

# SOME ASPECTS OF AUTISM INCLUDING IMMUNOLOGY

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## Abstrak

Bukti telah banyak menunjukkan bahwa serotonergik, imunologi, endokrinologi berinteraksi penting dalam predisposisi genetic, keadaan paska kelahiran, dan xenobiotik, berperan pada patogenesis autisme. Pengaruh umur, masa pubertas, jenis kelamin, ras, dan IQ telah diketahui sangat khusus pada data biologi. Terdapat bukti yang kian berkembang bahwa kompleksitas dan keragaman interaksi sangat mungkin berpengaruh pada keragaman fenotip tingkah laku dan biologi pada autisme. Faktor genetic, neuro-anatomi, dan neuro-fisik dilaporkan sesuai dengan relevansi anggapan faktor neuro-kemikal.

## Abstract

Evidences become to accumulate that serotonergic, immunological, endocrinological system and the interaction of these three pivotal systems with predisposing genetic and external (pre-, peri- and postnatal) conditions and xenobiotics play an important role in the pathogenesis of autism. The influence of age, pubertal stage, sex, race and IQ have been recognized very special on biological data. There is growing evidence that the complexity and variability of those interactions might be responsible for the heterogeneity of behavioral phenotypes and biological findings in autism. Genetic, neuroanatomical and neurophysiological data were mentioned according their relevance to neurochemical opinions.

*Keywords: Autism, serotonin, immunology, endocrinology, genetic, pathogenesis.*

## Introduction

Autism is a pervasive developmental disorder. Children of asthmatic mother and other allergic diseases during second trimester of pregnancy have 2-fold prevalence of autism if compared with non asthmatic mother.<sup>1</sup> This association has highlighted that immunology play a role in the pathogenesis of autism. The condition is highly prevalent (5–15 per 10 000) and is four times more common in boys than in girls.

During the last decade the idea of autism being a 'disease entity on his own right' has been dropped progressively. This evolution coincides with the discovery of a multitude of underlying or associated medical conditions with one or more common behaviours, called 'the autistic syndrome' or 'the autistic phenotype', suggesting that all those underlying diseases might share one or a few common final pathways responsible for the autistic behaviours.

Underlying diseases, determined by pathological variations in genes, enzymes, metabolic pathways, neurotransmitters, (neuro-)hormones and cellular and humoral immunity help to demonstrate some of the pathophysiological hypotheses that may apply in the autistic syndrome.<sup>2</sup> The attempt to find what subjects with autism actually do have in common, which results in their autistic behaviour pattern, was still not successful.

## **Biochemistry**

### *Molecules involved in neuro-transmission*

#### **Serotonin and tryptophan.**

Serotonin plays an important role in autism and other developmental disorders because of its role as a morphogenetic agent and a differentiation factor in the developing brain and as a neurotransmitter in the central nervous system (CNS). Blood concentrations of serotonin (5-HT) are increased in a subgroup of autistic children.<sup>3</sup> It has been found that when 5-HT was measured in whole blood, the autistic group showed a modest (15%) elevation in mean levels that might have proved statistically significant in a larger sample.<sup>4</sup> However, expressed as platelet volume, the mean level for the postpubertal autistic group was slightly lower than that for the control group, suggesting that hyperserotonemia is more prevalent in prepubertal than postpubertal autistic individuals. Hanley et al. found that about one-half of the children who are not autistic but severely retarded show hyperserotonemia. The discrepancy between the McBride et al. study and the Hanley et al. study might be explained by the lack of well-established methods for a systematic approach to exclusion of autism in the mentally retarded population when those subjects were seen in the 1960s.<sup>4,5</sup> Abnormal levels of serotonin are not specific for autism and are found in numerous other diseases.

Selective 5-HT reuptake inhibitors (SSRIs), have beneficial effects in the treatment of some individuals with autism.<sup>6</sup> Tryptophan depletion techniques result in a significant increase of autistic behaviors.<sup>7</sup> PET-scan studies of autistic boys revealed decreased 5-HT synthesis in the frontal cortex and thalamus, but elevated 5-HT synthesis in the contralateral dentate nucleus. High functioning autistic girls had a similar pattern, but low-functioning girls did not.<sup>8</sup> The normal developmental process that humans undergo a period of high brain 5-HT synthesis capacity until the age of 5 years may be disrupted in autistic children.<sup>9</sup> There is some preliminary evidence of association between the 5-HT transporter gene and autistic behavior, although there exist discrepancies between Cook et al. and Klauck et al, who found association with the longer rather than the short allele of the 5-HT transporter gene in autistic subjects. It has remained elusive, however, whether platelet paroxetine binding characteristics are altered in autism.<sup>10,11</sup>

Autistic subjects with elevated whole blood serotonin levels have been shown to have elevated 5-HT transport into platelets.<sup>3</sup> Weizman et al, on the other hand, were unable to find any significant differences in platelet imipramine binding characteristics in autistic patients.<sup>12</sup>

In a study group consisting of 13 postpubertal, male, Caucasian autistic subjects with an IQ > 55 and an identically matched control group could not be found hyperserotonemia, which was reported to occur in autism.<sup>13</sup> Previous studies have already showed that whole blood 5-HT is not significantly different between postpubertal, Caucasian males with autism and normal controls, and that hyperserotonemia may be confined to prepubertal children with autism.<sup>4</sup> One study

showed that the affinity of platelet [<sup>3</sup>H]-paroxetine binding sites is significantly decreased in postpubertal, male, Caucasian autistic subjects with an IQ > 55. These findings may suggest that autism is accompanied by defects in the peripheral (platelet) and perhaps the central 5-HT transporter system.<sup>13</sup> Peripheral 5-HT and 5-HIAA values were normal and that there were no significant correlations between those peripheral markers and the [<sup>3</sup>H]-paroxetine K<sub>d</sub> values suggest that the peripheral serotonergic turnover is probably not involved in the changes in the 5-HT transporter system in postpubertal, male, Caucasian autistic subjects with an IQ > 55. Findings concerning tryptophan, the precursor of 5-HT, the competing amino acids and the ratio of tryptophan to amino acids known to compete for the same cerebral uptake site (competing amino acids, CAA) have been inconsistent.<sup>6,14</sup> It has been found significantly lower serum concentrations of total tryptophan in a group of autistic children, although the tryptophan/CAA ratio was not significantly lowered in autism.<sup>13</sup>

Total plasma tryptophan as well as the tryptophan/CAA ratio are indicators for the availability of tryptophan to the brain and, hence, for 5-HT synthesis in the brain. Tryptophan circulates in the blood with a minor fraction free, while 70–90% of this amino acid is loosely bound to serum albumin.<sup>15</sup> It is thought that the blood–brain barrier transport site of tryptophan and its competing amino acids strips off albumin as it passes through the brain capillaries and, consequently, that the availability of tryptophan to the brain depends on serum concentrations of free and total tryptophan as well as on the concentrations of albumin and the competing amino acids.<sup>16</sup> The measurement of free in addition to total tryptophan as a measure of the tryptophan availability to the brain would have been more informative.

In addition, as the tryptophan/CAA ratio is not decreased it remains unclear whether the availability of tryptophan to the brain is decreased in autism. The lack of any changes in the tryptophan/CAA ratio in a study is explained by the significant decreases in the sum of CAA which, in turn, is attributable to lowered serum concentrations of phenylalanine, leucine, isoleucine and valine. These findings may indicate a more generalized disorder in protein metabolism in autism. In accordance with previous hypotheses, the results of study suggest that serotonergic mechanisms may play a role in the pathophysiology of autism.

Another major finding is that serum tryptophan and 5-HIAA in 24-h urine are significantly and positively correlated with age in both autistic subjects and normal controls.<sup>13</sup> These findings may indicate that the serotonergic system undergoes significant changes during the lifespan, e.g. from the postpubertal period to adulthood.<sup>17</sup> Recent findings suggest that humans undergo a period of high brain 5-HT synthesis capacity during childhood, and that this developmental process is disrupted in autistic children.<sup>9</sup> Given the fact that all but one of the autistic subjects in this study belong to the high-functioning group we cannot exclude whether the findings are specific for the entire autistic population or might be confined to the higher 30% of the overall population of autistic subjects. It remains unclear if, or to what degree, disturbances in the serotonergic system may contribute to the overall phenotype of autism or only to the degree of severity of autism and/or the presence and severity of associated features. Although there is increasing evidence of the role of 5-HT in the pathophysiology of autism, we must remain very cautious when interpreting abnormal peripheral 5-HT levels in autistic subjects. Correlations between peripheral and central findings are not yet established and the 5-HT values are probably influenced by variables such as age, sex, race, stage of puberty, IQ,

presence of seizures, drug state of the patients, dietary factors, associated medical features, platelet characteristics, measuring techniques and 5-HT values in parents.

### **Catecholamines.**

Patients with autism show higher plasma norepinephrine (NE) concentrations than normal controls.<sup>18</sup> This elevation was not found by Minderaa et al. Urinary NE and 3-methoxy-4-hydroxyphenyl glycol (MHPG), the major NE metabolite, have been inconsistent and inconclusive.<sup>14</sup> A significantly higher homovanillylic acid (HVA)/MHPG ratio was found in the CSF of autistic subjects, suggesting an imbalance between the dopaminergic and noradrenergic systems.<sup>19</sup> Findings concerning serum dopamine- $\beta$ -hydroxylase have been inconsistent and inconclusive. However, to the best of our knowledge, no research in autism has demonstrated significant alterations in platelet [<sup>3</sup>H]-rauwolscine binding characteristics. Serum tyrosine, the precursor of NE, was elevated in autistic patients with neurological signs but not in those without.<sup>20</sup> There is evidence that the synthesis of brain NE is determined, in part, by brain tyrosine concentrations, which are reflected in the serum by the molar ratio of tyrosine to the competing amino acids tryptophan, phenylalanine, leucine, isoleucine and valine.<sup>21</sup> A PET study using regional FDOPA ratios demonstrated low medial prefrontal dopaminergic activity in a group of 14 13-year-olds with autism.<sup>22</sup>

No significant differences in the measurements of the catecholaminergic variables. The [<sup>3</sup>H]-rauwolscine binding  $B_{max}$  values and  $K_d$  values were not significantly different between autistic patients and normal volunteers. There were no significant differences in the NE, epinephrine and dopamine values in 24-h urine between autistic patients and healthy volunteers.<sup>13</sup>

### **Other amino acids.**

Aspartic acid, glutamine, glutamic acid and gamma aminobutyric acid were found to be significantly decreased inside the platelets of 18 people with autism compared to controls.<sup>23</sup> Variation in the levels of of excitatory amino acids were reported in the plasma of 14 children with autism.<sup>24</sup>

### **Pterins.**

Pterins are cofactors in the production and release of monoaminergic neurotransmitters. Significant variations in plasma- and urinary levels of the pterine compounds tetrahydrobiopterin (THB), neopterin and monapterin have been found.<sup>24,25</sup> Other studies showed no difference in plasma and urinary levels of THB.<sup>27</sup> Administration of THB during 12 weeks had beneficial effects in a group of 84 autistic children.<sup>28</sup>

### **Gangliosides, GFA-protein.**

The release of gangliosides increases with increased synaptic activity. Gangliosides were found to be increased in the CSF of autistic children.<sup>29</sup> Glial fibrillary acidic protein (GFA), a putative marker of the degree of gliosis and neuronal density also appeared to be significantly elevated in the CSF of autistic children.<sup>30</sup> Those findings fit into a growing body of evidence from brain imaging and neuropathological studies in favor of prenatal disturbances of brain morphogenesis. All the aspects of cortical formation (proliferation, migration and organization) have

found to be disturbed in at least one study of autistic children, highlighting once more the heterogeneity of this disorder.<sup>31</sup>

Serotonergic and (auto-)immune mechanisms might be responsible for those early disorders of brain development. Chugani et al hypothesize that neuropathological findings such as increased neuronal cell numbers (e.g. in the hippocampus) might result from serotonergic depletion during critical fetal developmental periods<sup>9,32</sup> This might explain the megalencephalic brains and macrocrania reported in autistic children.<sup>32,33</sup> This macrocrania is not, or to only a minor degree, found in older autistic patients.<sup>32,34</sup> This might be another argument in favor of the idea that autism is a developmental disorder, ongoing from childhood into adulthood. These findings concur with findings of an abnormal developmental pattern in brain 5-HT-synthesis capacity in autistic subjects during their lifetime.<sup>9</sup>

### **Neuropeptides.**

Neuropeptides are a perfect illustration of the complexity of neuroendocrine–immune communication. A primary deficit within the brain opioid system (and/or in pro-hormones processing) has been suggested as a possible biological factor in the pathophysiology of autism. Opioid peptides play an important role in brain development and all brain areas that have been described to be incompletely developed or dysfunctioning in autism contain high concentrations of opioids. The presence of opioid receptors in cells of the immune system and the capacity of immune cells to produce opioid peptides demonstrate that opioid substances play an important role in neuroendocrine–immune communication.<sup>35</sup>

Several studies link endogenous opioids to autism but the results are complex and not yet interpreted in a coherent way. Plasma beta-endorphins were found to be lower and to be correlated to self-injury and to the severity of stereotypies in children with autism.<sup>36</sup> On the other hand, Tordjmans et al mention higher levels of plasma beta-endorphins in the autistic group and Leboyer et al found that the medial C-terminal directed beta-endorphin fraction was higher and the N-terminal directed fraction was lower in the group of autistic children.<sup>37,38</sup> Fifty-three per cent of the mothers of subjects with autism had elevated levels of the C-terminal directed beta-endorphin protein immunoreactivity.<sup>39</sup> Conflicting results were found in the CSF, beta-endorphin being elevated, decreased and normal.<sup>40,41,42</sup>

A dimorphic pattern of plasma endorphins correlating with specific alterations in immune parameters has been found, in particular with the NK cell population.<sup>43</sup> Therapeutic studies using the opioid antagonist naltrexone showed an influence on behavioral alterations present in the autistic population, e.g. on self-injurious behavior stereotypies, verbal production and social contact.<sup>44,45</sup> Clinical trials with ORG 2766, a synthetic analog of the ACTH (4–9) fragment showed an improvement in social behavior and information processing.<sup>46</sup> The majority of the above-mentioned findings suggest the important role of opioid peptides as potential molecular messengers in neuroendocrine–immune cross-talk communication and as an important element in the pathophysiology of autism. Oxytocin, another neuropeptide involved in species-typical social behavior, communication and rituals in animals was found to be decreased in the plasma of autistic children.<sup>47</sup>

### **Metabolic pathways.**

End products of several metabolic pathways are useful in identifying disease entities underlying autism and/or mental retardation (e.g. phenylketonuria), but generally not significant for the autistic disorder per se. Only in the case of abnormal

levels of uric acid, the end-product of all the purine pathways, in blood and/or urine, does there seem to exist a separate autistic disease entity, given the frequent association between abnormal urine acidic levels, classical symptoms of autism, seizures, self-injury, psychomotor delay, constipation and megastools. Gout is found frequently in family members of those children.<sup>48</sup> Adenylosuccinate lyase (ASL) has been found at deficient levels. A mutation in the human ASL gene was linked to mental retardation with autistic features in three affected siblings, but the prevalence of mutations on the ASL gene in a larger autistic population appeared to be rare.<sup>49,50</sup> Adenosine de-aminase (AD) general allele, which confers lower enzymatic activity, has been found in a large autistic population compared to a control group.<sup>51</sup> It is interesting to mention the link between the inherited purine enzyme deficiencies and immune system abnormalities.<sup>52</sup> Age seems to play a role, as hyper- or hypouricosuria could be detected only during childhood. In some autistic patients, however, the phosphoribosylpyrophosphate (PRPP) synthetase, the rate-limiting step of purine metabolism, remained abnormal after puberty.<sup>48</sup> Diet with a purine restriction and pharmacotherapy with drugs affecting the puridine pathway resulted in an improvement of the behavioural symptoms of some autistic children.<sup>53</sup>

### **Immunology**

There is now some evidence that autism may be accompanied by abnormalities in the inflammatory response system (IRS). Products of the IRS, such as proinflammatory cytokines, may induce some of the behavioural symptoms of autism, such as social withdrawal, resistance to novelty and sleep disturbances. One study showed signs of an inflammatory system response in autism, as shown by a significantly increased IFN- $\gamma$ , IL-6 and IL-1-rA and an increase, albeit not significant, in IL-10 and TNF- $\alpha$  in unstimulated culture supernatant. There was a significant and positive correlation between serum IL-1RA and unstimulated culture supernatant IL-1RA and between serum IL-2R and unstimulated culture supernatant IFN- $\gamma$ . There was a trend towards a positive correlation between serum IL-6 and unstimulated culture supernatant IL-6. The findings of this study therefore suggest that autism is probably accompanied by an activation of the monocyte–macrophage and lymphocytic lineage of CMI. These results are in accordance with previous findings reporting abnormalities in substances known as markers of cellular immunity activation, including: (i) increased urinary levels of neopterin; (ii) elevated interferon- $\alpha$  levels in plasma; (iii) increased levels of T8 antigen (sT8) and soluble interleukin-2 (sIL-2), but not soluble interleukin-2 receptor (sIL-2R) nor sIL-1, and increased levels of IL-12 and IFN- $\gamma$  in unmedicated autistic children;<sup>25,26,54,56,57</sup> Croonenberghs et al measured the production of interleukin (IL)-6, IL-10, the IL-1 receptor antagonist (IL-1RA), IFN- $\gamma$  and TNF- $\alpha$  by whole blood and the serum concentrations of IL-6, the IL-2 receptor (IL-2R) and IL-1RA. Results of this study showed a significantly increased production of IFN- $\gamma$  and IL-1RA and a trend toward a significantly increased production of IL-6 and TNF- $\alpha$  by whole blood of autistic children. There were no significant differences in the serum concentrations of IL-6, IL-2R and IL-1RA between autistic and normal children. These results suggest that autism may be accompanied by an activation of the monocytic (increased IL-1RA) and Th-1-like (increased IFN- $\gamma$ ) arm of the Inflammatory Response System. It is hypothesized that increased production of proinflammatory cytokines could play an important role in the pathophysiology of autism.<sup>58</sup>

The pathophysiology and meaning of the increased CMI and cytokine production in autism and the relation between an increase of cytokines and certain behavioral aspects of the autistic disorder have remained elusive. The following factors might be of importance in this context (either causal or as a consequence of the increased CMI):

- Symptoms typical for the autistic disorder, such as social withdrawal and suppression of exploratory behaviour (reluctance to novelty and insistence on sameness), and common features in autism such as sleep disturbances and mood alterations, are possibly in caused part by abnormalities of products of cell-mediated immunity, i.e. IFN- $\gamma$ , IL-1 and IL-6. Administration of ORG 2766, a synthetic analog of the ACTH fragment, showed an improvement in social behaviour and information processing.<sup>59</sup> Withdrawal and loss of interest have also been observed in children receiving interferon therapy for cancer.

- Increased CMI might be the expression of an underlying autoimmune disorder. Numerous findings concerning cellular and humoral immunity suggest a high incidence of autoimmune disorders and an increased susceptibility to viral infections in the autistic population.<sup>60,61</sup>

Abnormal reactivity against proteins extracted from human cortex is found frequently in autistic subjects. Increased levels of cytokines might be the result of brain cell destruction due to an autoimmune disease. Weisman et al reported an inhibition of macrophage migration in response to human myelin basic protein (a specific CNS antigen implicated in myelination).<sup>62</sup> Antibodies against myelin basic protein (MBP) and neuron-axon filament-protein (NAFP) are found frequently in children with autism.<sup>63,64</sup> Immunotherapy resulted in a clinical improvement in terms of speech, learning abilities, attention span and sleep patterns in six of eight autistic patients with antibodies against NAFP.<sup>65</sup> Abnormal reactivity against cerebellar neurofilaments is found in autistic subjects.<sup>66,67</sup> Abnormal reactivity against frontal cortex could not be detected.<sup>66,68</sup> Children with autism spectrum disorders have a greater frequency of serum antibodies to brain endothelial cells.<sup>69</sup> Autoantibodies against nerve-growth factor have been reported in subject with Kanner's and Asperger's syndrome.<sup>70</sup>

Autoimmune diseases are triggered frequently by micro-organisms and it is thought that an immune response to brain antigens resulting from pre- or postnatal viral infections plays a role in the development of the autistic phenotype.<sup>63</sup> An association between virus serology and brain antibody (anti-MBP and anti-NAFP) has been demonstrated by Singh et al.<sup>71</sup>

Auto-antibodies against frontal cortical 5-HT<sub>1a</sub> receptors have been described in a group of autistic children.<sup>64</sup> Cook et al, however, found that inhibition of several serotonergic receptor binding sites by immunoglobulins was not specific for children with autism.<sup>72</sup>

A positive relationship was observed between elevated 5-HT levels and the major histocompatibility complex (MHC)-types associated with autism.<sup>73</sup>

The rate of D8/17 (monoclonal antibody identifying a B-lymphocyte antigen) is high in patients with autism.<sup>74</sup> High levels of antibodies against casein and lactalbumin were found in autistic subjects and a cow's milk-free diet resulted in an improvement of the behavioural symptoms, suggesting a relationship between food allergy and autism.<sup>75</sup>

Genetic factors may influence the immune responses to an infectious agent, which can trigger autoimmunity, e.g. alterations in the complement systems. Warren et al reported that the major histocompatibility complex (MHC), including the null allele of the C4B- gene, the extended haplotype B44-C30-DR4 and two alleles of the

DR beta 1 gene is associated with autism.<sup>76</sup> Incomplete or partial T-cell activation were inversely correlated with a decreased level of the C4B-protein.<sup>77</sup> This might reflect the genetic predisposition for immunological abnormalities in the autistic disorder.

Pro-inflammatory cytokines have profound effects on the peripheral and brain serotonergic systems. Peripheral and central administration of IL-1, IFN- $\gamma$  and TNF- $\alpha$  increase extra-cellular 5-HT concentrations in several brain areas, such as the hypothalamus, hippocampus and cortex.<sup>78</sup> IL-1 modulates the activity of the 5-HT transporter,<sup>79</sup> which plays a central role in serotonergic neurotransmission by reuptake of 5-HT.

The immune effects of SSRIs, tricyclic and heterocyclic antidepressants may be explained in part by their serotonergic activities, such as depletion of intracellular 5-HT stores, increased extra-cellular 5-HT and/or 5-HT<sub>2A/2C</sub> receptor blockade.<sup>80</sup> T-lymphocytes express 5-HT receptors, such as 5-HT<sub>1A</sub> and 5-HT<sub>2A/2C</sub> receptors, as well as a high-affinity 5-HT transporter. Macrophages have a specific active 5-HT uptake system similar in affinity to that of platelets.<sup>81</sup> There are now some reports that depletion of intracellular 5-HT stores, increased extra-cellular 5-HT and/or 5-HT<sub>2A/2C</sub> receptor blockade may result in negative immunoregulatory effects.<sup>82</sup> An inverse relationship is shown between lower plasma tryptophan, the precursor of 5-HT, and various indicators of the inflammatory response in depression.<sup>83</sup> The effects of SSRIs on the immune system and on certain symptoms of the autistic disorder may be explained in part by their serotonergic activities.

It remains elusive to what degree changes in (HPA)-axis function and 5-HT turnover are responsible for the immunological changes we found in our study. Inversely it is unclear what the influence is of the immunological changes on the serotonergic abnormalities found frequently in previous autism studies. In a study measuring auto-antibodies against nine different neuron-specific antigens and three cross-reactive peptides in the sera of autistic subjects and healthy controls by means of enzyme-linked immunosorbent assay (ELISA) testing. The antigens were myelin basic protein (MBP), myelin-associated glycoprotein (MAG), ganglioside (GM1), sulfatide (SULF), chondroitin sulfate (CONSO4), myelin oligodendrocyte glycoprotein (MOG), alpha,beta-crystallin (alpha,beta-CRYS), neurofilament proteins (NAFP), tubulin and three cross-reactive peptides, Chlamydia pneumoniae (CPP), streptococcal M protein (STM6P) and milk butyrophilin (BTN). Autistic children showed the highest levels of IgG, IgM and IgA antibodies against all neurologic antigens as well as the three cross-reactive peptides. These antibodies are specific because immune absorption demonstrated that only neuron-specific antigens or their cross-reactive epitopes could significantly reduce antibody levels. These antibodies may have been synthesized as a result of an alteration in the blood-brain barrier. This barrier promotes access of pre-existing T-cells and central nervous system antigens to immuno-competent cells, which may start a vicious cycle. These results suggest a mechanism by which bacterial infections and milk antigens may modulate autoimmune responses in autism.<sup>84</sup>

Searching for a mechanism underlying autoimmunity in autism, it has been postulated that gliadin peptides, heat shock protein 60 (HSP-60), and streptokinase (SK) bind to different peptidases resulting in autoantibody production against these components. We assessed this hypothesis in patients with autism and in those with mixed connective tissue diseases. Associated with anti-gliadin and anti-HSP antibodies, children with autism and patients with autoimmune disease developed anti-dipeptidylpeptidase I (DPP I), anti-dipeptidylpeptidase IV (DPP IV [or CD26])

and anti-aminopeptidase N (CD13) auto-antibodies. A significant percentage of autoimmune and autistic sera were associated with elevated IgG, IgM, or IgA antibodies against three peptidases, gliadin, and HSP-60. These antibodies are specific, since immune absorption demonstrated that only specific antigens (e.g., DPP IV absorption of anti-DPP IV), significantly reduced IgG, IgM, and IgA antibody levels. For direct demonstration of SK, HSP-60, and gliadin peptide binding to DPP IV, microtiter wells coated with DPP IV were reacted with SK, HSP-60, and gliadin. They were then reacted with anti-DPP IV or anti-SK, anti-HSP, and anti-gliadin antibodies. Adding SK, HSP-60, and gliadin peptides to DPP IV resulted in 27 to 43% inhibition of the DPP IV-anti-DPP IV reaction, but DPP IV-positive peptides caused 18 to 20% enhancement of antigen-antibody reactions. It is proposed that (i) superantigens (e.g., SK and HSP-60) and dietary proteins (e.g., gliadin peptides) in individuals with predisposing HLA molecules bind to aminopeptidases and (ii) they induce auto-antibodies to peptides and tissue antigens. Dysfunctional membrane peptidases and auto-antibody production may result in neuro-immune dysregulation and autoimmunity.<sup>85</sup>

### **Endocrinology**

Investigations into the neuro-endocrinology of autism have provided inconclusive data concerning values at baseline and after loading tests and diurnal rhythms.

Cases of improvement after therapy with tri-iodothyronine (T3) have been reported, and a high rate of abnormal response to thyrotropin-releasing hormone challenge has been demonstrated.<sup>86</sup> Marginal changes in circadian rhythms of thyroid-stimulating hormone and an abnormal circadian rhythm of melatonin have been seen in severely autistic young adults.<sup>87</sup> Despite normal basal hormonal levels, subgroups of autistic children have been shown to have a blunted or delayed growth hormone response to levodopa,<sup>88</sup> a blunted prolactin response to fenfluramine, and a high rate of cortisol non-suppression after dexamethasone administration.<sup>89,90</sup> Plasma levels of testosterone and dehydroepiandrosterone (DHEA) has found not to be a common feature in autism. Tordjman et al observed precocious puberty in four of 12 prepubertal children with autism.<sup>91</sup> Some studies about autism emphasize the role of dysfunctions in the HPA-axis.<sup>92</sup>

### **Neuroendocrine disorders and IRS activation**

Contemporary neuro-endocrine models of autism do not incorporate the effects of the inflammatory response system (IRS) and the turnover of serotonin (5-HT), even though there exists an explicit and mutual relationship between those three elements.

There exists an important and mutual regulation between the hypothalamic–pituitary–adrenal (HPA) axis and the turnover of serotonin (5-HT), on the one hand, and the immunesystem on the other hand. IRS activation involves not only specific immune but also neuroendocrine changes such as hyperactivity of the HPA axis, and alterations in the peripheral and central turnover of 5-HT.

IL-1 and IL-6 exert a potent enhancing effect on HPA-axis by stimulating hypothalamic corticotropin-releasing hormone (CRH), pituitary adrenocorticotrophic hormone (ACTH) and adrenal steroidogenesis, e.g. cortisol.<sup>93</sup>

## **Pre-, peri- and postnatal damages to the developing CNS**

There is a body of evidence that genetic factors play a most important role as a base for the diseases underlying the autistic phenotype. Evidence shows that the disorder is genetically heterogeneous.<sup>94</sup> Higher functioning individuals with autism may have another genetic background than lower functioning ones. Earlier in this review, it has been mentioned many such genetic factors determining abnormalities of the serotonergic, immunological and endocrinological system. The most interesting chromosome regions concerning the aetiology of autism are chromosomes 7q31–35, 15q11–13 and 16p13.3 which have been suggested by different lines of genetic research.<sup>95</sup>

Non-genetic factors, however, may also be relevant. There are several hypotheses stating that a 'cascade' of inopportunities might be responsible for the final autistic phenotype. The primary factor might be a predisposing genetic error. Secondary factors might be 'traumatic events' in the largest sense, e.g. a prenatal infection, perinatal inopportunities, background radiation, food allergy or xenobiotics such as heavy metals in a multitude of substances. Interaction between one or more modifying genes and exogenous factors might result in widespread impairment of multiple areas or circuits in the CNS leading to the typical disturbed functions and behaviours in autism. The presence or severity of autistic symptoms depends possibly on the strength or the load of genetic predisposition, individuals with a strong genetic predisposition perhaps always presenting symptoms and individuals with a low genetic load presenting symptoms depending on the presence of harmful environmental factors.

The timing of an insult, harmful to the developing brain, also defines the nature of the CNS damage and the consecutive phenotypical stigmata and behavioural problems. Most cases seem to have an origin in the first and second trimester of pregnancy and perinatal complications seem to derive mainly from previous abnormalities in the fetus.<sup>94</sup> Early infections can occur by chance but can also be caused by and/or result in an altered auto-immune system. The role of pitocin, a synthetic oxytocin administered during labour and delivery, in the genesis of autism needs further elaboration. Postnatal changes, however (regression from normal but also cases of spontaneous recovery and amelioration as a consequence of therapy) demonstrate that environmental factors can probably be of great influence on the ongoing plasticity of the developing brain, e.g. on an adequate cortical pruning.<sup>96,97</sup>

The complexity of environmental factors hampers the study of putative postnatal harmful events. In order to determine the possible influence of each event we need an extensive questionnaire concerning the genetic (e.g. allergic) predisposition of the parents' illnesses and medical or dental treatment during pregnancy, the medical antecedents, vaccinations, allergies and other medical conditions of the child, environmental circumstances such as place of residence, professional occupancies of the parents, smoking, drinking and eating habits (actual and during pregnancy).

It has since long been known that there is a subgroup of autistic children with gastrointestinal problems presenting with diarrhea and steatorrhea.<sup>98</sup> The pathophysiology of this autism/steatorrhea entity has not yet been elucidated. There are repeated findings of children to have both autism and celiac disease but other studies have failed to demonstrate a causal relationship between celiac disease and the autism/steatorrhea entity.<sup>99</sup>

The supposition of abnormal intestinal permeability (leading to incomplete breakdown of food proteins and the absorption of putative harmful peptides) and food intolerance led to the therapeutic use of different diets. A milk-eliminating diet (inspired by the repeated findings of abnormal immuno-reactivity against casein) resulted in behavioural improvement of some subjects with autism.<sup>75</sup> The elimination of wheat products (gluten and gliadin) from the diet, inspired by the above-mentioned celiac findings, also resulted in behavioural improvement of some autistic children. The use of secretin, an enzyme stimulating the pancreaticobiliary secretion, could not be confirmed as a valuable therapy in large-scale, controlled studies.<sup>100</sup>

Another group of xenobiotics consists of the heavy metals present in an astonishing number of sometimes beneficial but often harmful products of our 'increasingly artificial environment'.<sup>101</sup> Preventive (in most countries, vaccinations) and therapeutic agents often contain concentrations of heavy metals several times higher than the generally accepted toxic concentrations. Repeated or chronic exposure can induce autoimmune mechanisms in genetic predisposed individuals. Genetic predisposition regulates the detoxifying capacity and sensitivity to metals.<sup>102</sup> However, the epidemiological evidence on the association of the measles-mumps-rubella vaccine and the occurrence of autism, is consistently negative.<sup>103</sup>

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