Stem Cell Therapy in Autism

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Autism (αὐτός [aw’tos], self)

- Unlike tuberculosis and polio, two infectious diseases that were nearly eradicated in the twentieth century as a result of scientific advances, many neurological disorders are actually on the rise. One prominent example is autism, a disorder that results in excessive fear and anxiety, extreme aloneness, and limited social communication skills.

- Frequency of these disorders, recognized as public health problem, is dramatically increasing: 23% reported increase since 2009 and 78% increase since 2007; until to present rates of 11.3 per 1,000 (one in 88) children aged 8 years in US, according to Center for Disease Control (Siniscalco D., Antonucci N. Curr Prot Pept Sci, 2013).

- This striking growth has renewed public dialogue about the causes of autism and the subjectivity of its diagnosis.
Autism diagnosis

Incidence Cumulative Growth
[ U.S. School Years 1992 - 2003 ]

Graph Source: www.fightingautism.org
Data Source: www.ideadata.org and www.cdc.gov/nchs/
Figure 1. Percentage of children aged 6–17 years with parent-reported autism spectrum disorder, by age group and sex: United States, 2007 and 2011–2012

Diagnosis: rising

By some counts, autism diagnoses have climbed steadily since the 1970s. Some research has found explanation for more than half of the rise (right).

Reasons: unclear

- 46% Unknown
- 25% Diagnostic accretion*
- 15% Greater awareness
- 4% Spatial clustering
- 10% Parental age

*Children who formerly would have been diagnosed solely with mental retardation

Study publication date:
- 1 in 5,000 (1975)
- 1 in 2,500 (1985)
- 1 in 500 (1995)
- 1 in 250 (2001)
- 1 in 160 (2004)
- 1 in 110 (2007)
- 1 in 100 (2009)
Autism pathogenesis

Genetics + Environment =

Digestion
Immunity

Brain
Heavy Metals
Autism: multifactorial disorders

- Multiple interacting genetic factors could contribute to autism;
- Environment factors, such as toxics, perinatal toxic stimuli, virus, bacteria, ...
Autism

Executive summary

Genetics of autism

• There is strong evidence for a genetic basis of autism, with inherited and de novo genetic causes.
• Linkage studies have shown a high level of disagreement but have identified several regions with evidence for linkage in multiple samples.
• Cytogenetically visible chromosomal alterations are identified in 3–5% of subjects with autism.
• Microdeletions/microduplications as defined by current technology are increasingly recognized as causal approximately an additional 10% of cases.
• Candidate genes with high penetrance have been identified – neuroligins, NRXN1, SHANK3, PTEN and CNTNAP2.
• Molecular genetic diagnostic characterization will become an integral part of clinical evaluation and consequent therapeutic trials of ASD.

• Multigenic pathology with epigenetic factors!

Brkanac, 2008
Immune transcriptome alterations in the temporal cortex of subjects with autism

Krassimira Garbett¹, Philip J. Ebert¹, Amanda Mitchell¹, Carla Lintas²,³, Barbara Manzi⁴, Károly Mirnics¹,⁵,*, and Antonio M. Persico²,³,*

Autism is a severe disorder that involves both genetic and environmental factors. Expression profiling of the superior temporal gyrus of six autistic subjects and matched controls revealed increased transcript levels of many immune system related genes. We also noticed changes in transcripts related to cell communication, differentiation, cell cycle regulation and chaperone systems. Critical expression changes were confirmed by qPCR (BCL6, CHI3L1, CYR61, IFI16, IFITM3, MAP2K3, PTDSR, RFX4, SPP1, RELN, NOTCH2, RIT1, SFN, GADD45B, HSPA6, HSPB8 and SERPINH1). Overall, these expression patterns appear to be more associated with the late recovery phase of autoimmune brain disorders, than with the innate immune response characteristic of neurodegenerative diseases. Moreover, a variance-based analysis revealed much greater transcript variability in brains from autistic subjects compared to the control group, suggesting that these genes may represent autism susceptibility genes and should be assessed in follow-up genetic studies.

Elevated cytokine levels in children with autism spectrum disorder

Cynthia A. Molloy, Ardythe L. Morrow, Jareen Meinzen-Derr, Kathleen Schleifer, Krista Dienger, Patricia Manning-Courtney, Mekibib Altaye, Marsha Wills-Karp

This study compared production of IL-2, IFN-γ, IL-4, IL-13, IL-5 and IL-10 in peripheral blood mononuclear cells from 20 children with autism spectrum disorder to those from matched controls. Levels of all Th2 cytokines were significantly higher in cases after incubation in media alone, but the IFN-γ/IL-13 ratio was not significantly different between cases and controls. Cases had significantly higher IL-13/IL-10 and IFN-γ/IL-10 than controls. Conclusion: Children with ASD had increased activation of both Th2 and Th1 arms of the adaptive immune response, with a Th2 predominance, and without the compensatory increase in the regulatory cytokine IL-10.
The Expression of Caspases is Enhanced in Peripheral Blood Mononuclear Cells of Autism Spectrum Disorder Patients

Dario Siniscalco · Anna Sapone · Catia Giordano · Alessandra Cirillo · Vito de Novellis · Laura de Magistris · Francesco Rossi · Alessio Fasano · Sabatino Maione · Nicola Antonucci
Figure 1. Autism disease induced an over-expression of caspase-1, -2, -4, and -5, but not of caspase-8 in ASD-PBMCs. The measured mRNA levels were normalized with respect to GAPDH (housekeeping gene) and gene expression values were expressed as percentage of arbitrary units±S.E.M.

°P values<0.05

Siniscalco et al., JADD 2012
Figure 2. Representative western blot analysis of Caspase-7 and Caspase-12 protein levels in the PBMCs obtained from the ASD patients respect to the healthy controls, respectively. The histograms indicate percentage variations in Caspase-7 and -12 protein levels normalized with respect to the signal obtained for β-actin housekeeping protein in the PBMCs of ASD patients respect to the healthy controls (CTL). °P<0.05.

Siniscalco, JADD 2012
Figure 3. Representative fluorescent photomicrograph of PBMCs showing immunocytochemistry for active Caspase-3. Arrows indicate active form Caspase-3 positive staining (green fluorescent). Cell nuclei were counterstained with bisbenzimide (blue fluorescence). (A): control subjects; (B): ASD pts.

Siniscalco et al., JADD 2012
Dario Siniscalco, Nicola Antonucci, Sabatino Maione, Laura De Magistris

Receptor/regulatory molecules pattern changes: a focus on caspases in autism spectrum disorders. In
A COMPREHENSIVE GUIDE TO AUTISM. BIOCHEMICAL ASPECTS IN AUTISM SPECTRUM DISORDERS. Springer, 2013. in press.
CONCLUSIONS-1

- Caspases up-regulation in ASD children:
  
  Inflammation
  Immune imbalance

Grant: Autism Research Institute (ARI).
The Promise of Stem Cell Research

- Drug Development and Toxicity Tests
- Cultured Pluripotent Stem Cells
- Experiments to Study Development and Gene Control
- Tissues/Cells for Therapy

- Bone Marrow
- Nerve Cells
- Heart Muscle Cells
- Pancreatic Islet Cells
Stem Cell Applications

- Stem Cell
- Drug Testing on Human Heart Cells
- Treating Birth Defects

Types of cells:
- ECTODERM: Neurons
- MESODERM: Blood Cells
- ENDODERM: Liver Cells

Creating cells and tissue for transplant
Hierarchy of Stem Cells

- **Totipotent cell**: Capable of dividing and developing to form a complete, mature organism.
- **Pluripotent cell**: Capable of developing into many different cell types.

**Blood Stem Cells**
- **Red Blood Cells**
- **White Blood Cells**

**Other Stem Cells**
- **Muscle**
- **Nerve**
- **Bone**
- **Other Tissues**
Stem Cell Applications in Regenerative Medicine for Neurological Disorders.

LIU SP, FU RH, HUANG SJ, HUANG YC, CHEN SY, CHENG CH, LIU CH, TSAI CH, SHAO WC, LIN SZ

Abstract

Stem cells are capable of self-renewal and differentiation into a wide range of cell types with multiple clinical and therapeutic applications. Stem cells are providing hope for many diseases that currently lack effective therapeutic methods, including stroke, amyotrophic lateral sclerosis, Alzheimer's disease, and Parkinson's disease. Embryonic stem (ES) cells were originally targeted for differentiation into functional dopamine neurons for cell therapy. Today, induced pluripotent stem (iPS) cells are being tested for such purposes as generating functional dopamine neurons and treating a rat model of Parkinson's disease. In addition, neural stem cell and mesenchymal stem cells are also being used in neurodegenerative disorder therapies for stroke and Parkinson's disease. Although stem cell therapy is still in its infancy, it will likely become a powerful tool for many diseases that currently do not have effective therapeutic approaches. In this article we discuss current research on the potential application of neural stem cells, mesenchymal stem cells, ES cells, and iPS cells to neurodegenerative disorders.

PMID: 23127757 [PubMed - as supplied by publisher]
The Promise of Regenerative Medicine and Stem Cell Research for the Treatment of Autism

Dario Siniscalco, James Jeffrey Bradstreet and Nicola Antonucci

able to affect immune system cells, likely through secretion of large amounts of several biomolecules with anti-inflammatory properties (paracrine activity). In this way, they are able to counterbalance the immune system aberrant alterations and activate endogenous restorative mechanisms within damaged tissues contributing to recovery of function lost [10].

Before stem cell transplantation can become a successful reality for ASDs, researchers need to more complete and exhaustive investigations. Some issues on stem cell biology have to be further clarified: proliferative capacity, life span, senescence, exact stem cell

New perspectives for ASDs therapy are provided by stem cells [9]. Indeed, novel findings on the molecular, cellular, neuroimmunological, and environmental of ASDs suggest that stem cell therapy could be a unique and potent tool for the treatment of autistic syndromes [10,11].
Three properties defining stem cells make them potential therapeutic agents for ASDs:

- **Self-renewal ability with the capacity to generate more identical stem cells,**
- **Capacity to give rise to more differentiated cells,**
- **Paracrine regulatory functions.**


Neurodevelopmental Diseases - Laboratory and Clinical Research
Mesenchymal Stem Cells:
The basis for new cell-based therapies.
M1 pro-inflammatory macrophages

Switching to M2 anti-inflammatory macrophages

hMSC systemic treatment

CD206 over-production

IL-10 over-production

IL-1β and IL-17 down-production

Siniscalco et al., Front Int. Neurosci, 2011
### Table 1. Summary of criteria to identify MSC

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<th>Adherence to plastic in standard culture conditions</th>
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<td>2</td>
<td><strong>Phenotype</strong></td>
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<td>CD105</td>
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3. *In vitro* differentiation: osteoblasts, adipocytes, chondroblasts (demonstrated by staining of *in vitro* cell culture)

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**Siniscalco, Methods Mol Biol, 2010**
Review Article

Autism Spectrum Disorders: Is Mesenchymal Stem Cell Personalized Therapy the Future?

Dario Siniscalco,1,2 Anna Sapone,3,4 Alessandra Cirillo,5 Catia Giordano,1 Sabatino Maione,1 and Nicola Antonucci6

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Figure 1: Paracrine and immunomodulatory effects as possible mechanisms of action of mesenchymal stem cells (MSCs) in autism spectrum disorder (ASD) treatment. In humans, ASDs are associated with immune alterations and pro-inflammatory cytokines (i.e., IL-1β) overproduction. These cytokines are able to trigger pro-inflammatory cellular events. Data from *in vitro* models show that MSCs are able to affect not only T cells, but also other cells of the immune system (i.e., NK cells). Immunoregulatory properties of MSCs are through secretion of large amounts of several bioactive molecules (paracrine activity), that is, PGE-2, IL-10. These molecules cause the inhibition or the unresponsiveness of T-cell mediated responses.
Stem Cell Research: An Opportunity for Autism Spectrum Disorders Treatment

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How stem cell could work in ASDs requires further investigations. Beneficial effects of stem cells could not be only restricted to cell restoration, but also paracrine and, above all, immunomodulatory effects may represent the possible mechanisms of action of stem cells in ASD pathology [6]. Indeed, the extraordinary characteristics of stem cells are: i) strong immunosuppressive activity that renders them a useful tool for successful autologous, as well as heterologous, transplantations without requiring pharmacological immunosuppression [8]; ii) paracrine actions [9]. Stem cells have the capability to produce a huge array of trophic and growth factors [10]. Through this natural paracrine activity, stem cells are able to produce molecules that activate endogenous restorative mechanisms within injured tissues contributing to recovery of function lost [6].
Fetal stem cells (FSCs) have immune-regulatory functions found in mesenchymal stem cells, but in addition, exhibit a potent expansion capacity and plasticity, showing a great potential for clinical use.
1. Fetal stem cells (FSC) have the highest proliferative potential (ability to multiply).
2. Administration of these cells helps to avoid histocompatibility problems. HLA expression is either absent or minimal. Besides, immunological tolerance, total or selective, develops upon administration of these cells, thus immunosuppression is unnecessary and, as a rule, these cells engraft well in the recipient's body.
3. Pluripotency is preserved until week 9.
4. These cells are no longer capable of uncontrolled growth.

Neural crest cells in culture
Rationale for the use of FSCs in ASD therapy

FSCs are derived from ectodermal, mesodermal and endodermal layers, and thereby retaining their regulatory instructions, unlike primitive ESCs.

Cell or organ specific FSCs could restore the brain, gut and immune system, thereby increasing their appeal for use in ASDs.

Fetal stem cell therapy in autism:
18 autistic patients (9 males age: 7.11±4.40, 9 females age: 6.78±3.31) diagnosed with ASDs in according to Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) were enrolled.

**Autism Treatment Evaluation Checklist score**
- speech
- sociability
- sensory
- health

**Aberrant Behaviour Checklist score**

evaluated before cell treatment, after 1, 3, 6, 12 months (work in progress).
FSC Methods
On day 1, we used liver stem cells harvested from tissues of 5-8 weeks old human fetuses. Suspension containing cryopreserved fetal liver stem cells was administered via drip-feed IV in the amount > 1.6 ml with nucleated cell count > 30.68x10^6/ml per transplantation.

On day 2, we administered ectodermal stem cells (nervous cell precursors) subcutaneously, in the amount >1.0 ml with nucleated cell count >8.7x10^6/ml per transplantation.

FSC were harvested from 5-8 weeks old legally aborted (for family planning reasons) normally developed and infection-free embryonic cadavers. Women were tested for viral and TORCH-infections before abortions. FSC were isolated by homogenization of fetal internal organs, then cryopreserved with cryoprotector in 3 stages with the initial speed of 1ºC/min and seeding point. After cryopreservation, the samples were stored in liquid nitrogen at -196ºC. Suspensions were screened for bacterial and viral safety and approved for clinical application. All patients signed the informed consent for treatment. This study is a scientific research registered (0113U004140) in the State Agency of Science, Innovations and Informatization of Ukraine of the Ukrainian Institute for Research and Development and Economic Information.
Fetal stem cell therapy in autism
Fetal stem cell therapy in autism
Results

Children treated with FSCs observed no adverse effects.

There were no significant safety issues related to the treatment.

Statistically significant differences were shown on ATEC and AB-C scores evaluation in the treatment group compared to the control at several time-points post-treatment (p<0.05).

Transplantations of FSCs were effective in improve autistic syndrome, as autistic children showed improved in behaviour, cognitive and communication deficits.
Hematopoietic Stem Cells:

- Characterized by specific Cluster of Differentiation markers (CD34, 59, 90, 117)

- Self-renewal, mobilization and multipotent differentiation capacities (including monocytes and macrophages)

- Paracrine activity

- Homing in the inflammation sites.
Warnings: are HSCs pro-inflammatory?
The proper cytokine signaling as deterministic of the end-effect of stem cell responses

Siniscalco et al., Frontiers Immunol 2013
Patient-specific somatic cell reprogramming could have a large impact on medicine by providing a source of cells for disease modelling and regenerative medicine.
KLF4, SOX2, c-Myc, Nanog, Oct-3/4, LIN-28

Adult Fibroblast Cell

Reprogram Cells

iPS cells

Cardiomyocytes

Adipocytes

Dopaminergic Neurons

Neural Cells

Motoneurons

Hematopoietic Progenitor Cells

Pancreatic β-Cells
Patient-Specific Stem Cell Therapy

1. Biopsy
2. Nuclear transfer
3. Nuclear reprogramming
4. Culture to blastocyst stage
5. Isolate and propagate pluripotent stem cells
6. Transplant
   - Blood
   - Nerve
   - Heart
   - Muscle
iPSC patient specific therapy. From an autistic subject it is possible to obtain an examination on their behavior, brain structure and network, together with genetics for an attempt to identify the type of autism.

Beatriz C.G. Freitas, Cleber A. Trujillo, Cassiano Carromeu, Marianna Yusupova, Roberto H. Herai, Alysson R. ...

**Stem cells and modeling of autism spectrum disorders**

Experimental Neurology 2012
Adipose-Derived Mesenchymal Stem Cells

-abundant in adipose tissue in humans, and are easy to harvest with minimally invasive procedures.
-differentiation processes into cell lineages apart from adipocytes have not yet been conclusively demonstrated in human subjects

Umbilical Cord-Derived MSCs
-placentas discarded after delivery
-low immunogenicity

Neural Stem Cells
-integrate into the tissue, replace damaged cells, and reconstruct neural circuitry
  1) reliable sources of sufficient autologous NPCs;
  2) how to regulate neural plasticity;
  3) the means to control differentiation of NSCs in the adult nervous system


Neurodevelopmental Diseases - Laboratory and Clinical Research
Clinical Trials

Safety and Efficacy of Stem Cell Therapy in Patients With Autism
human cord blood mononuclear cells and human umbilical cord mesenchymal stem cells
Shenzhen Beike Bio-Technology Co., Ltd.
Collaborators: Shandong Jiaotong Hospital

Autologous Cord Blood Stem Cells for Autism
Sutter Health
Michael Chez, MD, Sutter Health

Autologous Bone Marrow Stem Cells for Children With ASDs
The purpose of this study is to determine whether the plasticity of autologous intrathecal hematopoietic cells would improve the neurologic and the social skills of pediatric patients with autism spectrum disorders.
Hematology Service, Hospital Universitario Monterrey, Nuevo Leon, Mexico

Adipose Derived Stem Cell Therapy for Autism
Ageless Regenerative Institute, Mexico
Autism: finding a cure ... 

Dario Siniscalco (Second University of Naples, Italy),

Nataliia Sych (Cell Therapy Center EmCell, Kiev – Ukraine),

James Jeff Bradstreet (International Child Development Resource Center, Cumming, Georgia, USA),

Nicola Antonucci (Biomedical Centre for Autism Research and Treatment, Bari, Italy).
hMSC administration in neuropathic mice reduces mechanical allodynia and thermal hyperalgesia until 90 days neuropathic pain induction, without affecting neurological function.

Transplantation procedure: $2 \times 10^6$ cells/100µl in tail vein.

*Siniscalco et al., Front Int. Neurosci, 2011*