The Ban on US Government Funding Research Using Human Fetal Tissues: How Does This Fit with the NIH Mission to Advance Medical Science for the Benefit of the Citizenry?

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Introduction

Tissues and organs are composites of many different types of cells organized into precise microarchitectural domains, including blood vessels and stromal cells. No cell culture or organoid culture captures this architecture and the functions of such organs and organized tissues. Yet, human diseases arise in the organs and human regenerative biology can still only be accomplished in these organs and tissues within the body. Systems such as blood formation and immune functions include cells that migrate out of or into bone marrow, thymus, spleen, lymph nodes, Pey-er’s patches, etc., generating and emphasizing the phrase, “in vivo veritas” (reviewed in Weissman, 2010).

Thirty-one years ago, we led an effort to figure out how HIV causes AIDS, and how to identify and isolate human blood-forming “hematopoietic” stem cells (HSCs). Solutions came from the development and optimization of the severe combined immunodeficiency humanized (SCID)-hu mouse, an immunodeficient mouse implanted with the hematolymphoid organs obtained from recently aborted fetuses, namely fetal bone, liver, thymus, spleen, intestine, skin, and lymph node (McCune et al., 1988; Namikawa et al., 1990). Because this mouse model has human blood-forming cells and the human immune organs in which these cells mature and function, it has provided a critical tool for research on the biology of the blood-forming system and diseases affecting it, including cancer and AIDS. At the same time, implantation of pediatric or adult postmortem tissue has been found to not be useful for these purposes. To ensure that fetal tissue was procured in an ethical and legal manner, we sought advice from bioethicists, clergy, and government officials, establishing strict guidelines: the decision to have an elective abortion preceded any decision to use fetal tissues from the abortion for biomedical research, the abortion procedure was not altered in any manner as the tissue was obtained, and neither the woman making the decision nor the physician or any other personnel involved in the procedure or collecting, preparing, or delivering the tissues to the research labs profited from these endeavors (summarized in Weissman, 1989). That is the standard we accepted then and that exists today, despite false reports to the contrary.

Here, we elaborate on discoveries from our labs made possible by using these tissues, the translation of these discoveries to clinical trials, and objective early phase clinical successes. Thirty-one years later, we report that there is still no substitute for the use of the SCID-hu mouse (and/or successive generations of related models using human fetal tissue [Shultz et al., 2019]) for many kinds of discoveries and translation of these discoveries to human therapies. Although we do not address the multiple other fields of scientific inquiry that benefit from human fetal tissue research, it is likely that most if not all of them would also find no substitute.

Analysis of HIV Pathogenesis

At the time that the SCID-hu mouse was developed, infection with HIV was a death sentence and there was no animal model in which the virus and AIDS, the disease it causes, could be studied (Weissman, 1988). It was precisely to fill this crippling blind spot that the SCID-hu model was developed. It immediately became apparent that the HIV stocks being studied in tissue culture around the world were the wrong viruses to use for studies in vivo: only “primary isolates” obtained directly from patients were capable of replicating in the vascularized human organs of the SCID-hu mouse (Namikawa et al., 1988). This observation led to the definition of subgenomic regions present in
primary isolates that were lost or that became defective upon tissue culture passage of HIV (Su et al., 1997), regions that are essential for infectivity and/or pathogenesis in vivo. Given an experimental living model in which the infective process could be carefully monitored and systematically explored, subsequent studies in the SCID-hu mouse also taught us multiple other lessons about HIV pathogenesis, including the mechanisms by which the virus abrogates T cell production in the thymus (Aldrovandi et al., 1993; Bonyhadi et al., 1993; Kaneshima et al., 1994; Stanley et al., 1993; Su et al., 1995) and the causes and immunologic consequences of HIV-mediated induction of interferon-α (IFN-α) pathways (Berkowitz et al., 1998a, 1998b; Favre et al., 2011; Keir et al., 2002a, 2002b; Stoddart et al., 2010). Each of these elements of pathogenesis has been found to also occur in HIV-infected humans. Many have also been shown to be counteracted in the SCID-hu model on provision of select therapeutic agents. Reciprocally, it has been found that multiple candidate antiviral compounds that appear to inhibit lab-adapted isolates of HIV in vitro do not inhibit primary isolates of the virus in vitro (see, for instance, Daar et al., 1990) or in vivo (see, for instance, Van Dis et al., 2016). As a result, findings in humanized mice—made only because human fetal tissue was engrafted into the SCID mouse—have led to the conceptualization and analysis of immunotherapies (e.g., provision of interleukin-7 [IL-7] or IFN-α blockade) (Fry and Mackall, 2002; Swainson et al., unpublished data) that might reverse T cell depletion and IFN-α-induced inflammation in HIV-infected humans. To the extent that further exploration of HIV pathogenesis in this model will no longer be possible, the restrictions imposed by the Trump administration rob future generations of progress and health benefits that might otherwise accrue.

Preclinical Evaluation of Antiviral Drugs against HIV

Test systems that can be used preclinically to reveal the efficacy and toxicity of candidate drugs in the development pathway can vastly accelerate and focus the entry of new therapies for human disease states. Though studies in the test tube and in silico can contribute to this selection process, animal models have historically proven to be the most predictive of toxicity and, at least in some settings, efficacy. The most useful animal models are those that demonstrate relevance to the known physiology and pathophysiology of the disease in humans, that are reproducible from one experiment to the next, that can be deployed in large enough numbers to generate statistically meaningful data, and that are practical in terms of cost and ease of use (Shultz and McCune, 2014). At the time that the SCID-hu mouse was developed (1987), there was no animal model with anything close to these qualifications that could be used to figure out how to keep people with HIV alive.

After extensive studies to confirm that the structure and function of the human thymus of the SCID-hu mouse was related to that of the normal human thymus, concerted efforts were made during the 1988–1993 time frame to optimize and to standardize the model for the preclinical analysis of drugs against HIV (Rabin et al., 1996). As had been shown in humans (Chaissen et al., 1986), the nucleoside analog azidothymidine (AZT) inhibited HIV replication in the model. Presaging future clinical applications of antiviral drugs in humans, AZT was also found in the humanized mouse model to prevent infection when given before infection (i.e., pre-exposure prophylaxis) (McCune et al., 1990), as well as immediately after infection (i.e., post-exposure prophylaxis) (Shih et al., 1991). After considerable empiric work, procedures and practices were put into place that enabled the construction of up to 50 SCID-hu mice from the thymic and liver tissues of a single aborted fetus (which would have otherwise been discarded), allowing for a cohort of animals that could be sub-divided into distinct and relatable experimental groups providing data that could be analyzed statistically for significance. Given the ability to test candidate drugs in such a model and under the auspices of a contract from the NIH, a “Humanized Mouse Models for HIV Drug Development” facility was established that enabled the preclinical evaluation of multiple lead candidates being developed by biotech/pharma as well as by academic investigators. A few of these compounds were later advanced to show efficacy and to be relatively non-toxic in HIV-infected humans (Stoddart et al., 2007). Most, however, were shown to exert their “antiviral effect” by virtue of the fact that they were toxic to most if not all living cells. Given these results gathered in the SCID-hu mouse, the advancement of many candidate treatments through the pipeline was aborted, meaning that HIV-infected humans (including the fetuses of pregnant, HIV-infected mothers, as well as HIV-infected babies) were not subjected to their toxicity. After almost 30 years of continual service, this is the NIH contract that was just discontinued by the Trump administration.

Preclinical Evaluation of Other Medically Important Viruses

As is the case for HIV, most other viruses (and infectious agents, in general) are exquisitely adapted for growth in some but not all species. For those causing disease in humans, it is typically only in humans that the virus can be studied. Given the human tissue microenvironments of the SCID-hu mouse (including not simply thymus and liver but also lymph node, spleen, lung, bone, intestine, and skin) (Kim et al., 1992; Kyoizumi et al., 1992; Maidji et al., 2012; McCune et al., 1988; Mocarski et al., 1993;
Moffat et al., 1995; Namikawa et al., 1990; Sampson-Johannes et al., 1996; Wang et al., 2017), multiple investigators have found it to be a rich discovery tool for the analysis of the infective cycle, pathogenesis, and/or treatment of a variety of important viruses, including cytomegalovirus (Mocarski et al., 1993), measles (Auwaerter et al., 1996), human herpesvirus 6 (HHV-6) (Gobbi et al., 1999), varicella/zoster (Baiker et al., 2004), and Kaposi’s sarcoma-associated herpesvirus/HHV-8 (Dittmer et al., 1999). Building on the experience with HIV, further development and standardization of these models could facilitate the rational development and down-selection of drugs to combat these and other viruses. Notably, the fetus and the newborn are particularly vulnerable to infection by these viruses. The actions of the Trump administration suggest that it is either oblivious to or unconcerned about the fact that fetal tissue research might provide benefit to young lives.

Analysis of Immune Ontogeny
As detailed below, the SCID-hu mouse was a critical in vivo tool for identification of human adult HSCs. In later work, the same model was used to show that fetal HSCs are phenotypically similar to adult HSCs but give rise to mature lineages that are dramatically different. In particular, the fetal HSC generates human CD4+ T cells that are “regulatory” (or “tolerogenic”) in nature and that, among other attributes, prevent the fetal immune system from responding against (and rejecting) the body of the genetically distinct mother during pregnancy (Mold et al., 2008, 2010). Likewise, myeloid-lineage cells are poised to be less effective at augmenting a fetal immune response against the mother (Krow-Lucah et al., 2014). Sometime in the second to third trimester of pregnancy, fetal-like T and myeloid cells are replaced by cells emanating from an adult HSC such that—after birth—the newborn is endowed with an immune system that can instead react against foreign entities (e.g., infectious agents) in the environment. Of note, this transition does not occur at precisely the same time in all newborns: some babies are born with an immune system that is completely fetal (and, hence, tolerogenic), others are born with an immune system that is completely adult (and, hence, immunoreactive), and most are born with varying admixtures of the two (D. Bunis et al., unpublished data). In ongoing work, attempts are being made to understand how such inter-individual variation might arise, how it impacts on the propensity of the newborn to develop allergic responses to environmental antigens, and how it might distinguish those newborns who can from those who cannot respond to vaccines or infectious agents. It is also entirely conceivable that aberrations in the fetal program of immune ontogeny could lead to disruptions in the normal course of pregnancy and labor. The latest move by the Trump administration has halted this work, effectively stripping potential health benefits from future unborn generations.

Discovery and Clinical Use of Human Hematopoietic Stem Cells
In 1988, we completed the first comprehensive enrichment of mouse hematopoietic stem cells by surface markers using fluorescence-activated cell sorting isolation techniques, followed by intravenous transplantation into lethally irradiated mice (Spangrude et al., 1988); these cells were 1/2,000 of bone marrow cells and were 2,000-fold enriched in survival of reconstituted lethally irradiated mice with all blood cell types (Spangrude et al., 1988). Key to that study was the full multilineage engrafment and expansion and life-saving activities of HSCs alone, and these required several anatomical microenvironments present in the mice (bone marrow, spleen, and thymus), but not then and not now replicated by any in vitro culture systems. Single-cell transplants into irradiated hosts showed that, while most regenerations were multilineage, the frequency of long-term self-renewing HSCs was lower than that of short-term multilineage progenitors (called ST-HSCs or MPPs) in the same isolate (Smith et al., 1991). Self-renewal could be measured by serial transplantation of these cells (Morrison and Weissman, 1994).

In 1992, we reported the first isolation of HSCs from human bone marrow or mobilized peripheral blood (MPB), testing the cells in irradiated SCID-hu mice (Baum et al., 1992; Peault et al., 1991). Purified HSCs were almost entirely depleted of contaminating T cells that could cause graft-versus-host (GvH) disease in allogeneic hosts and contaminating cancer cells if isolated from marrow or mobilized blood of patients with widespread metastatic breast cancer, recurrent non-Hodgkin’s lymphoma, and multiple myeloma (Weissman, 2000). This led to clinical trials in patients with these diseases and, in all cases, there was a dose-dependent recovery of blood granulocytes, B cells, T cells, erythrocytes, and platelets in these patients (Negrin et al., 2000). Long-term follow-up of metastatic breast cancer patients (who at last testing before transplantation were still chemoresponsive) treated with high-dose chemotherapy with carmustine, cisplatin, and cytoxan then rescued with either unfractionated MPB (cancer-contaminated) or purified HSCs (cancer-free) was reported in 2012 (Muller et al., 2012). As of that time, MPB-transplanted patients showed ~26-month median survival, while HSC recipients had 10-year median survival; overall survival 14–16 years was 7% of MPB-rescued patients, and 33% of HSC-rescued patients (Muller et al., 2012). The company that purchased the rights to this treatment never continued any HSC trials of autologous transplants in cancer patients or allogeneic transplants for correction of mutant blood systems, e.g., sickle cell anemia,
thalmia, SCID, type 1 diabetes, or systemic lupus erythematosus, the latter two established experimentally (Beilhack et al., 2003; Smith-Berdan et al., 2007; Uchida et al., 1998b, 1998a). In addition, tests and clinical trials for gene therapy of enriched or purified HSCs were carried out on and tested in the humanized SCID-hu mouse to make sure they could engraft and safely develop into corrected blood and immune systems (Uchida et al., 1998a, 1998b). As described above, HIV pathogenesis results in the loss of thymic production of new immune competent T cells and, with SCID-hu mice, it was first shown that the thymic precursors of both CD4 and CD8 T cells are immature CD4 cells (Kraft et al., 1993) susceptible to HIV infection (Su et al., 1995). Human immune responses can be tested with SCID-hu mice, even against HIV (Lavender et al., 2013). The pathogenesis of lymphomas arising in patients with organ transplants who are heavily immunosuppressed was first clarified in SCID-hu mice (Waller et al., 1993). Finally, human multiple myeloma cancer stem cells were discovered when tested in the human fetal bones of SCID-hu mice, while mouse bone engraftment did not work (Kim et al., 2012a, 2012b). All of these studies required the initial discovery of purified HSC in SCID-hu mice, a discovery that would not have been made had something like the Trump ban on fetal tissue been in effect at the time that the mouse model was developed.

Discoveries Made with Purified Human Hematopoietic Stem Cells and Diseases, Including Leukemias, Caused by Genetic Lesions in Them

The identification and isolation of human HSCs enabled the demonstration that the myelodysplastic syndrome (MDS) and myeloproliferative neoplasms (MPN) result from somatic mutations or chromosomal anomalies in a single HSC that replaced most or all identifiable normal HSCs (Jamieson et al., 2006; Pang et al., 2013, 2019), presumably by competition for normal HSC bone marrow niches (Chao et al., 2010a, 2010b; Feng et al., 2015, 2018). CD47 was then shown to be a “don’t eat me” signal on all tested human cancers (Advani et al., 2018; Edris et al., 2012; Kim et al., 2012a, 2012b; Liu et al., 2015; Sikic et al., 2019; Takimoto et al., 2019; Willingham et al., 2012). Therapeutic monoclonal blocking antibodies to CD47, alone and in combination with the anti-cancer antibodies rituximab for NHL, cetuximab and panitumumab in EGFR+ solid tumors, and trastuzimab in Her2+ breast cancers tested preclinically (Chao et al., 2010a, 2010b; Liu et al., 2015) and in phase 1/2 clinical trials have activity in some early phase trials (Advani et al., 2018; Sikic et al., 2019; Takimoto et al., 2019). Linking CHiP and atherosclerosis, independent studies showed that human and mouse atherosclerosis results from proliferative expansions of arteriolar and arterial smooth muscle cells that have calreticulin but resist phagocytosis by resident macrophages due to expression of dominant “don’t eat me” CD47 (Chao et al., 2010a, 2010b; Kojima et al., 2016). Other preclinical studies have demonstrated that not only cancer cells, but pathogenic fibroblasts in idiopathic pulmonary fibrosis, systemic sclerosis, renal and hepatic fibrosis, and peritoneal adhesions are CD47+ and calreticulin+, and respond to blocking anti-CD47 antibodies alone or in combination with other antibodies to these pathogenic cells (Tsai et al., 2018; Wernig et al., 2017). Several other “don’t eat me” molecules and their macrophage receptors have been discovered the past 2 years, each expressed only on a subset of cancers and subsets of scavenger macrophages, potentially opening the door to combined blocking antibodies to cover therapeutic trials in all human cancers (Bar-kal et al., 2018, 2019; Gordon et al., 2017). All of these studies required the initial discovery of purified HSCs in SCID-hu mice which, again, the Trump ban would have prevented.
Autoimmune Diseases Can Be Cured by Transplanted Healthy HSCs in Experimental Models

Transplantation of normal HSCs not only end the autoimmune phases of type 1 diabetes mellitus (Beilhack et al., 2003) and systemic lupus erythematosus (Smith-Berdan et al., 2007) models in mice, but also induce immunological tolerance to organ grafts from the HSC donor in allogeneic transplants (Gandy and Weissman, 1998; George et al., 2019; Shizuru et al., 1996); and the absence of T cells in the graft prevents the development of GvH disease (Uchida et al., 1998a, 1998b). For preparation of the recipient for transplantation, antibodies can replace irradiation or chemotherapeutic conditioning in SCID (Czechowicz et al., 2007) and normal mice (Chhabra et al., 2016) by removing recipient HSCs, T cells, and natural killer cells. The transplanted mice become immunologically tolerant to heart grafts from the HSC donor strain (George et al., 2019). Again, all of these studies required the initial discovery of purified HSCs in SCID-hu mice.

The Use of the SCID-hu Mouse to Identify Other Organ-Specific Stem Cells

Heart muscle precursors have been identified, enriched, and isolated by experimental differentiation from pluripotent human stem cell lines; such cells cannot be shown by transplantation into mice, as they cannot sustain viability in mouse hearts that beat >400 times per minute, but they readily engraft, fuse into existing heart myocardium, and beat synchronously in human fetal heart tissue transplants in SCID-hu mice (Ardehali et al., 2013; Loh et al., 2016). Human skeleton stem cells and multiple myeloma cancer stem cells were discovered using human fetal tissues and SCID-hu mice (Chan et al., 2018; Kim et al., 2012a, 2012b). Again, all of these studies required the initial discovery of purified HSCs in SCID-hu mice and/or testing in SCID-hu mice bearing human fetal tissue grafts.

Human fetal brain slices in vitro undergo the initial steps of multilayer formation of fetal brain architecture, giving clues to which steps might go wrong in diseases of neurodegeneration, brain cancer development, and inherited or acquired psychiatric disorders (Subramanian et al., 2017). Human CNS stem cells were isolated from human fetal brains, tested for function in SCID-hu mice, and revealed site-appropriate human CNS stem cell self-renewal, daughter cell migration, and site-appropriate differentiation to neurons, astrocytes, and oligodendrocytes (Kelly et al., 2004; Tamaki et al., 2002; Uchida et al., 2000, 2012). These CNS stem cells are mid-trial as therapies for two congenital human disorders first tested in SCID-hu mice, for thoracic and cervical spinal cord injury tested first in SCID-hu mice, and for dry, age-related macular degeneration. All of these studies required the use of human fetal brain tissues, including the initial discovery of purified CNS stem cells and their behavior in SCID-hu mice; they would have not occurred had the Trump ban on fetal tissue research been in place.

Conclusion and Recommendations

At a meeting at the National Institutes for Allergy and Infectious Diseases (NIAID) in December 2018, after hearing scientific evidence comparing the use of human fetal tissue in research (including in SCID-hu mice) and any other methods to study many central biomedical issues of human physiology, pathology, and infectious disease, Admiral Brett Giroir (Assistant Secretary for Health in the Department of Health and Human Services, or HHS) opined that, for the many instances cited at the meeting, the use of fetal tissue for such research was the gold standard and that, at present, no other methods could substitute. Despite this judgment by top officials at this evidentiary workshop, on June 5 by executive order, Secretary Azar of HHS announced a ban on intramural funding of such research by NIH, and declared that laboratories doing AIDS/HIV research at the Rocky Mountain Lab of NIAID in Hamilton, Montana, and at UCSF in California were to receive no further funding through pre-agreed contracts from NIAID. A report on events leading up to this executive order was recently published in Atlantic magazine (Nicholas, 2019). Even more recently, NIH announced restrictions on the use of human fetal tissues in research for all extramural grants including many barriers—restrictions that we believe are an effective ban—as well as banning students and postdocs funded by NIH from using human fetal tissue in any circumstance. Insofar as most academic labs rely on students and postdocs to do research, this is a ban.

This departure of policy based on biomedical research evidence goes against the mission of the NIH to advance medical research and its translation for the benefit of the citizenry. To the extent that it was motivated by the personal religious beliefs of those in the chain of authority leading to the ban, it appears that it also transgresses the separation of church and state: citizens who do not adhere to the same religious belief systems will nonetheless be denied the opportunity to access life-saving therapies that have been or could be shown completely reliant on the use of fetal tissue for their discovery and development. Each patient with an incurable disease that eventually requires the use of fetal tissue for their discovery and development. Every patient with an incurable disease that eventually could be treated by therapies derived from the described research has, had, or will have only a short window of time to receive these therapies; delay or abandonment of such therapies dooms them. In our view, those who blocked or delayed such therapies are responsible for their failure to be treated (Weissman, 2007).

We hold these convictions strongly because we have witnessed the entire evolution of work in humanized mice and we find that their application to problems in human disease
has gone well beyond that which we could have imagined 30 years ago. As described above, the advent of humanized mouse technology and the availability of primary human fetal cells (e.g., to test for candidate progenitor cells, such as HSCs and intrathymic T progenitor cells) newly enabled multiple broad platforms of investigation, all of which are relevant to our understanding of the pathophysiology and treatment of significant human disease states, many of which affect unborn children, their mothers, and newborns. Recent reviews highlight the fact that multiple new and improved generations of humanized mouse models have been developed and deployed during this time frame, and now comprise the basis for research in many NIH-funded labs in this country alone (Brehm et al., 2014; Marsden and Zack, 2015; Shultz et al., 2019). It is notable that, within days of the publication describing the first humanized mouse made with human fetal tissue (the SCID-hu mouse) (McCune et al., 1988), a second mouse model using non-fetal tissue (the hu-peripheral blood lymphocyte-SCID mouse) (Mosier et al., 1988) entered the scene. In part because of the relative ease of its construction, this latter model was studied extensively and found to not be applicable for the exploration of many of the above lines of research, particularly those necessitating the analysis of human cell maturation in vivo (e.g., from HSCs and intrathymic T progenitor cells); rather, it has been used most extensively to study GvH disease and lymphomagenesis induced by the Epstein-Barr virus (Ahmed and Baiocchi, 2016; McCune, 1991; Murphy et al., 1996). The comparative experiment suggested by Dr. Giroir, in other words, was largely completed three decades ago.

Research with human fetal tissue breaks no federal law, and those who support the ban are endangering the health and lives of those in the future who could benefit from discoveries and the resulting therapies emanating from such research. Whether if by intent or by neglect, such real or potential endangerment of many US citizens prompts us to call on the judicial, legislative, and executive branches of our government to decide the consequences to those in the chain of authority who have promulgated this funding ban.

In the interim, we believe that those who have acted or who will act to deprive biomedical researchers from using mouse models constructed with human fetal tissue should take a pledge not to accept any therapy derived from discoveries made when such research was allowed and funded, nor to avail themselves of subsequent discoveries that necessarily used these mice or variants of them.

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