will require further exploration. Despite the cell type specificity of monoclonal antibodies, as compared to the pleiotropic action of radiation or chemotherapy, the former is not without toxicity. For instance, an anti-CD40L antibody was found to induce thromboembolic events (Kawai et al., 2000, Robles-Carrillo et al., 2010) in humans by cross-linking platelets. However, this effect was found to be Fc-mediated and newer anti-CD40L therapies that lack Fc-domains or contain an Fc-dead domain (Shock et al., 2015, Xie et al., 2014) have proven to be immunosuppressive without inducing thrombosis. Therefore, further studies to better understand the human expression of the proteins targeted here are needed for clinical translation.

Importantly, the ability to induce immunological tolerance to foreign organs could increase opportunities for all patients requiring lifesaving organ transplants. Today the donor of an organ, tissue or HSC transplant is a living or recently deceased person. The ultimate goal of regenerative medicine will be to differentiate a pluripotent (embryonic or induced pluripotent) stem cell line into HSCs and other needed tissue stem cells (such as neural (Uchida et al., 2000), bone and cartilage (Chan et al., 2015), or liver^{-} (Wang et al., 2015)), either *in vitro* (Loh et al., 2014, Loh et al., 2016) or *in vivo* within a large-animal host, such as a pig (Rashid et al., 2014, Yamaguchi et al., 2017). Our approach may enable the use of these methods to result in more robust, rapid, and gentler transplant schemes reducing the burden on an organ donation system that is currently dramatically underserving the patients most in need.

STAR METHODS

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Dr. Irving L. Weissman (irv@stanford.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

For HSC transplantation experiments, CB6F1/J (Jax: 100007) and DBA/1J (Jax: 670) mice were purchased from Jackson laboratories, while AKR/J x C57Bl/6 F1 (AB6F1) mice were bred at Stanford University. At the time of transplant, all donors and recipients were 8–12 weeks in age and female unless otherwise noted on Supplemental Table 1. For ear-heart transplantation, untimed pregnant DBA/1J mice were purchased from Taconic Biosciences. Mice for all experiments were immunocompetent and group housed; littermates of the same

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sex were randomly assigned to experimental groups. All experiments were performed according to guidelines established by the Stanford University Administrative Panel on Laboratory Animal Care.

METHOD DETAILS

Pre-Transplantation Conditioning—For antibody conditioning, all antibodies were suspended in phosphate buffered saline (PBS) and injected intraperitoneally unless otherwise noted. When antibodies were withheld or animals were not conditioned, equivalent volumes of PBS were given intraperitoneally instead. Day 0 corresponds to the day of transplantation. For anti-CD47 (clone mIAP410), mice received 100μg on Day −8 and 500μg daily from Day −6 through Day −2. For anti-KIT (clone ACK2), which is injected retro-orbitally, 500μg was given on Day −6. Thirty minutes prior to anti-KIT injections, mice received 400μg of diphenhydramine intraperitoneally. Both anti-CD4 (clone GK1.5) and anti-CD8 (clone YTS169.4) were given as 100μg injections daily from Day −2 through Day 0 (Chabbra et al., 2016). For anti-CD122 (clone Tm-β1), 250μg was given on Day −2 (Tanaka et al., 1993, Seung et al., 2003). For anti-CD40L (clone MR-1), 500μg was given on Day 0 (Wekerle et al., 2000). Irradiation control mice were exposed to two doses of 6.5Gy x-ray radiation on the day of transplantation.

Graft Preparation and Transplantation—Whole bone marrow was extracted from tibias, femurs, hips, and spines of donor mice. Bones were crushed with a mortar and pestle, filtered, and subsequently underwent red blood cell (RBC) lysis. For LSK cell transplants, RBC lysed whole bone marrow was bound to the Miltenyi Lineage Cell Depletion kit cocktail as per the manufacturer's instructions. Flow-through from the magnetic separation columns was collected and stained in PBS with 2% fetal bovine serum (FBS) and the following antibodies: CD3 PE (clone 17A2; 0.66μg/mL final concentration), CD4 PE (clone GK1.5; 0.66μg/mL final concentration), CD5 PE (clone 53–7.3; 0.66μg/mL final concentration), CD8a PE (clone 53–6.7; 0.66μg/mL final concentration), B220 PE (clone RA3–6B2; 0.66μg/mL final concentration), Gr-1 PE (clone RB6–8C5; 0.5μg/mL final concentration), Mac-1 PE (clone M1/70; 0.5μg/mL final concentration), Ter119 PE (clone TER119; 0.66μg/mL final concentration), SCA1 Pe-Cy7 (clone D7; 1.0μg/mL final concentration), and CD117 APC (clone 2B8; 1.0μg/mL final concentration). Propidium iodide was added as a viability stain just prior to sorting on a BD Aria. All cells for transplant were re-suspended at the desired concentration in PBS with 2% FBS. All mice were anesthetized using isoflurane and then transplanted with 100uL of cell suspension via retro-orbital injection.

Peripheral Blood Analysis—Mice were periodically bled retro-orbitally into EDTA coated tubes. Blood was then incubated in 1% dextran with 5mM EDTA at 37C for 1 hour. The supernatant from each tube was extracted, lysed, and then stained with the following antibodies for peripheral blood chimerism: CD3 APC (clone 17A2; 2.0μg/mL final concentration), CD19 PE-Cy7 (clone ebio103; 2.0μg/mL final concentration), Gr-1 eFluor-450 (clone RB6–8C5; 1.0μg/mL final concentration), Mac-1 APC-Cy7 (clone M1/70; 1.0μg/mL final concentration), CD45.1 FITC (clone A20; 2.0μg/mL final concentration), and CD45.2 PE (104; 2.0μg/mL final concentration). To assess Vβ6 TCR

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expression, peripheral blood was processed as above and stained with Vβ6 APC (RR4–7; 2.0μg/mL final concentration). Propidium iodide was added as a viability stain just prior to analysis. Samples were analyzed on a BD Fortessa. Donor versus host chimerism was distinguished based on CD45 allelic differences. For complete blood counts, 20μl of peripheral blood was analyzed on a Heska HemaTrue Veterinary Hematology Analyzer.

Spleen and Bone Marrow Population Analysis—Spleens were harvested from conditioned mice and directly mashed in a 70-μm filter. Hips, femurs and tibia were crushed as described above for bone marrow analysis. Single cells from spleen and bones were lysed, filtered, and stained with the following antibodies for immune cell analysis: CD4 APC (clone GK1.5; 2.0μg/mL final concentration), CD8 FITC (clone 53–6.7; 5.0μg/mL final concentration), CD19 PE (clone 1D3; 2.0μg/mL final concentration), NK1.1 PE-Cy7 (clone PK136; 2.0μg/mL final concentration), Mac-1 APC-Cy7 (clone M1/70; 0.5μg/mL final concentration), and Gr1 eFluor-450 (clone RB6–8C5; 1.0μg/mL final concentration). For long-term HSC chimerism, cells were stained with the following antibodies: CD3 PE (clone 17A2; 0.66μg/mL final concentration), CD4 PE (clone GK1.5; 0.66μg/mL final concentration), CD5 PE (clone 53–7.3; 0.66μg/mL final concentration), CD8a PE (clone 53–6.7; 0.66μg/mL final concentration), B220 PE (clone RA3–6B2; 0.66μg/mL final concentration), Gr-1 PE (clone RB6–8C5; 0.5μg/mL final concentration), Mac-1 PE (clone M1/70; 0.5μg/mL final concentration), Ter119 PE (clone TER119; 0.66μg/mL final concentration), SCA1 Pe-Cy7 (clone D7; 1.0μg/mL final concentration), CD117 APC (clone 2B8; 1.0μg/mL final concentration), CD150 Pacific Blue (clone TC15–12F12.2; 5.0μg/mL final concentration), CD34 FITC (clone RAM34; 5.0μg/mL final concentration), CD45.1 FITC (clone A20; 2.0μg/mL final concentration), and CD45.2 PE (clone 104; 2.0μg/mL final concentration). Propidium iodide was added as a viability stain just prior to analysis. Samples were analyzed on a BD Fortessa.

Ear-Heart Graft—Neonatal mice were euthanized 1–2 days after birth and their hearts were harvested and placed in ice cold PBS. Recipient mice were prepared by making a small incision on the dorsal side of their ear near the skull. Afterward, using a trocar, a pouch was created by tunneling from the incision site to the tip of the pinna. Neonatal hearts were delivered at the distal end of the pouch with the trocar. The tunnel was closed by gently pushing the lifted skin back to the dermis. Heart viability was monitored for beating by visualizing the graft through a dissecting microscope. At the noted time points, ears were dissected from representative animals and embedded in optimal cutting temperature (O.C.T.) compound. Tissue was sectioned and stained with hematoxylin and eosin, as well as cardiac troponin I. For troponin staining, sections were blocked with PBS containing 10% FBS for 1 hour, and then stained overnight at 4 degrees Celsius in 0.5% bovine serum albumin (BSA) with 10μg/mL of anti-cardiac troponin I antibody. Secondary staining was performed with 10μg/mL of goat anti-rabbit IgG Alexa Fluor 488 for 30 minutes, and then subsequently stained with DAPI. Images were collected on a Leica DMI6000B epifluorescence-equipped inverted microscope.

KLH immunization and monitoring of KLH antibody production in serum—KLH (Sigma-Aldrich) were mixed with an equal volume of the complete Freund adjuvant

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(Sigma-Aldrich) for primary immunization or incomplete Freund adjuvant (Sigma-Aldrich) for secondary immunization to form an emulsion by vortexing in 15 mL tube. KLH emulsion (100 μg KLH in 100 μl emulsion/mouse) was intraperitoneally injected into transplanted mice 4 weeks post-transplantation for primary immunization. Two (2) weeks after primary immunization, secondary immunization was performed. Serum was collected by retro-orbital bleeding of KLH-immunized mice 2 weeks after secondary immunization. Anti-KLH antibody titer in the serum was determined using KLH IgG mouse ELISA kit (Abnova) and a SpectraMax i3x plate reader (Molecular Device) according to the manufacturer's instructions.

QUANTIFICATION AND STATISTICAL ANALYSIS

Analyses were performed on GraphPad Prism. One- and two-way ANOVA, log-rank test, and unpaired and multiple t-tests were used where appropriate; n signifies the number of animals used.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Six antibodies suppress a mouse's HSCs, T cells and NK cells in 8 days
- Antibody treatment enables HSC transplants without radiation/chemotherapy
- Transplants can be performed with fully MHC mismatched donors
- Following HSC transplants, animals are tolerant to solid organs from the same donor

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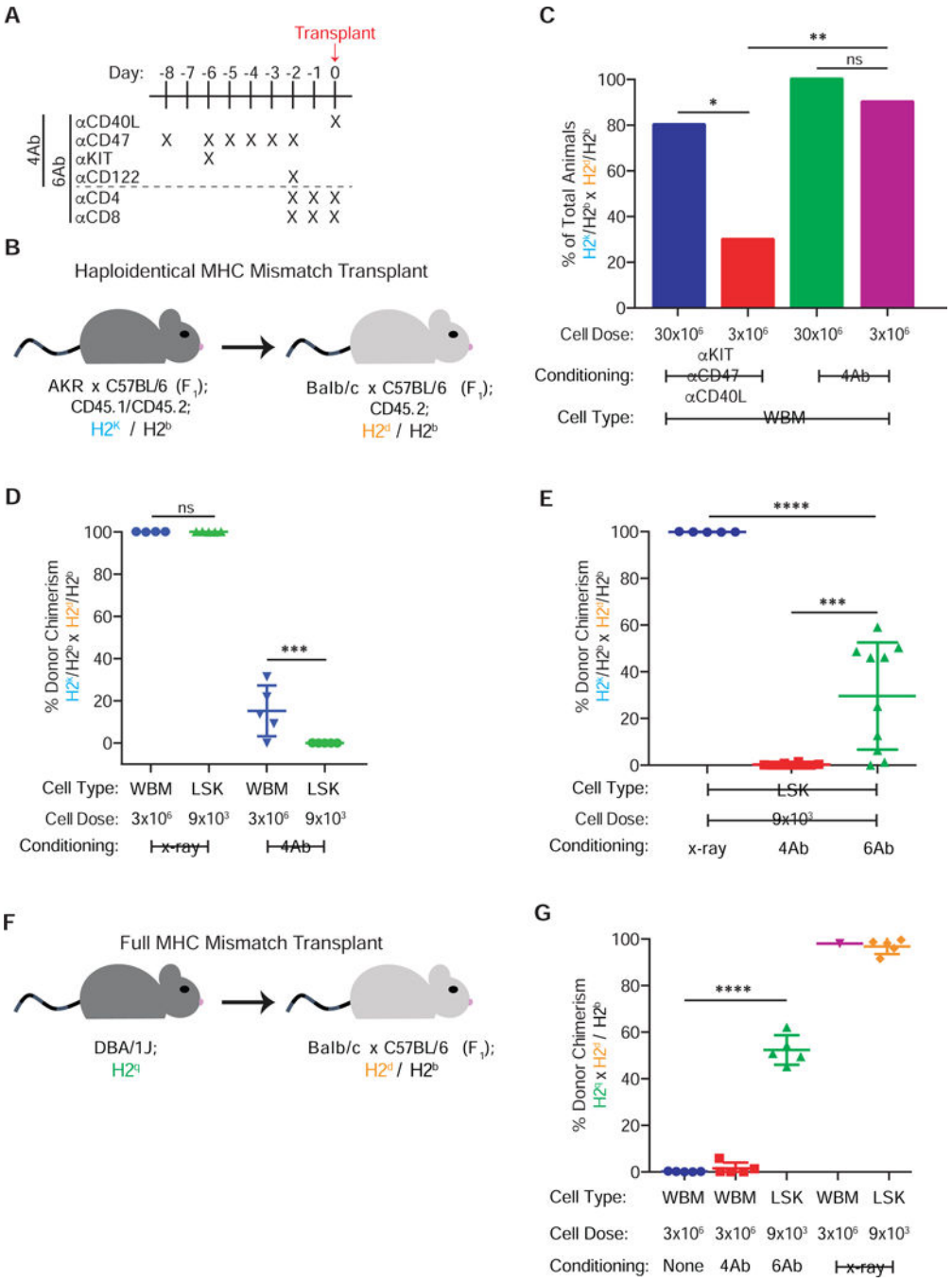

Figure 1. A monoclonal antibody cocktail can induce long-term multi-lineage hematopoietic reconstitution.

(a) Dosing schedule for 4Ab and 6Ab conditioning regimen, where α refers to antibody. (b) Haploidentical transplantation schema using AKRB6F$_1$ donors and CB6F$_1$ recipients. (c) Percentage of animals which are chimeric at 14–16 weeks after transplanted 30×10^6 or 3×10^6 haploidentical WBM cells, with or without NK cell depletion (pooled data from two replicate experiments, pooled n=5–10). (d) Donor granulocyte chimerism at 16 weeks following haploidentical transplantation of 3×10^6 WBM or 9×10^3 LSK in the setting of 4Ab conditioning or irradiation (n=4–5). (e) Donor granulocyte chimerism following 9×10^3

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haploidentical LSK transplantation at 16 weeks in irradiated, 4Ab- and 6Ab-conditioned animals (pooled data from two replicate experiments, pooled n=5–10). (f) Transplantation schematic using DBA/1J donors and $CB6F_1$ recipients. (g) Donor granulocyte chimerism at 8 weeks following WBM and LSK fully MHC-mismatched transplantation in irradiated, 4Ab- and 6Ab-conditioned animals (n=5). Data in (c) represent total percentage; one way ANOVA was performed where *P≤0.05 and **P≤0.01. Data and error bars in (d), (e) and (g) represent means ± SD; one way ANOVA was performed where *P≤0.05, **P≤0.01, and ***P≤0.001.

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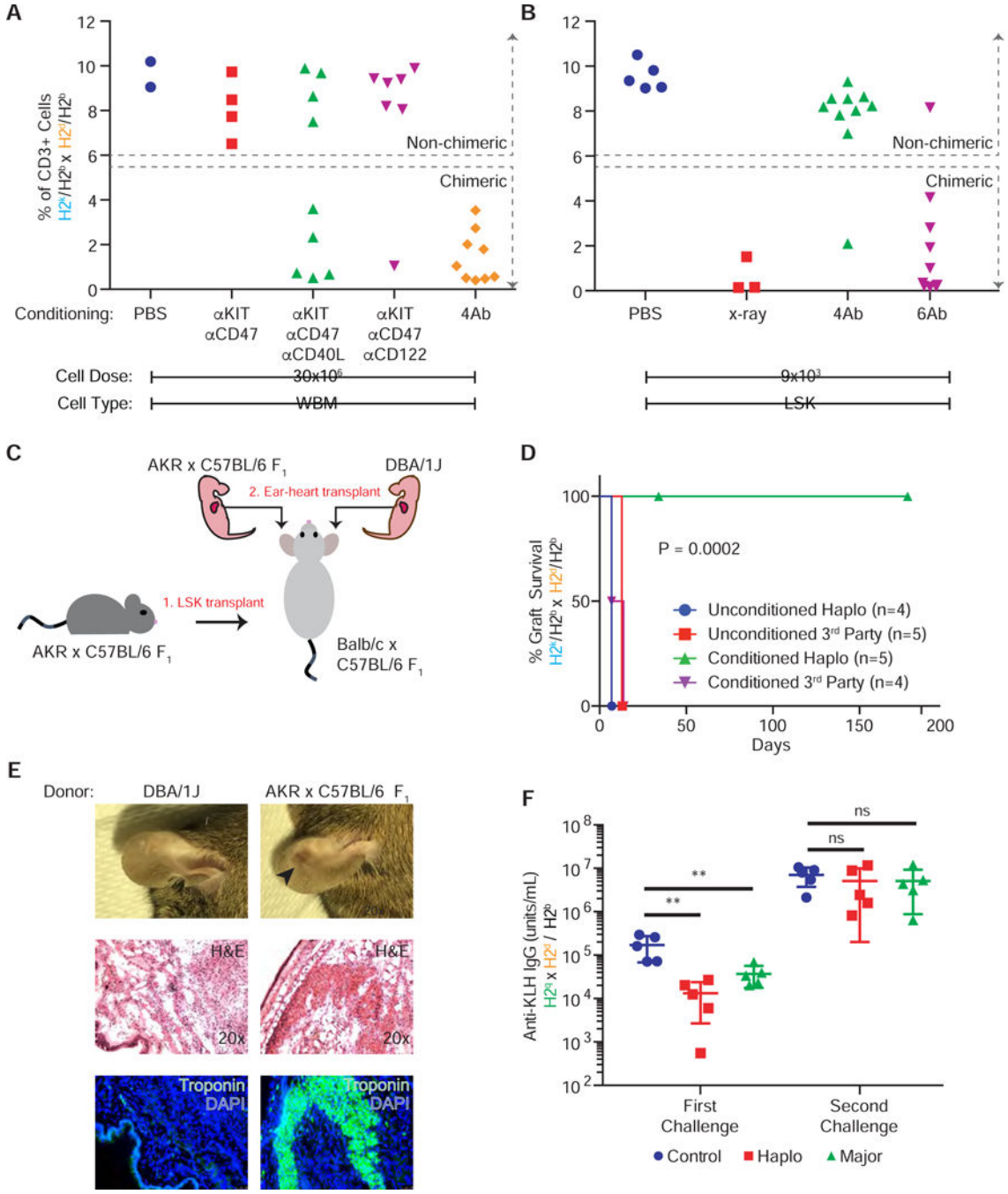

Figure 2. Antibody conditioned animals have intact immune systems and display tolerance to matched organ grafts.

(a-b) Abundance of host Vβ6 $^{+}$ T cells in peripheral blood following (a) WBM and (b) LSK haploidentical transplantation (pooled data from two replicate experiments, pooled, n=2–10). (c) Fetal heart into ear transplantation schematic. (d) Kaplan-Meier curve showing donor heart survival in haploidentical chimeras (n=5). (e) Gross examination, H&E, and IF of representative HSC-donor and 3rd party heart tissue in ears at 34 days following tissue transplant in haploidentical chimeras. (f) Anti-KLH IgG production following KLH immunization six (first challenge) and eight (second challenge) weeks after fully-MHC

mismatched HSC transplantation (n=5). Data in (d) was subjected to a log-rank (Mantel-Cox) test and yielded P = 0.0002. Data and error bars in (f) represent means ± SD and one way ANOVA was performed where ****P≤0.0001.

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KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse monoclonal anti-CD47	BioXCell	RRID: AB_2687806
Rat monoclonal anti-CD117	BioXCell	RRID: AB_2687818
Rat monoclonal anti-CD122	BioXCell	RRID: AB_2687820
Armenian hamster monoclonal anti-CD40L	BioXCell	RRID: AB_1107601
Rat monoclonal anti-CD4	BioXCell	RRID: AB_1107636
Rat monoclonal anti-CD8	BioXCell	RRID: AB_10950145
Rat monoclonal anti-CD3 PE	BioLegend	RRID: AB_312662
Rat monoclonal ant-CD4 PE	BioLegend	RRID: AB_11152678
Rat monoclonal anti-CD5 PE	Thermo Fisher Scientific	RRID: AB_2539168
Rat monoclonal anti-CD8 PE	Thermo Fisher Scientific	RRID: AB_465529
Rat monoclonal anti-B220 PE	Thermo Fisher Scientific	RRID: AB_10371899
Rat monoclonal anti-Gr1 PE	Thermo Fisher Scientific	RRID: AB_10376319
Rat monoclonal anti-Mac1 PE	Thermo Fisher Scientific	RRID: AB_11154207
Rat monoclonal anti-TER199 PE	Thermo Fisher Scientific	RRID: AB_2535273
Rat monoclonal anti-Sca1 PE-Cy7	Thermo Fisher Scientific	RRID: AB_469669
Rat monoclonal anti-CD117 APC	Thermo Fisher Scientific	RRID: AB_469429
Rat monoclonal anti-CD3 APC	Thermo Fisher Scientific	RRID: AB_2536039
Mouse monoclonal anti-CD19 PE-Cy7	BD Biosciences	RRID: AB_394495
Rat monoclonal anti-Gr1 Pacific Blue	Thermo Fisher Scientific	RRID: AB_10376182
Rat monoclonal anti-Mac1 APC-Cy7	BD Biosciences	RRID: AB_396772
Mouse monoclonal anti-CD45.1 FITC	Thermo Fisher Scientific	RRID: AB_2534248
Mouse monoclonal anti-CD45.2 PE	Thermo Fisher Scientific	RRID: AB_2534922
Rat monoclonal anti-Vb6	BioLegend	RRID: AB_2564056
Rat monoclonal anti-CD4 APC	Thermo Fisher Scientific	RRID: AB_11152647
Rat monoclonal anti-CD8 FITC	Thermo Fisher Scientific	RRID: AB_11153636
Mouse monoclonal anti-CD19 PE	Thermo Fisher Scientific	RRID: AB_465579
Mouse monoclonal anti-NK1.1 PE-Cy7	Thermo Fisher Scientific	RRID: AB_469665
Rat monoclonal anti-Mac1 APC-Cy7	Thermo Fisher Scientific	RRID: AB_2534404
Rat monoclonal anti-Gr1 eFluor-450	Thermo Fisher Scientific	RRID: AB_1548788
Rat monoclonal anti-CD150 Pacific Blue	BioLegend	RRID: AB_2187962
Rat monoclonal anti-CD34 FITC	Thermo Fisher Scientific	RRID: AB_465021
Rabbit polyclonal anti-Cardiac Troponin I	Abcam	RRID: AB_869982
Goat polyclonal anti-Rabbit IgG Alexa Fluor 488	Thermo Fisher Scientific	RRID: AB_143165
Chemicals, Peptides, and Recombinant Proteins		
KLH	Sigma-Aldrich	Cat# H7017
Complete Freund’s Adjuvant	Sigma-Aldrich	Cat# F5881

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Incomplete Freund's Adjuvant	Sigma-Aldrich	Cat# F5506
Diphenhydramine	APP Pharmaceuticals	Cat# 63323066401
Critical Commercial Assays		
KLH IgG (Mouse) ELISA Kit	Abnova	Cat# KA2460
Experimental Models: Organisms/Strains		
CB6F1	Jackson Laboratories	Cat# 100007
DBA/1J	Jackson Laboratories	Cat# 670
DBA/1J Untimed Pregnant Mice	Taconic Biosciences	N/A
AKR x C57Bl/6 F1 (AB6F1)	Bred in the Weissman Laboratory, Stanford University	N/A
Other		
Lineage Cell Depletion Kit, mouse	Miltenyi Biotec	Cat# 130–110-470