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Review Article

The Biochemical Etiology of Autism

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Abstract

As early as 1988, Insulin-Like Growth Factor-1 (IGF1) and truncated IGF1 were identified in bovine colostrum. Subsequent studies, to be reviewed here, suggested links between autism arising in young children and deficient IGF1 in their biochemical constitution. With prolonged maternal breast feeding as an IGF1 source for the neonate, the incidence of autism is reduced. It was also reported that ingested vitamin D3 increases the circulating level of IGF1.

Many new mothers are unable to breastfeed their neonates exclusively for more than a few weeks after delivery. Other obligations and activities often curtail a mother's commitment to continue this for several months. Medical studies support the conclusion that enhanced IGF1 ingestion to prevent autism should last 6-12 months. A literature review here describes some synthetic methods that might soon lead to industrial preparation of supplementary IGF1 for addition to the baby's feedings to substitute for maternal lactation.

Human breast milk is a positive promoter of this biosynthetic function and is encouraged for feeding the newborn for the first 6-12 months. Means for manufacturing IGF1 which can be added to formulas or bovine milk are reviewed here as well.

Keywords: Autism; Colostrums; Myelination; Interleukin; Inflammatory

Abbreviations

ASD: Autism Spectrum Disorder; **BP:** Binding (carrier) Protein; **cGP:** cyclo-Glycyl-Proline; **DSM:** Diagnostic & Statistical Manual of Mental Disorders; **IGF:** Insulin-Like Growth Factor; **IL:** Interleukin; **JAK/STAT:** Cytosolic Protein Tyrosine Kinase/Transcription Factor; **MERS:** Middle East Respiratory Syndrome; **PCR:** Polymerase Chain Reaction – to produce polynucleotides; **SARS:** Severe Acute Respiratory Syndrome.

Introduction

"My first-born child, Mikey, was fine during his first 18 months of infancy. He was growing normally and was cheerful within a loving attentive home environment. Mikey played with his toys and liked to share them with playmates. However, nearing his second birthday, we began to notice that he was not developing socially similar to other children his age. He now tended to avoid interaction with other children and seemed to prefer playing by himself. Mikey would spend hours arranging his blocks in a row, disassembling them, and then repeating the same action over and over again. We expected him to be speaking single words and two-word phrases by this time, but he only babbled incoherently without answering us when we called him by name. He acted as though we were not in the same room with him.

"When our pediatrician was approached about this situation, he suggested that our son should be evaluated by a neuropsychologist. I told the specialist that I had no medical difficulties before or during my pregnancy with Mikey. None of the children in our extended families had problems comparable to what we were witnessing with our son. We lived in a comfortable, middle-class, pollution-free neighborhood. Mikey weighed 7 pounds at his 40th-week birth and cried soon after delivery, following an 8-hour, uncomplicated labor. My husband and I were both 28 years old at the time of Mikey's birth.

"After a detailed evaluation, the specialist concluded that our son was autistic." - Signed, distressed mother (fictitious)

Since the first report of a seemingly new psycho-behavioral malady in 1943 [1], multiple attempts have been made to define the etiology of autism and autism-like conditions (ASD) in children and adults. Among sufferers of this condition there were found several degrees of severity and seemingly variable presentations of it between different patients. Initially, it was deduced that their variations actually represented different diseases or multiple causes, but recently the range of symptoms appears to be degrees of involvement and complication of the same disease [2].

Autism is highly variable among those affected by it. The disorder in general is characterized in the patient bearing it as a neurodevelopmental malady marked by deficiencies in social interaction, communication, outside interests, and behavior. Usually, no signs or symptoms in the gravida during the pregnancy or in the early months following birth give a warning in the child that autism will develop.

Prior to the release of the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), much confusion existed with differentiating Asperger's Syndrome, Pervasive Development Disorder-Not Otherwise Specified (PDD-NOS), Childhood Disintegrative Disorder, and Autistic Disorder. The designation of PDD was often applied when all criteria for Asperger's and/or Autism Disorder were not met.

Based on scientific advances, targeted research, and clinical experience, the classification scheme for these conditions was modified in 2013. The nomenclature in DSM-5 differentiated all

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cases deemed to be autism primarily based on severity. The general term of "Autism Spectrum Disorder" (ASD) includes all of the earlier named maladies in this group and is classified by degree of behavioral involvement (but not intellectual abilities). In particular, ASD is seen as a psychological condition which had presentations ranging from mildly to severely affected (the "Spectrum").

As defined in the DSM-5 and ranked by degree, ASD is further diagnosed by at least three deficits in childhood and is caused by significant impairment in the customary social or occupational areas of:

1) Social and emotional reciprocity;

2) Non-verbal communication behaviors;

3) Developing and maintaining appropriate relationships;

4) Repetitive speech, movements, or employing of objects;

5) Excessive maintenance of routines, formalized patterns of verbal/nonverbal behavior, or excessive refusal to change;

6) Highly restricted, unusual, fixated interests (e.g., strong preoccupation with atypical objects); and

7) Hyper- or hypo-reactivity to sensory input or extraordinary interest in appreciated aspects of the environment [3,4].

With this definition of ASD, it was found that impairment of normal motor control, behavior, and learning skills in autistic children characterize this neurological disorder. In test animals with ASD-like characteristics, synaptic plasticity, especially in the cerebellum, appears to be at the core of at least some of the cases of such behavior. For example, dysfunctional cerebellar Purkinje cells contribute to autism-like behavior in Shank3-deficient mice. It is proposed that the release of GABA (gamma-aminobutyric acid) by these cerebellar cells reduces nerve impulse transmission, as also observed in some children with autism or with Niemann-Pick disease type C. However, these changes are not seen in all autistic children, thereby challenging the premise that such genetic or anatomic changes can be primary causes of all cases of autism. Both possibilities will now be examined in more detail.

Therefore, in evaluating a child with behavioral problems, it is essential to carefully consider observations, analyze pertinence and credibility of actions, and distinguish between correlations and cause-and-effect associations. As noted in the opening maternal letter above, usually no signs or symptoms in the mother during the pregnancy or in the neonate in the early months following birth give a warning that autism may develop in the child [2]. Later in this discussion, the means for reducing the chance of autism development in the youngster, especially when the mother experienced a serious febrile course during the pregnancy, will be reviewed.

Confusion often results if a distinction is not clearly made between correlation and causation. Coincidence or association does not necessarily imply causation. It is important to question such terms as "appears to be", "implicated in", "associated with", "linked to", and "suggesting." The weakest of all such mental exercises is "unsubstantiated intuition." This is especially risky if a psychological overtone or a false sense of urgency is subjectively implanted into the analysis of research, diagnosis, or emotional findings. Uncorroborated claims advanced before a specific outcome is realized should be suspect. As a result, caution must be used in claiming the definitive etiology of a complex medical condition such as autism.

In 1883, Robert Koch proposed three essential requirements to define the basis of particular medical maladies:

1) Suspected causal facts must be constantly and recurrently associated with the disease;

2) Dysfunction must be identified within an affected individual;

3) When a susceptible host is modified appropriately, such as with genetic silencing, the same symptoms of the spontaneous disease must develop in the test case when occurring naturally.

Requirement #3 is especially difficult to realize in psychosocial conditions such as autism if a cause other than genetic alteration is the key factor [2].

Methods

Subject literature review

Prior publications have emphasized the central role played by ***insulin-like growth factor-1*** (IGF1) in attempting to prevent autism [5-12, 3,4,13-17]. IGF1, a major multipurpose growth factor, is produced primarily by the liver. In particular, this factor promotes the myelination of new nerves as the babies develop their fetal/neonatal central and peripheral nervous systems. Some published reports have proposed etiologies of autism emphasizing diminished development of active nerve circuits, especially in the newborn. The level of IGF1 in human milk is higher than cow's milk, thus enhancing neural development and maturation [15,17].

IGF1 is produced in many tissues, especially the liver. As early as 2001, Riikonen and then Patterson began drawing attention to juvenile cases with neurochemical deficits of cerebrospinal IGF1, which appeared to coexist with autism. For example, in obstetrical cases where the mother suffered from antepartum fever-inducing viral illnesses (e.g., coronavirus, influenza), increases in inflammatory interleukins and a decrease in IGF1 were frequently found (Figure 1). This may be followed by birth of offspring destined to become autistic [12,16,17,19-28].

This biochemical approach to explaining the etiology of autism is in marked contradistinction to often incorrectly cited genetic errors. For example, Rett Syndrome has been discussed as an example of an autism-like condition. The key differences are illustrated in Table 1.

However, over 100 various nuclear defects have been reported but have occurred in fewer than 10% of cases of autism [28,29]. In another review of 3871 autism patients, loss-of-function genes were found in only 5% of the affected individuals [30]. In a third study, about 10% of the test group with ASD had phenotypes which were directly associated with identifiable monogenic disorders such as PTEN hamartoma tumor syndrome [31]. In contradistinction, a biochemical error such as deficient IGF1 is central to inadequate myelination of neo-neurons in the developing fetus and neonate. This results in reduced velocity (down to as little as 0.1% compared to similar myelinated neurons) of normally functioning nerve impulses in the brain and subnormal neurologic function in key brain centers and synapses [32,22].

Although the genetic approach to elucidating the etiology of autism has been less emphasized recently, several studies have reported epidemiological examinations linking ASD with exposure to various viruses such as rubella, measles and mumps, polyomarviruses, cytomegalovirus, and influenza [33-35]. This will be explained more fully later in the discussion about the interactions between IGF1 and IL-6 in particular.

As early as 1988, bovine colostrum and milk were found to contain insulin-like growth factor-1 [36]. Supplying IGF1 via human breast milk exclusively for the first postpartum year has been reported in several large meta-analytical studies to reduce the incidence of autism significantly. After this, when autistic traits appear overtly in children (typically at ages 1-4 years old), it would be too late to correct the biochemical/behavioral problems, within the current limitations of available therapy. Statistically significant lower levels of IGF1 occur in the cerebrospinal fluid much more often in this group of affected young children with autism than in normal individuals. Hence, it would be desirable to test all umbilical cord bloods at birth for insufficient IGF1 to identify the most at-risk neonates, in particular [5,8,37-44].

In babies born preterm, the serum IGF1 concentration often correlates with brain volume, unmyelinated white matter volume, cerebellar volume, and subnormal mental development [45]. Treating children already with idiopathic autism with IGF1 supplement induces transcriptional responses in their neurons, although complete cure is usually unattainable [46]. Worldwide, almost 70% of all infants are not breastfed exclusively for at least 6 months postpartum.

Potentially more reliable would be to treat all newborn with milk rich in IGF1. Such is the case with human lactation. Many infants are not fed human milk, which has a higher natural provision of IGF1 than cow's milk [47]. Children nursed on human milk exclusively for 6 months have 75% lower chance of developing autism than those fed by other means. To be discussed here in detail is the relatively equal dietary benefit for human milk to be derived from bovine milk or formula which has been augmented with synthetic IGF in this important regard. IGF1 is a linear polypeptide with 70 amino acid units joined together by peptide bonds. Although it is a polypeptide orally ingested, IGF1 apparently survives passage through the stomach [48,49]. In the current market, this polypeptide when isolated from synthetic or natural sources is expensive to purchase. This makes it unreachable by the broad market of women looking to feed their babies with this beneficial additive to food forms other than human milk. The historical trend to follow is the increasing ability to produce synthetic, effective IGF1 at a reasonable cost.

Beginning in 2012, a series of nine reports appeared with statistics detailing the overall value of these meta-analytical studies, have shown the connection between neonatal breast-feeding and the reduction of autism in young children [37,38,50-54,39-44]. The key to this was apparently the higher level of IGF1 in human milk than in bovine milk or formula. The longer the mother continued exclusive breast-feeding, the lower was the typical occurrence of autism in the neonate. For example, a multi-center study in China of the benefit of breast-feeding for 6 months reported an autism percent prevalence of 2.4 in those with no breast-feeding and 0.7 with exclusive breast-feeding [45,46,42]. These results are comparable to those found in several other comparative studies. [Reported overall rates in a given population may depart from the exact value because of idiosyncratic characteristics of each community or country, and the reliability and completeness of methods used to collect the data. An additional essential factor for data analysis in a particular population is the medical definition of the condition under investigation.

Gravidas who experience a severe febrile disease, such as corona virus, during the pregnancy are often low in vitamin D3 and IGF1. As a result, babies fed formula much of the first year of life display a significantly higher occurrence of autism spectrum disorder later in childhood and beyond for the same reason. For those women unable to provide sufficient breast milk to their neonates, human milk supplies are commercially available. Similarly, milk augmented with commercially prepared IGF1 added to milk or formula should serve the same purpose [11,12,16,55]. If one child born to parents is autistic, the chance of a subsequent birth also yielding an affected child is 20%. If the maternal and neonatal diets were supplemented with extra vitamin D, the affected sibling rate dropped to as little as 5% [56]. These studies would support the advice of providing neonates with enough vitamin D3 and IGF1 from maternal milk for a year to reduce the chance of developing autism. This is especially important for babies with growth hormone deficiency [46]. Alternatively, a way needs to be developed for women who cannot breastfeed the full postpartum year to be able to provide the equivalent IGF1 and D3 nourishment by an alternative mode.

Vitamin D enhances circulating IGF1 and helps reduce the level of interleukins in the presence of coexistent inflammatory processes. An example is treatment of otherwise normal individuals with 5000-7000 iu of vitamin D3/week; control volunteers received no D supplement. As a result, IGF1 was raised in the test group by 12.7-13.1 ng/ml, compared to no change in the control group. Thus, vitamin D was shown to increase IGF1 in these participants [57].

Deficient biosynthesis

The antepartum placenta has the capacity to generate a wide range of needed metabolic substances. By 1977, it had been determined that a large number of biochemicals necessary for fetal development were produced by the placenta [58]. By 1980, the biosyntheses of additional placental proteins and peptide hormones were reported [59]. Cultured placental cells are now employed commercially to prepare needed agents in specific medical situations.

Among these controlling biochemicals, IGF1 was studied for its many known functions and applications. Beginning in 2001, this agent was evaluated for its effect on the development of the nervous system in particular. Type I IGF1 receptors are found in much of the neonatal body, but especially in the vicinity of neural tissue. IGF1 is an important factor in axonal regeneration, astrocyte proliferation, and neurite sprouting, especially with neural myelination. The velocity of impulse transport in nerves may vary by whether they are myelinated or not [32]. IGF1 directly affects the rate at which oligodendrocytes promote myelination and neural circuitry development in the infantile CNS. The level of IGF1 in the neonate may be reduced because of inadequate nutrition, advanced parental age, or immune activation. Insufficient myelination may be the result of genetic alterations, polymorphisms, or mutations in the baby's nuclear makeup, but this accounts for neurologic problems in only a

small percentage of abnormal cases [60].

Although juvenile autism was first observed in 11 children and reported in 1943 as a psychological disturbance and social withdrawal from one year of age by Leo Kanner [1], the identification of IGF1 deficiency as the apparent cause was not expressed until the 1990s by D'Ercole [61]. Since the general definition and characterization of autism in the early 1940s by as a unique psychopathological entity with varying presentations, little attention was paid to it by the general public until the last 20 years or so.

In 2001, Riikonen and coworkers began reporting a series of studies on IGF1 deficiency in the cerebrospinal fluid of autistic youngsters compared to neurologically normal children [18-24]. In 2011, Patterson studied maternal infection and immune involvement in autism generation [25]. He detailed the reactions in the placenta in the presence of maternal inflammatory reactions which raised the level of IL-6 (interleukin 6) and activated JAK/STAT3, thereby causing a reduction of IGF1 production [62,55].

The level of IGF1 at birth can also be proportional to the baby's size, apparently due to an underactive placenta or ante partum maternal infections. Therefore, more attention must be paid to underweight neonates specifically for possible inadequate IGF1 levels, which are often an early warning sign of potentially developing autism later.

As adults grow older, their serum IGF1 levels decrease. Thus, older birthing mothers would have reduced blood IGF1 in comparison to younger mothers. This may account in part for why older parents are more likely to give birth to babies who develop autism.

Another factor which has been found to be related to the statistics of autism occurrence in young children is vitamin D supplementation. This is apparently because of the increased level of IGF1 and reduced levels of interleukins in the presence of coexistent inflammatory processes in children receiving this vitamin augmentation. This supports the contention that this vitamin in particular can be a stimulus to IGF1 enhancement [63,64]. To contemplate ways to produce IGF1 artificially, peptide synthesis methods need now to be reviewed for their utility.

Extracorporeal peptide synthesis

The first successful method for amino acid polymerization in the laboratory was the solid phase synthesis of a tetra peptide by RB Merrifield, published in 1963. The approach utilized protected amino acids joined to an enlarging peptide chain, attached covalently to a resin particle. For the ten years before this, the method at that time involved amino acid residues which created polymers that became increasing insoluble and unreactive with enlarging size as the reactions preceded. The advantage of the Merrifield technique is that the polymer is attached to an insoluble resin component, thereby allowing for washing of unused reactants as the polypeptide [65]. This would shorten the time to complete the peptide polymer production compared to earlier methods.

The next improvement in methodology was completed by Paul Berg in 1972. This process, named genetic engineering, involved removing DNA from a bacterium and placing it in a virus (a recombinant cell). A variation of this was to fuse a tumor cell with a cell able to produce proteins, now called a hydrioma [66]. By 1977, evidence for the production by the human placenta was reported for chorionic gonadotropin, lactogen, thyrotropin, follicle stimulating hormone, relaxin, oxytocin, vasopressin, alkaline phosphatase, and renin. By 1982, this recombinant method was approved for producing insulin in E. coli for use in human patients.

In 2014, Simon, Pentelute, and coworkers published a solid-based peptide synthesis technique which was rapid flow-based. Another amino acid could be added to the growing polymer every 2-3 minutes. Reagents and solvents were delivered to a solid support at high flow rate, thereby accelerating the production of polymer. The process was continuously monitored by UV detectors. Polypeptides as large as 130 residues could be produced by this method in relatively short time [67].

By 2016, Polymerase Chain Reaction (PCR) had been applied in several biochemical studies of DNA. Using this technique small amounts of DNA could be rapidly reproduced to yield significant quantities of specific nucleic acids. Now linear DNA expression templates synthesized directly from PCR could be used to promote cell-free protein syntheses [68].

Automated flow-based accelerated peptide synthesis was published by Mijalis, Pentelute, and associates in 2018 to execute rapid solid-phase polypeptide synthesis. The system was fully automated and created new peptide bonds every 40 seconds. In less than one hour, it can form a complete peptide polymer with up to 60 amino acids linked together with 99% efficiency. The mixture of reactants is briefly heated to 90 degrees Celsius to make them more reactive [69].

Polypeptide syntheses in the presence of competing factors in a water/dichloromethane biphasic system was reported in 2019. The aqueous phase was incorporated to extract impurities of the reaction. Polymerization of N-carboxyanhydrides in this reaction environment led to low-cost, large-scale synthesis of polypeptides [70].

Currently, functional peptides can be produced with processed placental cells (see website of Pluristem Therapeutics for details). Also, beneficial peptides may now be induced with modified placental enzymes.

IGF1 is biosynthesized primarily in the liver and is under the control of growth hormone for the quantity to be secreted into the general circulation. By IGF1 stimulating oligodendrocytes, myelination of neo-neurons is promoted in the baby. Axonal regeneration is induced by local deposition of the polypeptide. In addition, IGF1 is synthesized in peripheral tissues as an autocrine/paracrine agent for local needs. The hormone promotes its effects via IGF1-specific, cell membrane-bound receptors [71].

Increasing IGF1 availability *in situ*

There are six different IGF-Binding Proteins (BP) in the human body, usually holding/storing 99% of the available but inactive IGF1 in the circulation. Binding protein #3 (BP_3) carries the largest amount of IGF1. The potential release of IGF1 attached to the bonding protein (BP_3) by cGP (cyclic glycyl-proline) may explain the mechanism ameliorating its deficiency. In the presence of added cGP, IGF1 is released. IGF1 attached to one of the six binding factors is more stable over time than free IGF1 [3,4,16, 72,73,74].

$$IGFBP_3\text{-}IGF1 + cGP \rightarrow IGFBP_3\text{-}cGP + IGF_1$$

Therefore, it appears possible to utilize laboratory-based methods for mass-producing IGF1, IGF1-promoting agents, or IGF1-mimicking drugs economically. The product itself, IGF1, could be added by the caregiver to bovine milk or formula to reach the nutritional level afforded by human milk, once such a product is available at an acceptable price [16].

The desire to proceed with exclusive breast-feeding for six months or more is often impeded by family size, work commitments outside the home, or maternal illness, among several reasons. Many parents circumvent these obstacles by feeding their infants bovine milk or commercial formula. As noted earlier, these sources typically have significantly less IGF1 and/or vitamin D than mother's milk. One approach to resolving this deficiency would be to secure human milk from marketable sources. Secondly, many lactating mothers use manual pumps to obtain and store their milk while away from the home. A third solution would be to add IGF1 powder to cow's milk or formula. However, this source of the biochemical is commonly used for laboratory research or treatment of childhood growth inadequacy, and is currently quite expensive.

Milk from humans is similar in most parameters to that from cows. Human milk averages 4.1% fat totally and from cows 2.5%. Furthermore, bovine milk is 1.1% unsaturated fat and human milk is 2.3% unsaturated fat, on average. As a result, lipids in human brain development play a greater role than in cattle. Furthermore, this is consistent with cow's whole milk containing, on average, 3.8 iu/L (international units/liter) of vitamin D, whereas human milk has 15 iu/L. The major source of this vitamin D is skin exposure to sunlight. It may be ingested from the diet and supplements as well. The highest level of IGF1 in the milk of cows, sheep, and goats is soon after parturition [75], whereas human IGF1 production remains active as long as breast-feeding continues.

If a method could be developed to make possible bulk commercial manufacturing of IGF1, the financial problem caused by inadequate, expensive supply of this factor (now about $600/50 microgram) could be avoided. For example, earlier in this report a method for producing biochemicals using placental cells was described. If such a method could be developed to the point where IGF1 would be available in bulk for a reasonable price, it might serve to reduce the incidence of autism in young children. Autism in their child now can cause the parents a lifelong health-related excess expenditure of $3,000,000 or more for therapy, supervision, and medications [3].

It is apparent that children who are breast-fed generally do not suffer from conditions resulting from excess IGF1 intake. However, if the missing IGF1 is available and added to cow's milk, care must be taken to measure the supplementary amounts so as to avoid abnormal growth of the skeleton. In children, gigantism causes bones to grow longer, resulting in very tall people with large feet and hands, or an error in lipid metabolism. A defect in lipid metabolism is also observed in some patients with acromegaly. The high level of IGF1 in acromegaly is related to an increased risk of some malignancies, particularly colon cancer and thyroid cancer. Excess administered IGF1 can lead to abnormal glucose regulation, causing diabetes mellitus. However, these side-effects are uncommon and may result from lack of proper care and caution using this dietary factor. In contradistinction, the added IGF1 may be enough to prevent autism

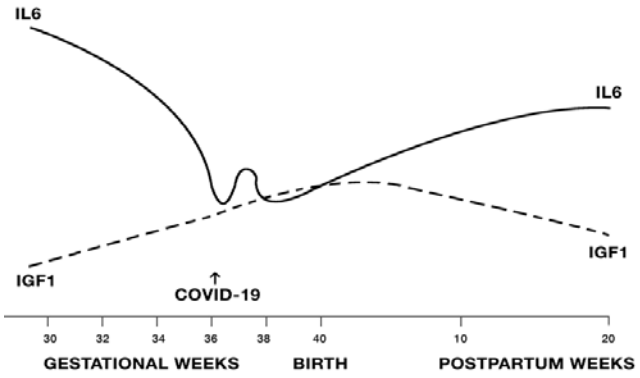

Figure 1: Relationship between fetal/neonatal serum IL6 and IGF1 before and after a febrile Covid-19 attack.

spectrum disorder, a lifelong disability [76].

IGF1 availability is commonly reduced during and after an infectious cytokine storm, such as during pregnancy (Figure 1).

It would appear that the generation of some (but not necessarily all) cases of autism begin with the intrauterine fetus exposed before birth to elevated maternal temperatures (e.g., caused by influenza, SAR-CoV-1, H5N1, MERS-CoV, and groups of interleukins due to these diseases). In the developing baby, pre- and postpartum, rising interleukin levels are matched with falling IGF1 [55]. In selected laboratory animals, a link has been demonstrated between maternal immune activation and autism-like outcomes. In a post-mortem study of human brains, elevated cytokines and infection states have been observed. Children with inflammatory diseases typically have reduced IGF1 and elevated IL-6. Overall, a balance between pro-inflammatory and anti-inflammatory functional cytokines is needed for good health [8,77,78].

Antepartum maternal infection can promote the release of specific cytokines such as IL-6 into the mother's bloodstream. In a meta-analysis of >40,000 autism cases, maternal infection during pregnancy was correlated with autism in the babies. In contrast, IL-10 is anti-inflammatory. In a study of 69 severely affected corona virus patients, where IL-6 was used as a monitoring marker, elevated levels of LDH, C-reactive protein, ferritin and D-dimer were commonly found. Use of anti-pyretics reduced the incidence of autism in these cases [79,77].

Results/Discussion

Conclusions

Recent investigations have come to emphasize a search for a biochemical etiology of autism. Rather than looking for genetic abnormalities within sufferers of this condition, many postpartum studies since about 2001 have been on a hunt for congenital biochemical aberrations in autism causations. Special attention has been given to the catabolism of IGF_1, as noted earlier in this review. Once overt behavioral problems become identifiable at 1-4 years of age, the cure of autism is essentially gone, within the current limitations of available therapy.

One prominent attack on this problem in humans has centered on the chemistry of insulin-like growth factor-1 (IGF_1). This polypeptide

Table 1: Key differences between Rett Syndrome and Autism [19,18].

Trait	Rett Syndrome	Autism
Cranial abnormalities	Microcephaly	Macrocephaly
Primary effects	Cholinergic neurons	Serotonergic neurons
Brain abnormalities	Frontal cortex path	Cerebellum-hippocampus
Age of onset	Postnatal	Early prenatal
Mutation site	MECP2*	Undetermined
Gender association	Almost all female	Male/female (4/1)

*MECP2 = methyl CpG binding protein-2

is a linear polymer with 70 amino acid residues with very few differences between humans and animals. In the human blood stream, approximately 99% of the IGF_1 molecules are inactive when attached to one of six specific bonding (BP) proteins, to yield $IGFBP_{1\text{-}6}$. The greatest single proportion of the six is typically $IGFBP_3$ in humans. The initial *in vivo* step in the inactivation of IGF_1 is the hydrolysis of the N-terminal tripeptide (glycyl-prolyl-glutamate). This is soon followed by removal of the C-terminal glutamate by hydrolysis, to yield cyclic glycyl-proline (cGP). Both peptides have been found to be neuroprotective in early hypoxic reactions [19,13].

As noted earlier, IGF_1 itself is functional in promoting the synthesis of myelin, the insulating material which acts to protect new neural pathways and to accelerate the passage of electrical messages within the brain of the developing fetus. In the normal human physiologic situations, the ratio of $IGFBP_{1\text{-}6}$-IGF_1 to free IGF_1 is 99/1 in the blood [2]. Increasing the amount of free cGP present can promote the release of IGF_1 from the carrier protein (BP). Non-autistic children typically display 3-4 times higher blood levels of free IGF_1 than autistic youngsters [42].

As noted in previous reports, only about 30% of the babies in the world are fed human milk, even though it typically contains a higher level of IGF_1 than bovine milk. The overall occurrence of autism is higher in the group using cow's milk than the human, for various medical, agricultural, or social reasons. Babies worldwide are usually fed milk from cows, sheep, or goats, or with commercial "formula" products. As it relates to autism, the preferred diet in infants should be least six continuous months of breast-feeding with vitamin D3 supplement, especially with babies whose mothers had a febrile event during gestation or gave birth previously to an autistic child [17]. A variation of this is the addition of cGP to cow's milk to reach the preferred human milk (anti-autism) level of free IGF_1.

It has been proposed that IGF_1 or its derivatives, such as the dipeptide (cyclic glycyl-proline) or the tripeptide (glycyl-prolyl-glutamate), could be effective in treating other neurological conditions such as Parkinson's Disease and ischemic brain injury as well [80,81]. Therefore, it appears possible to utilize already available laboratory-developed methods for mass-producing IGF1 economically. Bovine milk typically has lower concentrations of IGF1 than human milk [47]. An IGF1 supplement could be added by the caregiver to bovine milk or formula to reach the nutritional level afforded by human milk, once such a product is available at an acceptable price. One should expect better long-term therapeutic results in babies who have not yet displayed symptoms than those who are overtly symptomatic with autistic characteristics.

Another approach to treating/preventing autism spectrum disorder is to employ the truncated terminal dipeptide, cGP, in particular, found in bovine colostrum or in modified forms isolated from synthetic sources [82]. The redesigned dipeptide in particular has medicinal benefits similar to IGF1 in the case of autism-related maladies and is more stable than the tripeptide, once isolated. Studies are underway using recombinant IGF1 format on children with Rett Syndrome [83]. [This syndrome resembles autism in some aspects but differs from it in others -see Table 1.

Phelan-McDermid Syndrome, another autism-like neurologic disorder with some characteristics in common with Rett Syndrome, has been traced to a segmental deletion of chromosome 22q13.3. Promising clinical trials are now underway using a derivative of cGP previously uncovered and developed in the laboratory to treat this syndrome [84,85,86,73,74].

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Chronology of Pertinent Discoveries

1988-IGF1 and glycyl-prolyl-glutamate identified in bovine milk.

1991-Control of IGF1 level in cow's milk detected.

2001-Patterson hypothesized that in a febrile infection, the production of interleukin-6 depresses IGF1 levels, thereby promoting the appearance of autism.

2001, 2006-Riikonen announced that cerebrospinal fluid in young children has low IGF1 levels up to age 4 years than in unaffected children.

2011-Patterson reported on immune involvement in maternal infection.

2013, 2015-Guan proposed the use of IGF1 to treat neurological conditions.

2014-Guan noted that cGP can regulate IGF1 homeostasis via binding of IGFBP3 to IGF1

2016-Yune observed that cGP improves brain glutamatergic neuroplasticity; Singh-Mallah reported that administered IGF1 increases cGP level.

2018-Guan promoted the use of cGP to regulate the level of IGF1; Glass registered US Patent #9,867,823 – use of bi-cyclics to treat ASD

2019-Fan used the cGP/IGF1 ratio to monitor stroke patients.

2020-Glass described use of bi-cyclics to treat autistic disorders in US Patent Publication #2020/0163961A1.

2021- Singh-Mallah reported use of cGP in infancy to improve memory; Fan measured levels of IGF1 and the associated risk of Covid-19.

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