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Journal of Stem Cell Research & Therapy

ISSN: 2157-7633

Review Article

Live Fetal Stem Cells Therapy, Anti-Neu5Gc Responses and Impact on Human Heart, Brain and Immune System

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ABSTRACT

Fetal stem cells used for clinical applications in humans can cause xenogenic immune reactions and impact vital organs by immune dysregulation. Animal stem/fetal cells express glycan antigens, such as Neu5Gc. Humans do not produce these antigens. Two major sialic acids are described in mammalian cells, Neu5Gc, the N-glycolylneuraminic acid, and Neu5Ac the N-acetylneuraminic acid. Neu5Gc synthesis starts from the N-acetylneuraminic acid (Neu5Ac) precursor modified by a hydroxylic group addition catalyzed by cytidinemonophospho-N-acetyl-neuraminic acid hydroxylase-Neu5Ac hydroxylase enzyme (CMAH). CMAH was inactivated by a 92 base pairs deletion over 2 million years ago and is non-functional in humans, Neu5Gc as well as the peptides derived from fetal cells is remarkably immunogenic for humans and promotes inflammation, arthritis, cancer. Accumulating evidence shows that xeno-transplantation of animal stem cells results in inflammation autoimmune responses and immune-rejection and may cause death. Here we highlight the serious deleterious effects of the presence of Neu5Gc antigen in animal fetal cells and the effects of presence and absence of Neu5Gc antibodies in humans acquired through the consumption of animal products. There are many reports of immune reactions to animal stem cells and their derivatives. Insect bite-related anti-alpha-gal and anti-Neu5Gc antibodies, and those induced by animal cells, cause "immune enhancement" and serious allergic reactions and or immune-pathology leading to irreversible damage to vital organs in the human body. Therefore needless to say that the use of animal stem cells and their derivatives being marketed as nutritional supplements are harmful for human use and should not be used either as oral supplements or as peptide injections. There is no evidence of their safety or efficacy for treating disease conditions or for their anti-aging effects. Neu5Gc (xeno-antigen), are produced by Animal Stem/fetal Cells and the peptides derived from them. Remarkably, Neu5Gc is a xeno-antigen for humans, and promote inflammation, arthritis, cancer, and xeno-transplantation of animal stem cells can result in inflammation autoimmune responses and immune-rejection (GVHD) and death.

Keywords: Neu5Gc; Animal stem cells; Fetal stem cells; Live stem cell therapy; Heart; Brain; Immune-enhancement

INTRODUCTION

Stem cells

Stem cells are undifferentiated cells found in all tissues of the body. Although these cells are normally kept in a quiescent, non-dividing state, they proliferate and differentiate to replace naturally dying cells (senescent cells) within tissues and to perform the repair to injury [1-5]. Human Embryonic Stem Cells (hESCs), due to their proliferative nature and their implicit ability to regenerate tissue, have the potential to treat a variety of degenerative diseases as well as aging. Several embryonic stem cell lines generated from blastocyst stage (4 to 5 days post gamete fusion) be self-renewing and able to produce approximately 298 types of the cells in the body through the process of differentiation and are exemplary pluripotent stem cells. Human

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Received date: April 29, 2020; **Accepted date:** April 30, 2020; **Published date:** June 01, 2020

Citation: Bhogal B, Royal D, Boer R, Phillips J, Knight A (2020) Live Fetal Stem Cells Therapy, Anti-Neu5Gc responses and Impact on Human Heart, Brain, and Immune System. J Stem Cell Res Ther 10:459. doi:10.35248/2157-7633/20.10.459.

Mesenchymal Stem Cells (MSCs) are present in bone marrow, adipose tissue, and birth tissues [4,5] as adult stem cells (multipotent).

An ideal stem cell for human clinical applications should not:

(1) Induce an immune response; and

(2) Should not require any immunosuppression. Both, immune responses to stem cells and immune-suppression pose clinical risks

Autologous stem cells are likely to meet the above safety profile, although some reports suggest that autologous stem cells show immune reactions (reasons discussed below). Allogeneic stem cells (bone marrow stromal cells, hematopoietic stem cells, and cells from umbilical cord blood/tissue), and an alternative source of stem cells, inducible Pluripotent Stem Cells (iPSCs), also present immune reactivity in human clinical applications [6,7]. It appears stem cells, in general, may induce some degree of immune reactivity [8,9] and may require at least transient immune suppression. There is evidence that animal stem cells (xenogeneic stem cells) express glycan antigens like Neu5Gc [10,11] and therefore can potentially induce an overwhelming Xeno reactivity in humans [12,13]. Therefore, Neu5Gc like glycan antigens, make the animal cells very harmful, and certainly, the human immune system does not consider these cells as friendly as suggested [14]. The presence of Neu5Gc in animal cells is a perfect example of the proverbial saying wolf in a sheep's clothing. Animal stem cells with the constitutive Neu5Gc antigen can be nothing but a source of inflammation if and when injected into humans and cause collateral damage in terms of morbidity and mortality on both short-term and long term basis (see below).

Xeno-antigens corrupt human embryonic stem cells

In as much as hESCs, iPSCs and human MSCs have the unique abilities for unlimited proliferation and differentiation and potential for human therapeutics and are believed to have the game-changing impact on strategies for disease prevention and treatment as well as for anti-aging strategies, the use of these stem cells has been curtailed due to their contamination by animal cell products used to grow these cells *in vitro*. The utility of these cells is currently limited for drug screening, study effects of stem cells *in vitro*, and studies of cell therapy applications in animal models of human diseases. The problems specifically with hESCs for their clinical use are twofold:

(1) Direct or indirect effects of xeno-contamination during their derivation and propagation in culture media containing animal-derived materials, such as Fetal Bovine Serum (FBS) and or inactivated Mouse Embryonic Fibroblasts (MEFs), or animal feeder cell layers [15-19], xenogeneic antigens/components, as well as the pathogen, retroviral oncogene transmission, and toxic protein transfer to human hosts [20-22], and

(2) Spontaneous teratogenesis [20]. Thus, stem cells (hESCs or iPSCs) exposed to conventional culture conditions, have increased risks of immune rejection. If the xeno-corrupted/contaminated hESCs are unsuitable for clinical applications in their current format, beyond doubt, injection of animal fetal stem cells into humans would create a massive immunological storm and autoimmune problems. The animal fetal cells express Neu5Gc (a well-characterized xeno-antigen) in hundreds of millions of glycan chains per cell [23]. There is a tremendous effort to eliminate animal-derived materials from hESCs and some progress in producing xeno-free culture conditions to reduce risks of transmitting non-human pathogens and immunogenic molecules have been on the horizon. Fetal Bovine Serum (FBS) has been replaced with Knock Out Serum and replacement with human-derived feeder cells.

The human immune system can be a double-edged sword when provoked by xeno antigens and animal stem cells

The immune system has evolved to seek out and eliminate or neutralize non-self-immunogens and pathogens. As such, transplantation of cells or tissues from genetically non-identical individuals activates immune responses that reject the transplanted cells and tissues. To prevent rejection immunosuppressive drugs are often used in clinical practice. The side-effects of long-term use of immunosuppressive drugs include such reactions as opportunistic infections, drug-related toxicities, cancer and immune dysregulation/autoimmunity/immune enhancement with associated cytokine storm, and diseases like diabetes and Graft Versus Host Disease (GVHD). Thus, the immune rejection of transplanted stem cells remains a hurdle and this could be a much bigger problem in a xenogeneic situation. One would think that embryonic stem cells may provoke less or no immune response; however, this may not be true of the differentiated tissue derived from the embryonic stem cells and for cells processed or exposed in *in vitro* culture media containing animal products. Stem cells are contaminated with animal components, such as Neu5Gc, when grown or processed in culture media containing animal products. Therefore, Major Histocompatibility Complex (MHC) antigen expression, and therefore, immunogenicity, will depend upon the cell type into which the stem cells differentiate, and their immunogenicity is increased in the presence of inflammation and an autoimmune response or signals that up-regulate co-stimulatory molecules and attract Antigen-Presenting Cells (APCs).

What is Neu5Gc, the relationship between Neu5Gc immunogenicity for humans and collateral damage that kills

Two major sialic acids are described in mammalian cells, (Neu5Gc) the N-glycolylneuraminic acid), and (Neu5Ac) the N-acetylneuraminic acid [24,25]. Neu5Gc synthesis starts from the N-acetylneuraminic acid (Neu5Ac) precursor modified by a hydroxylic group addition catalyzed by Cytidine Monophospho-N-Acetyl-Neuraminic Acid Hydroxylase-Neu5Ac Hydroxylase Enzyme (CMAH). Despite the ubiquity of Neu5Gc in most mammals, CMAH is non-functional in all humans [26,27] due to a deletion of CMAH exon 6 [28]. In humans, CMAH was inactivated by a 92 base pairs deletion that occurred over 2 million years ago. However, livestock species used for food production and as a source of biological materials for medical

applications carry Neu5Gc in hundreds of millions of glycan chains per cell [29]. Sialic acids play a special role in physiological processes and immune responses.

Consequently, it seems that it is technically impossible to avoid immune responses to Neu5Gc like antigens and immune rejection of injected animal stem/fetal cells because animal stem cells carry Neu5Gc like glycan antigens. Such antigens are inherently immunogenic to the human immune system unless there is a genetic manipulation to remove the Neu5Gc gene or insert the CMAH gene in the donor rabbits and sheep. Hence, due to an imminent elimination of transplanted fetal stem cells, it will make these cells ineffective for the intended clinical use to treat a disease condition and also for some reason if these cells establish in the human the immune complexes generated during the immune responses engendered against Neu5Gc may cause GVHD reaction and even death. In clinical practice, GVHD reactions are very common after cell transfusions and can range from barely noticeable to life-threatening. Acute GVHD starts soon after the transplant of cells and lasts a short time. Chronic GVHD starts later and lasts a long time. A person could have one, both, or neither type of GVHD and there are reports of many such instances. Neu5Gc and, therefore animal stem cells or their derivatives, is proving to be far more dangerous to far more parts of the body.

The pathological consequence of the presence of Neu5Gc glycan Vs absence and presence of anti-Neu5Gc antibodies in humans

The available large body of data suggests that Neu5Gc glycan cannot be viewed anything but a foe for human health and the immune system and the friendly face of Neu5Gc glycan has not yet been translated in regenerative medicine for its regenerative effects. There is some speculation that Neu5Gc may be a potential target to treat some malignancies [30]. However, the Neu5Gc targeting strategy is complicated by the absence of functional CMAH, the enzyme required for Neu5Gc sialic acid biosynthesis in humans. Neu5Gc has been overwhelmingly criticized for its adverse effects. A wealth of scientific evidence has been published to show that consumption of red meat (beef, pork, and lamb) causes the incidence of carcinomas, atherosclerosis, type 2 diabetes, brain tumors, and death. Humans carry natural, diet-induced, Anti-Neu5Gc antibodies, and when undertaking medical treatments or receiving transplants or devices that contain animal-derived products they can cause immunological reactions affecting pharmacology, immune tolerance, and severe side-effects like Serum Sickness Disease (SSD). Injections of animal cells expose human subjects to a large amount of Neu5Gc antigen and it is estimated that the average number of cells/g of tissue is 1.2×10^8 cells, and amount of Neu5Gc in animal cells is about 4 g to 5 g per 250 g of red meat and is likely to induce an immune response in humans [31].

Xenogeneic (Animal) stem cells and their derivatives

In addition to stem cells obtained from animal fetal tissues and used for human clinical applications, derivatives of such cells also pose serious safety issues. Animals cells are known to transmit various animal pathogens to humans [32], including the transmission of prions and retroviral oncogenes [33,34].

Even though stem cells obtained from Specific Pathogen-Free (SPF) animals may reduce the risk of transmission of certain pathogens to the host, such cells and their derivatives may still pose an increased risk of transmission of retroviral oncogenes [33]. Neu5Gc like glycans have been shown to result in cancer progression especially if the xenogeneic cells also migrate away from the site of transplantation. Already in humans, even allogeneic transplanted neural stem cells have caused a braintumor in at least one patient [34]. Furthermore, some types of iPSC-derived neural cells have an increased likelihood of tumor formation after transplantation into the brain or spinal cord. Efforts are being made with the hope that the selection of safe hESCs and iPSC-derived clones and removal of xeno-antigen contamination may overcome these issues [35-37].

In the context of immune responses to xeno-antigens (e.g., Neu5Gc and other glycan antigens) presented by animal stem cells, and the peptides derived from animal cells, a recent publication [14] advances an argument to justify the clinical use of animal stem cells in humans. These authors believe that the lack of dendritic cells in fetal tissue makes the animal fetal tissue less or non-immunogenic. Immunologically, this is a false and unsubstantiated argument to justify the use of animal stem cells in humans. The argument for a low number of dendritic cells in fetal tissue rendering these stem cells suited for human clinical use is flawed for the following reasons:

1. Clairace et al., [14] does not provide any data about the number of dendritic cells in rabbit or sheep fetal tissue

2. Clairace et al., also does not provide any data about the level of Neu5Gc or other similar glycan antigens in sheep or rabbit fetal tissue

3. Even though fetal tissue, as has been suggested, may have little or no MHC class I or II antigens, as well as dendritic cells, the intensity of immune responses to Neu5Gc antigen will be determined by the dendritic cells of the human host and its presentation to the human immune system, not the rabbit or sheep tissue

4. The human immune system and its antigen-presenting cells and the dendritic cell repertoire in the human will process the xeno-antigen (Neu5Gc) and determine the intensity of immune responses, not the rabbit or sheep fetal tissue and or the resident dendritic cell numbers in the donor animal. The human immune system is trained to recognize foreign antigens via a very precise system (incl., dendritic cells, Langerhans cells, antibodies, etc.).The presence or absence or low numbers of dendritic cells in the animal fetal tissue becomes irrelevant to the human immune system when the fetal cells, isolated from the donor animal and injected into a human as the Neu5Gc like xeno-antigens will be recognized as foreign. Thus, the relevance of presence or absence of dendritic cells in animal fetal tissue is a baseless argument and indicates a poor understanding on the part of the authors [14] of how the immune system works, and

5. Low numbers of antigen-presenting cells are naturally designed in fetal tissue to protect the fetus from immunological

recognition and rejection. Numerous fetal, maternal, and placental mechanisms work in concert during human placentation, and several changes occur in the uterus. For a successful pregnancy to occur, interactions between fetaltrophoblasts and maternal decidual immune cells, natural killer cells, immature dendritic cells, T cells, and macrophages are minimized, which allow the semi-allogeneic embryo and subsequent fetus to develop in the uterus while the mother's immune system remains largely intact.

Furthermore, the authors [14] quote various studies [33-38] to support their argument as examples for the acceptability of xeno-antigens. One study [33] shows the role of passenger leukocytes in an allogeneic response milieu and not a xenogeneic situation. In the other study [35], the results of a xenogeneic system have been reported where human mesenchymal stem cells engrafted in sheep are reported to persist in multiple tissues for as long as thirteen months after transplantation. Transplanted human cells undergo site-specific differentiation into chondrocytes, adipocytes, myocytes and cardiomyocytes, bone marrow stromal cells, and thymic stroma. Here, it should be pointed out that because the human cells do not express Neu5Gc, they do not present a challenge of Neu5Gc xeno-antigens to sheep immune system.

Given the foregoing, it seems, therefore, that the immunogenicity and xenoreactivity of animal stem cells seem to have been either overlooked or grossly misrepresented to desperate patients for financial gains by the promoters of animal stem cells. The applications of animals stem cells, as well as the exposure of humans to the Neu5Gc antigen, needs to be reviewed carefully in the light of the current information about xenogeneic antigenic pathological effects. Immune recognition of xeno-antigens and subsequent immune rejection of transplanted animal cells, are associated with serious pathological effects and may cause long term health issues. There are many reports of adverse events [39-65]. The claims about the safety and efficacy of animal stem cells made in non-peer reviewed publications and promotion of animal stem cells without any regulatory approvals pose a major hazard and challenge and bring the entire field into disrepute.

Oral consumption of Neu5Gc, oral tolerance, and auto-immunity

It is estimated that 250 g red meat per day equals to a daily intake of 4.5 g-7.5 g Neu5Gc [30,39,45]. Such large amounts of antigens exposure by oral route can induce oral tolerance and activation of systemic immune cells [66,67]. In a recent publication, Clairace et al. [14] show a long list of products manufactured for human as well as animal clinical use that are currently approved by the Food and Drug Administration (FDA). The list shows these products without Neu5Gc content. The authors do not show the Neu5Gc content perhaps to affirm their claim that the many animal cells, animal fetal stem cells, and derivatives of animal cells in the form of peptides being marketed by them are also acceptable if the FDA approved products in that list are good for clinical applications. The products being sold by these authors are not FDA approved and have no technical data to show their safety or efficacy. Instead, the animal stem cell derivatives are being promoted as nutritional supplements in many countries where regulatory conditions are lax or non-existent. These authors, Clairace et al., [14] do not show any reactivity to the Neu5Gc-like contaminants or list any side-effects.

Interestingly, these authors fail to show that all these products have Neu5Gc contamination as we show here in Table 1, according to Darius Ghaderi et al. [19]. All products in the FDA list, such as several protein classes, i.e., clotting factors, hormones, cytokines, anti-sera, enzymes, enzyme inhibitors, Ig-Fc-Fusion proteins, and monoclonal antibodies have serious side-effects listed for these products-even after they have undergone safety evaluations. Unfortunately, the safety tests performed in animal models have flaws in that the target animals express both the terminal Gal.1-3Galb1-(3)4GlcNAc (A-Gal) epitope, and Neu5Gc, a major mammalian sialic acid. Pre-clinical studies in animals to test the efficacy, potential side effects, and immunogenicity of drugs contaminated with alpha-Gal or Neu5Gc fail to show the adverse effects of Neu5Gc because animals, such as mice, rats, or rabbits have the natural occurrence of Neu5Gc. Clearly, these non-human glycan epitopes can potentially affect the immunogenicity and/or efficacy of therapeutic glycoproteins in a disadvantageous manner for the patient, as all humans tested have been found to have circulating antibodies against Neu5Gc (e.g., glycan antigens).

Table 1: Overview of currently FDA-approved therapeutic glycoproteins derived from different mammalian sources and the likelihood of Neu5Gc contamination.

Agent	Marketing Component	Source	Chance of Neu5Gc
Monoclonal Antibodies			
Actemra® Tocilizumab	Genentech Inc., Hoffmann-La Roche Ltd.	CHO	++
Avastj11® Bevacizumab	Genentech Inc., Hoffmann-La Roche Ltd.	CHO	++
Campath®, Mabcampath® Alemtuzumab	Genzyme Corp.	CHO	++
Herceptin® Trastuzumab	Genentech Inc., Hoffmann-La Roche Ltd.	CHO	++
Humira® Adalimumab	Abbott Laboratories	CHO	++
Prolia® Denosumab	Amgen	CHO	++
Rituxanf® Rituximab	Genentech Inc, Biogen Idec	CHO	++

Simponi® Golimumab	Centocor Ortho Biotech Inc., Merck and Co	CHO	++
Stelara™ Ustekinuman	Centocor Ortho Biotech Inc.	CHO	++
Veetibix® Panitumumab	Amgen	CHO	n.d.
Xolair® Omalizumab	GcnentcchInc.. Novartis Pharmaceut. Corp.Tanox Inc.	CHO	++
Yervoy® Ipilimumab	BMS	CHO	++
Zevalin® Ibritumomabtiuxetan	Biogen Idec.• Bayer Schering Pharma AG	CHO	++
Bexxar® Tositumomab-1131	Glaxo Smith Kline	Hyb.	+++
Orthoclone Okt3® Muromonab-CD3	Ceniooor Ortho Biotech Inc.	Murine ascitcs	+++
Soliris® Eculizumab	Alexion Pharmaceuticals, Inc	Mye.	+++
Arzerra® Ofatumumab	Glaxo Smith Kline	NS0	+++
Benlysta® Belimumab	Human Genome Sciences Inc.	NS0	+++
Mylotarg® Gemtuzumabozogamicin	Wyeth Pharmaceuticals	NS0	+++
Synagis® Palivizumab	Abbott Labs, Med Immune Inc.	NS0	+++
Tysabri® Natalizumab	ÉIan Pharmaceut., Biogen Idec	NS0	+++
Erbitux® Cetuximab	ImClone Systems, BMS	Sp2/0	+++
Ilaris® Canakinumab	Novartis Pharmaceuticals	Sp2/0	+++
Remicade® lnfliximab	Centocor Ortho Biotech Inc.	Sp2/0	+++
Repro® Abciximab	Centocor Ortho Biotech Inc. Eli Lilly and Co.	Sp2/0	+++
Simulect® Baxiliximab	Novartis Pharmaceuticals Corp.	Sp2/0	+++
Zenapax® Daclizumab	Hoffmann -La Roche Ltd., PDL BioPharma	Sp2/0	+++
Fc-Fusion Proteins			
Amevive® Alefacept	Astellas Pharma Inc.	CHO	++
Arcalyst® Rilonacept	Regeneron Pharmaceut. Inc.	CHO	++
Enbrel® Etanercept	Amgen, Wyeth Pharmaceutical	CHO	++
Nulojix Belatacept	BMS	CHO	++
Orencia® Abatacept	BMS	CHO	++
Hormones			
Follistim® Follitropin- β	Merck and Co	CHO	++
Gonal F® Follitropinalfa	EMD Serono, Inc.	CHO	++
Luveris® LH	EMD Serono. Inc.	CHO	++
OP-1 Putty Osteogenic protein- 1	Stryker Biotech	CHO	++
Ovidrel® Choriogonadotophin α	EMD Serono, Inc.	CHO	++
Thyrogen® Thyrotropin α	Genzyme Corp	CHO	++
Serostim®, Saizen®, Zorbtive™ Somatropin	EMD Serono. Inc.	Murine Cl27	+++
Emdogain® tooth enamel proteins	Staumann USA	Pig	+++
Cytokines			
Aranesp® Darbepoetin α	Amgen	CHO	++
Avonex® Interferon β -1a	BiogenIdec, lnc.	CHO	++
Neo Recormon Epoetin β	Hoffmann-La Roche Ltd.	CHO	++
Procrit®, Epogen® Epoetin α	Amgen, Centocor Ortho Biotech lnc.	CHO	++
Rebif® Interferon β -1a	Pfizer, Inc., EMD Serono, Inc.	CHO	++
Clotting Factors			
Xigris® Drotrecogin α	Eli Lilly and Co.	Hek293	+
Helixatc FS Factor VIII	ZLB Behring	BHK	++
Kogenate FS FactorVIII	Bayer Schering Corp.	BHK	++
NovoSeven® Factor VIla	Novo Nordisk	BHK	++

Advate® Antihemophilic Factor	Baxter InternationalJnc.	CHO	++
BeneFIX® Factor IX	Wyeth Pharmaceuticals	CHO	++
ReFacto® Antihemophilic Factor	Wyeth Pharmaceuticals	CHO	++
Xyntha® Factor VIII	Wyeth Pharmaceuticals	CHO	++
Enzyme Inhibitor			
ATryn® Antithrombin/ ATIII	GTC Biotherapeutics	Goat milk	+++
Enzymes			
Elaprase® ldursulfase	Shire Pharmaceuticals	HT-1080	+
Activase®, Cathflo® Activase®, Actilyse®Alteplase	Genentech Inc, Boehringer Ingelheim Pharma KG	CHO	++
Aldurazyme® Laronidase	Genzyme Corp	CHO	++
Certzyme® Imiglucerase	Genzyme Corp.	CHO	++
Fabrazyme® agalsidase- β	Genzyme Corp	CHO	++
Hylenex®, Cumulasc® Hyaluronidase	Medi Cult A/S, Mid Atlantic Diagnostics, Inc., Halozyme Baxter Healthcare	CHO	++
Myozyme®, Lumizyme® Alglucosidasealfa	Genzyme Corp	CHO	++
Naglazyme® GalNAc 4-sulfatase	Bio Marin Pharmaceutical Inc.	CHO	++
Pulmozyme® Human DNase	Genentech Inc., Hoffmann-La Roche Ltd.	CHO	++
TNKase® Tenecteplase	Genentech Inc	CHO	++
Amphadase® Hyaluronidase	Amphastar Pharmaceuticals	Bovine	+++
Creon® Pancrelipase	Abbott Products, Inc.	Pig	+++
Pancreaze® Pancrelipase	Ortho McNeil Janssen	Pig	+++
Vitrase® Hyaluronidase	ISTA Pharmaceuticals	Sheep	+++
Antisera			
Atgam® Anti-thymocyte globulin	Pfizer, Inc.	Equine Serum	+++
Thymoglobulin® Anti-thymocyte globulin	Genzyme Corp.	Rabbit Serum	+++
CroFab® Crotalidae Polyvalent Immune Fab	Savage Laboratories	Sheep Serum	+++
DigiFab® Digoxin Immune Fab	Savage Laboratories	Sheep Serum	+++

Meat allergy cases have increased in Sweden significantly over the past few years and all the patients presenting with such an allergy were found to have been bitten by a tick. The alpha-Gal present in the European tick *Ixodesricinus* is considered to be the cause of sensitization and explains the strong correlation between anti-alpha-Gal IgE due to tick bites, with development of red meat allergy as a secondary phenomenon [54,67]. Since Lyme disease is transmitted through tick bites, the immune reaction,along with the immunopathology of Lyme disease in 207 patients has also been correlated to alpha-Gal [68,69]. Similarly, the injection of a peptide derived from animal cells may pose a similar immune reaction as from a tick bite. We have observed (unpublished data) severe reactions in patients injected with the peptides manufactured by the company FCTI. Consequently, the Neu5Gc glycan antigen appears to be antagonistic to human health.Therefore, any products containing the Neu5Gc antigen should not be accepted for the treatment of human chronic conditions and anti-aging applications.

CONCLUSION

Given the problems discussed above to make the stem cells available for human clinical use they have to be:

- Safe
- Effective
- Affordable

To evaluate the safety of animal stem cells or human embryonic stem cells, grown in conventional culture medium containing animal products, and used in transplantation, it may help to screen the patients for Neu5Gc antibodies before transplantation or injection of any animal stem cell products (e.g., peptides). Patients should also be monitored for sero-conversion to glycan antigens (e.g., Nue5GC) post-injection. Xenogeneic (e.g., animal) stem cells and stem cells of unknown origin should not be injected into patients unless their definition, safety, and efficacy have been well-established. Regenerative Medicine offers many potential non-pharmacological opportunities for the successful treatment of chronic diseases and aging. Stem cell therapies represent only one of the many such opportunities. Therefore, false and we should not make false claims about safety and efficacy along with all such unscrupulous activities and claims made should be challenged if the stem cell field is to be saved from disrepute.

CONFLICT OF INTEREST

The authors report no conflicts of interest concerning the materials or methods used in this review or the findings specified in this paper. The authors have no competing financial interests related to this study.

REFERENCES

1. Caplan AI. The mesengenic process. ClinPlast Surg. 1994;21:429-435.
2. Dennis JE, Merriam A, Awadallah A, Yoo JU, Johnstone B, Caplan AI. A quadripotentialmesenchymal progenitor cell isolated from the marrow of an adult mouse. J Bone Miner Res. 1999;14:700-709.
3. Pittenger MF, MacKay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science. 1999;284:143-147.
4. Digirolamo CM, Stokes D, Colter D, Phinney DG, Class R, Prockop DJ. Propagation and senescence of human marrow stromal cells in culture: a simple colony-forming assay identifies samples with the greatest potential to propagate and differentiate. Br J Haematol. 1999;107:275-281.
5. Mauraglia A, Cancedda R, Quarto R. Clonal mesenchymal progenitors from human bone marrow differentiate in vitro according to a hierarchical model. J Cell Sci. 2000;113:1161-1166.
6. Okita K, Nagata N, Yamanaka S. Immunogenicity of induced pluripotent stem cells. Circ Res. 2011;109:720-721.
7. Zhao T, Zhang ZN, Rong Z, Xu Y. Immunogenicity of induced pluripotent stem cells. Nature. 2011;474:212-215.
8. Robertson NJ, Brook FA, Gardner RL, Cobbold SP, Waldmann H, Fairchild PJ. Embryonic stem cell-derived tissues are immunogenic but their inherent immune privilege promotes the induction of tolerance. Proc Natl Acad Sci USA. 2007;104:20920-20925.
9. Herberts CA, Kwa MS, Hermsen HP. Risk factors in the development of stem cell therapy. J Transl Med. 2011;9:29.
10. Varki A, Cummings RD, Esko JD, Stanley P, Hart GW, Aebi M, et al. Sialic acids and other nonulosonic acids. In: Essentials of Glycobiology. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. 2015:179-195.
11. MacKenzie KJ. Implications of xenoglycan sensitivity for increased cancer risk. Proc Natl Acad Sci USA. 2015;112:E1404.
12. Rémy S, Canova C, Daguin-Nerrière V, Martin C, Melchior B, NeveuI,et al. Different mechanisms mediate the rejection of porcine neurons and endothelial cells transplanted in to the rat brain. Xenotransplantation. 2001;8:136-148.
13. Apostolovic D, Tran TAT, Starkhammar M, Sánchez-Vidaurre S, Hamsten C, van Hage M. The red meat allergy syndrome in Sweden. Allergo J Int. 2016;25:49-54.
14. Anjum C, Chia YC, Chan MKS, Wong MBF, Pan SY. Presence of Neu5Gc in animal-derived products: Friend Or foe? Stem Cells Regen Med. 2020;4:1-7.
15. Grinnemo KH, Sylvén C, Hovatta O, Dellgren G, Corbascio M. Immunogenicity of human embryonic stem cells. Cell Tissue Res. 2008;331:67-78.
16. Martin MJ, Muotri A, Gage F, Varki A. Human embryonic stem cells express an immunogenic nonhuman sialic acid. Nat Med. 2005;11:228-232.
17. Padler-Karavani V, Varki A. Potential impact of the non-human sialic acid n-glycolylneuraminic acid on transplant rejection risk. Xenotransplantation. 2011;18:1-5.
18. Lu T, Yang B, Wang R, Qin C. Xenotransplantation: Current status in preclinical research. Front Immunol. 2019;10:3060.
19. Ghaderi D, Taylor RE, Padler-Karavani V, Diaz S, Varki A. Implications of the presence of N-glycolylneuraminic acid in recombinant therapeutic glycoproteins. Nat Biotechnol. 2010;28:863-867.
20. Blum B, Benvenisty N. The tumorigenicity of human embryonic stem cells. Adv Cancer Res. 2008;100:133-158.
21. Wang WH, Sun XF. Human embryonic stem cell lines are contaminated: What should we do? Hum Reprod. 2005;20:2987-2989.
22. Tanne JH. All approved US embryonic stem cell lines may be contaminated. BMJ. 2005;330:214.
23. Mahmood A, Ali S. Microbial and viral contamination of animal and stem cell cultures: Common contaminants, detection and elimination. J Stem Cell Res Ther. 2017;2:149-155.
24. Banda K, Gregg CJ, Chow R, Varki NM, Varki A. Metabolism of vertebrate amino sugars with N-glycolyl groups: Mechanisms underlying gastrointestinal incorporation of the non-human sialic acid xeno-autoantigen N-glycolylneuraminic acid. J Biol Chem. 2012;287:28852-28864.
25. Varki A, Schauer R. Essentials of Glycobiology. Cold Spring Harbor Laboratory Press. 2009.
26. Irie A, Koyama S, Kozutsumi Y, Kawasaki T, Suzuki A. The molecular basis for the absence of N-glycolylneuraminic acid in humans. J Biol Chem. 1998;273:15866-15871.
27. Chou HH, Takematsu H, Diaz S, Iber J, Nickerson E, Wright KL, et al. A mutation in human CMP-sialic acid hydroxylase occurred after the Homo-Pan divergence. Proc Natl Acad Sci USA. 1998;95:11751-11756.
28. Hayakawa T, Satta Y, Gagneux P, Varki A, Takahata N. Alu-mediated inactivation of the human CMP- N-acetylneuraminic acid hydroxylase gene. Proc Natl Acad Sci USA. 2001;98:11399-11404.
29. Lanctot PM, Gage FH, Varki AP. The glycans of stem cells. CurrOpinChem Biol. 2007;11:373-380.
30. Dorvignit D, Boligan KF, Relova-Hernández E, Clavell M, López A, Labrada M, et al. Antitumor efects of the GM3(Neu5Gc) ganglioside-specifc humanized antibody 14F7hT against Cmah-transfected cancer cells. Sci Rep. 2019;9:9921.
31. Tullis GE1, Spears K2, Kirk MD. Immunological barriers to stem cell therapy in the central nervous system. Stem Cells Int. 2014:507905.
32. Takeuchi H, Kobayashi R, Hasegawa M, Hirai K. Detection of latent infection by Epstein-Barr virus in peripheral blood cells of healthy individuals and in non-neoplastic tonsillar tissue from patients by reverse transcription-polymerase chain reaction. J Virol Methods. 1996;58:81-89.
33. Gilis L, Morisset S, Billaud G, Ducastelle-Leprêtre S, Labussière-Wallet H, Nicolini FE, et al. High burden of BK virus-associated hemorrhagic cystitis in patients undergoing allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant. 2014;49:664-670.
34. Le Berre L, Salama A, Evanno G, Rousse J, Nicot A, Semana G, et al. Increased IGM and IGG anti-NEU5GC antibodies in infectious mononucleosis (IMN): Link with multiple sclerosis (MS)? Xenotransplantation. 2015;22:S86-S86.
35. Meyer TJ, Rosenkrantz JL, Carbone L, Chavez SL. Endogenous Retroviruses: With us and against us. Front Chem. 2017;5:23.
36. Tannenbaum SE, Turetsky TT, Singer O, Aizenman E, Kirshberg S, Ilouz N, et al. Derivation of xeno-free and gmp-grade human embryonic stem cells-platforms for future clinical applications. PLoS One. 2012;7:e35325.

37. Afify SM, Seno M. Conversion of stem cells to cancer stem cells: Undercurrent of cancer initiation. Cancers. 2019;11:345.
38. Dorvignit D, Boligan KF, Hernández ER, Clavell M, López A, LabradaM, et al. Antitumor effects of the GM3(Neu5Gc) ganglioside-specific humanized antibody 14F7hT against Cmah-transfected cancer cells. Sci Rep. 2019;9:9921.
39. Samraj AN, Pearce OMT, Läubli H, Crittenden AN, Bergfeld AK, Banda K, et al. A red meat-derived glycan promotes inflammation and cancer progression. Proc Natl Acad Sci USA. 2015;112:542-547.
40. Ben-Arye SL, Schneider C, Yu H, Bashir S, Chen X, Gunten SV, et al. Differential recognition of diet-derived Neu5Gc-neoantigens on glycan microarrays by carbohydrate-specific pooled human IgG and IgA antibodies. Bioconjug Chem. 2019;30:1565-1574.
41. Engelking C. Red meat increases cancer risk because of toxic immune response. Discover. 2015;5:15.
42. Ghaderi D, Zhang M, Hurtado-Ziola N, Varki A. Production platforms for biotherapeutic glycoproteins: Occurrence, impact, and challenges of non-human sialylation. Biotechnol Genet Eng Rev. 2012;28:147-175.
43. Rémy S, Canova C, Daguin-Nerrière V, Martin C, Neveu I, Charreau B, et al. Different mechanisms mediate the rejection of porcine neurons and endothelial cells transplanted in to the rat brain. Xenotransplantation. 2002;8:136-148.
44. Coyne TM, Marcus AJ, Woodbury D, Black IB. Marrow stromal cells transplanted to the adult brain are rejected by an inflammatory response and transfer do no labels to host neurons and glia. Stem Cells. 2009;24:2483-2492.
45. Rose DP, Boyar AP, Wynder EL. International comparisons of mortality rates for cancer of the breast, ovary, prostate, and colon, and per capita food consumption. Cancer. 1986;58:2363-2371.
46. Willett WC. Diet and cancer. Oncologist. 2000;5:393-404.
47. Varki A. Are humans prone to autoimmunity? Implications from evolutionary changes in homininsialic acid biology. J Autoimmun. 2017;83:134-142.
48. Chou HH, Takematsu H, Diaz S, Iber J, Nickerson E, Kerry L, et al. A mutation in human CMP-sialic acid hydroxylase occurred after the Homo-Pan divergence. Proc Natl Acad Sci. 1998;95:11751-11756.
49. Tavani A, La VC, Gallus S, Lagiou P, Trichopoulos D, Levi F, et al. Red meat intake and cancer risk: A study in Italy. Int J Cancer. 2000;86:425-428.
50. Norat T, Lukanova A, Ferrari P, Riboli E. Meat consumption and colorectal cancer risk: Dose-response meta-analysis of epidemiological studies. Int J Cancer. 2002;98:241-256.
51. Zhang J, Kesteloot H. Milk consumption in relation to incidence of prostate, breast, colon, and rectal cancers: Is there an independent effect? Nutr Cancer. 2005;53:65-72.
52. Cross JA, Ferrucci LM, Risch A, Graubard BI, Ward MH, Park Y, et al. A large prospective study of meat consumption and colorectal cancer risk: An investigation of potential mechanisms underlying this association. 2010;70:2406-2414.
53. Amon R, Reuven EM, Ben-Arye LS, Karavani VP. Glycans in immune recognition and response. Carbohydr Res. 2014;389:115-122.
54. Samraj AN, Pearce OMT, Läubli H, Crittenden AN, Bergfeld AK, Banda K,et al. Increased IGM and IGG anti-NEU5GC antibodies in infectious mononucleosis (IMN): link with multiple sclerosis (MS)? Xenotransplantation. 2015;22:S86-S86.
55. Giovannucci E, Rimm EB, Colditz GA, Stampfer MJ, Ascherio A, Chute CG, et al. A prospective study of dietary fat and risk of prostate cancer. J Natl Cancer Inst. 1993;85:1571-1579.
56. Fraser GE. Associations between diet and cancer, ischemic heart disease, and all-cause mortality in non-Hispanic white California Seventh-day Adventists. Am J Clin Nutr. 1999;70:532S-538S.
57. Bosetti C, Micelotta S, Dal Maso L, Talamini R,Montella M, Negri E, et al. Food groups and risk of prostate cancer in Italy. Int J Cancer. 2004;110:424-428.
58. Tavani A, La Vecchia C, Gallus S, Lagiou P, Trichopoulos D, Levi F, et al. Red meat intake and cancer risk: A study in Italy. Int J Cancer. 2000;86:425-428.
59. Ganou CJ, Petrou C, Zografou I, Kotsa K, Varsamidou E, Sampanis C, et al. Prevalence of Anti-Insulin, Anti-IA2, Anti-GAD, Anti-IR Auto-antibodies and Anti-Neu5Gc Antibodies in Diabetic Type I And II Patients, Regional European Congress of Biomedical Laboratory Science and the 4th Greek Medical Laboratory Technologists Conference. 2018;49:50Z.
60. Finch CE, Stanford CB. Meat-adaptive genes and the evolution of slower aging in humans. Q Rev Biol. 2004;79:3-50.
61. Lopez-Garcia E, Schulze MB, Fung TT, Meigs JB, Rifai N, Manson JE, et al. Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. Am J ClinNutr. 2004;80(4):1029-1035.
62. Schulze MB, Hoffmann K, Manson JE, Willett WC, Meigs JB, Weikert C, et al. Dietary pattern, inflammation, and incidence of type 2 diabetes in women. Am J ClinNutr. 2005;82:675-684.
63. Esmaillzadeh A, Kimiagar M, Mehrabi Y, Azadbakht L, Hu FB, Willett WC. Dietary patterns and markers of systemic inflammation among Iranian women. J Nutr. 2007;137:992-998.
64. Taguchi R, Minami A, Matsuda Y, Takahashi T, Otsubo T, Ikeda K, et al. Preferential accumulation of 14C-N-Glycolylneuraminic acid over 14C-N-Acetylneuraminic acid in the rat brain after tail vein injection. PLoS One. 2015;10:e0131061.
65. Pham T, Gregg CJ, Karp F, Chow R, Padler-Karavani V, Cao H, et al. Evidence for a novel human-specific xeno-auto-antibody response against vascular endothelium. Blood. 2009;114:5225-5235.
66. Peri S, Kulkarni A, Feyertag F, Berninsone PM, Alvarez-Ponce D. Phylogenetic distribution of CMP-Neu5Ac hydroxylase (CMAH), the enzyme synthetizing the pro inflammatory human xenoantigen Neu5Gc genome biology and evolution. Genome Biol Evol. 2018;10:207-219.
67. Bhogal BS, Kariklanas YD, Bell MK, Sanchez P, Zemick G, Siskind GW, et al. Production of auto-anti-idiotypic antibody during the normal immune response: XII. Enhanced auto-anti-idiotypic antibody production as a mechanism for apparent B-cell tolerance in rabbits after feeding antigen. Cell Immunol. 1986;101:93-104.
68. Rodrigues CM, Martins-Filho OA, Vaz NM, Carvalho CR. Systemic effects of oral tolerance on inflammation: Mobilization of lymphocytes and bone marrow eosinopoiesis. Immunology. 2006;117:517-525.
69. Platts-Mills TAE, Commins SP, Biedermann T, van Hage M, Levin M, Beck LA, et al. On the cause and consequences of IgE to galactose- α -1,3-galactose: A report from the National Institute of Allergy and Infectious Diseases Workshop on Understanding IgE-Mediated Mammalian Meat Allergy. J Allergy ClinImmunol. 2020;145:1061-1071.