



HHS Public Access

Author manuscript

Acta Physiol (Oxf). Author manuscript; available in PMC 2018 February 14.

Published in final edited form as:

Acta Physiol (Oxf). 2016 January ; 216(1): 90–100. doi:10.1111/apha.12614.

Autoimmunity against dopamine receptors in neuropsychiatric and movement disorders: a review of Sydenham chorea and beyond

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Abstract

Antineuronal autoantibodies are associated with the involuntary movement disorder Sydenham chorea (SC) and paediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS) which are characterized by the acute onset of tics and/or obsessive compulsive disorder (OCD). In SC and PANDAS, autoantibodies signal human neuronal cells and activate calcium calmodulin-dependent protein kinase II (CaMKII). Animal models immunized with group A streptococcal antigens demonstrate autoantibodies against dopamine receptors and concomitantly altered behaviours. Human monoclonal antibodies (mAbs) derived from SC target and signal the dopamine D2L (long) receptor (D2R). Antibodies against D2R were elevated over normal levels in SC and acute-onset PANDAS with small choreiform movements, but were not elevated over normal levels in PANDAS-like chronic tics and OCD. The expression of human SC-derived anti-D2R autoantibody V gene in B cells and serum of transgenic mice demonstrated that the human autoantibody targets dopaminergic neurones in the basal ganglia and other types of neurones in the cortex. Here, we review current evidence supporting the hypothesis that antineuronal antibodies, specifically against dopamine receptors, follow streptococcal exposures and may target dopamine receptors and alter central dopamine pathways leading to movement and neuropsychiatric disorders.

Keywords

autoimmunity; chorea; dopamine; dopamine receptor; streptococci

Movement, behavioural and neuropsychiatric disorders affect millions worldwide, and there is a growing interest in the association between infections, autoimmunity and behavioural changes, and their impact on the genesis of neuropsychiatric disorders (Murphy *et al.* 2012). Studies suggesting that infection and antineuronal autoantibodies play a role in the

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Conflict of interest

Madeleine Cunningham is the chief scientific officer and co-founder with financial interest in Moleculera Labs, a commercial laboratory for diagnostic testing of antineuronal antibodies. Carol J. Cox declares financial interest in Moleculera Labs.

pathogenesis of movement and behavioural disorders began with the studies of Sydenham chorea (SC) and autoantibodies against the brain in rheumatic fever (Zabriskie 1967, 1985, Zabriskie *et al.* 1970, Husby *et al.* 1976, Bronze & Dale 1993).

Sydenham chorea is well established as the major neurologic sequelae of *Streptococcus pyogenes*-induced rheumatic fever (RF) (Stollerman 2001). It is characterized by involuntary movements and neuropsychiatric disturbances, including obsessive–compulsive symptoms which may predate the chorea, hyperactivity, emotional lability, irritability and distractibility (Marques-Dias *et al.* 1997).

Sydenham chorea is associated with streptococcal pharyngitis (Taranta & Stollerman 1956, Taranta 1959), while tics and obsessive compulsive disorders may be associated with streptococcal and other types of infections (Kurlan & Kaplan 2004, Kurlan *et al.* 2008, Gause *et al.* 2009, Murphy *et al.* 2010), as well as with autoantibodies against neuronal antigens (Swedo *et al.* 1989, 1993, 1997, 1998, Swedo 1994, Kirvan *et al.* 2003, 2006a,b, 2007, Brilot *et al.* 2011). These disorders have been identified as paediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS) (Swedo *et al.* 1998) or paediatric acute-onset neuropsychiatric syndrome (PANS) (Swedo *et al.* 2012) in the presence of other types of infections (Swedo *et al.* 1997, Swedo & Grant 2005).

To more specifically describe the discovery of PANDAS, Swedo and colleagues identified children who appeared with a sudden acute onset of obsessive compulsive disorder which had a relapsing–remitting course. Five diagnostic criteria were reported from the first 50 cases: (i) presence of obsessive–compulsive disorder (by DSM criteria) or a tic disorder; (ii) symptom onset between the ages of 3 years and puberty; (iii) episodic symptoms, with abrupt and substantial symptom exacerbations; (iv) symptom onset and exacerbations associated temporally with group A streptococcal infections; and (v) presence of neurologic abnormalities during symptom exacerbations (Swedo *et al.* 1998). These cases also displayed piano playing choreiform movements of the fingers and toes.

Murphy also described acute-onset OCD/tics with severe hyperactivity, loss of fine motor skills (handwriting deterioration) or choreiform movements (Murphy *et al.* 2012). Psychiatric symptoms described by Murphy *et al.* included irritability, frequent mood changes, separation anxiety, hyperactivity, late-onset attention problems, personality change, oppositional behaviours, sleep disturbances and deterioration in mathematical skills. Historical accounts from the first 50 cases of PANDAS indicate that at least some cases with PANDAS were immediately following or during a group A streptococcal infection (Swedo *et al.* 1998), but it is still questioned whether group A streptococcal infections are coincidental or causal, or whether PANDAS could be a variant of acute rheumatic fever (Kurlan *et al.* 2008).

Paediatric autoimmune neuropsychiatric disorders associated with streptococcal infections have been proposed to develop due to post-infectious autoimmune processes (Swedo *et al.* 1998, Kirvan *et al.* 2006b). Thus, as in acute rheumatic fever, antibodies against the group A streptococcus would cross-react with brain antigens as their neuronal targets in susceptible hosts, by the process of molecular mimicry (Kirvan *et al.* 2003). The pathogenesis of

PANDAS could be similar to Sydenham chorea where autoantibodies against neuronal cells in the basal ganglia may lead to neuropsychiatric and altered movement symptoms.

In the past, the basal ganglia has been implicated as a target of post-streptococcal immune responses (Swedo *et al.* 1993, Giedd *et al.* 1995). Sydenham chorea pathogenesis has been proposed to be an autoantibody-mediated disease with basal ganglia dysfunction where antibodies have an affinity for basal ganglia (Husby *et al.* 1976, Giedd *et al.* 1995), and anti-inflammatory treatments such as steroids, plasmaphoresis and intravenous immunoglobulin treatment are effective (Perlmutter *et al.* 1999). Plasmaphoresis has been found to reduce symptoms of Sydenham chorea and obsessive compulsive symptoms in PANDAS, which suggests that the removal of antineuronal autoantibodies abrogates symptoms (Swedo 1994, Perlmutter *et al.* 1999).

Antineuronal antibody titres have been associated with both severity and duration of choreic episodes in Sydenham chorea, and antibrain antibodies have been found in stained neurones in the basal ganglia (Husby *et al.* 1976, Bronze & Dale 1993). Husby *et al.* (Husby *et al.* 1976) found that chorea patient sera strongly reacted with cytoplasmic, but not nuclear, antigens in human caudate and subthalamic nuclei as well as cerebral cortex neurones. Autoantibodies, such as antilyso ganglioside (LGN) GM₁ (Kirvan *et al.* 2003, 2006b) and anti- β tubulin (Kirvan *et al.* 2006a), have been described in Sydenham chorea. Certainly, the removal of antibody by immunomodulatory therapies such as plasma exchange suggests that chorea may be caused by a pathogenic antibody response (Swedo 1994), and symptoms improved following plasmaphoresis (Allen *et al.* 1995, Perlmutter *et al.* 1999). PANDAS may also be associated with antineuronal antibody responses that may attack the basal ganglia (Singer *et al.* 2004, Kirvan *et al.* 2006b) and result in an abnormal brain MRI (Giedd *et al.* 2000).

The introduction has outlined the above evidence, suggesting that Sydenham chorea and potentially PANDAS are linked to autoantibodies against the brain. The antineuronal autoantibodies observed in acute episodes of Sydenham chorea and PANDAS are elevated during the acute stage of disease and decrease dramatically during the convalescent stages along with a reduction in symptoms as has been shown by Kirvan *et al.* in studies of human sera from Sydenham chorea (Kirvan *et al.* 2003) and PANDAS (Kirvan *et al.* 2006b). These studies also demonstrated IgG antineuronal antibody present in cerebrospinal fluid which reacted with human basal ganglia. The IgG antibodies reacted with lysoganglioside which is enriched in brain and present in the membranes and on the surface of human neuronal cells. Patients with the acute-onset type of PANDAS as well as Sydenham chorea respond positively to plasmaphoresis (Perlmutter *et al.* 1999), which also supports the hypothesis that antibodies may play a role in the disease. Intravenous immunoglobulin also led to a reduction in symptoms as well. Studies of human sera indicate that antineuronal antibodies including antilyso ganglioside, antitubulin and antidopamine receptor antibodies are elevated during disease and are reduced in convalescence (Kirvan *et al.* 2003). A study by Ben-Pazi *et al.* (Ben-Pazi *et al.* 2013) has shown that the symptoms in Sydenham chorea directly correlate with the ratio of antibodies against the D1 and D2 receptors.

The antineuronal antibodies have also been found to signal human neuronal cells through the calcium calmodulin protein kinase II (CaMKII) activation which potentially would lead to increased dopamine release. To prove that these types of antibodies are present in Sydenham chorea which react with brain and the group A streptococcus, we produced human mAbs derived from Sydenham chorea and used them to study the mechanisms and the *in vivo* targets in the brain. The studies of chorea-derived human mAb 24.3.1 are described below as well as the transgenic mouse created to express this human antibody. The Tg mouse was used to empathize the brain regions targeted by the human antibody from chorea which signalled CaMKII and also reacted with lysoganglioside and the dopamine receptors D1 and D2.

Animal models will also be discussed in our review and further support the hypothesis that the antibodies are linked to symptoms (Hoffman *et al.* 2004, Yaddanapudi *et al.* 2010, Brimberg *et al.* 2012, Lotan *et al.* 2014a,b). These animal models include streptococcal exposure and immunization which led to the production of antibodies that deposited in the brain and also led to movement and neuropsychiatric symptoms in the mice or rats. The models were also recapitulated in passive antibody transfer experiments. These studies also further support the molecular mimicry hypothesis where the antibodies produced against the group A streptococcus target the brain.

Sydenham chorea-derived human mAbs

The hypothesis that Sydenham chorea may develop due to autoantibodies and inflammation which affect neuronal signalling mechanisms has been supported from studies of chorea-derived human monoclonal antibodies (mAbs) (Kirvan *et al.* 2003). Choreaderived human mAb 24.3.1 reacted with group A streptococcal carbohydrate epitope *N*-acetyl- β -D-glucosamine (GlcNAc) and neuronal surface antigen lysoganglioside G_{M1} and alpha helical intracellular brain protein tubulin (Kirvan *et al.* 2003, 2007) in what is called cross-reactivity or molecular mimicry, where antigenic structures are similar between host and microbe (Cunningham 2012, 2014).

The human chorea-derived mAb 24.3.1 induced antibody-mediated neuronal cell signalling by activating Ca⁺⁺/calmodulin-dependent protein kinase (CaM kinase) II activity in a human neuronal cell line SK-N-SH (Kirvan *et al.* 2003), and intrathecal passive transfer of mAb 24.3.1 into Lewis rats induced elevated tyrosine hydroxylase in rat brain (Kirvan *et al.* 2006a). Sera and cerebrospinal fluid (CSF) IgG from Sydenham chorea demonstrated the same antigen cross-reactivities as well as similar neuronal cell signalling CaMKII activation *in vitro* (Kirvan *et al.* 2003). Our studies have shown Sydenham chorea and PANDAS IgG as well as chorea-derived mAb 24.3.1 all activated CaMKII in a human neuronal cell line, leading to tyrosine hydroxylase activation and subsequent dopamine release (Kirvan *et al.* 2003, 2006a,b). Tyrosine hydroxylase is the rate-limiting enzyme in dopamine synthesis, and was increased in the brain of Lewis rats after intrathecal infusion of mAb 24.3.1 (Kirvan *et al.* 2006a). Signalling properties of sera can be abrogated by the removal of the IgG (Brimberg *et al.* 2012), demonstrating that the CaMKII activation is associated with serum IgG.

Our evidence demonstrated that human choreaderived mAb 24.3.1 was similar to the IgG reactivity of Sydenham chorea sera not only from the mAb donor, but also from other cases of Sydenham chorea as well. Antibody-mediated cell signalling of the human neuronal cell line SK-N-SH appeared to cause excess dopamine release as shown in tritiated thymidine release assays from chorea-derived mAb and chorea serum-treated human SK-N-SH neuronal cells (Kirvan *et al.* 2006a). These data suggest that a possible alteration of central dopamine pathways may be involved in the immunopathogenesis of SC. Dopamine involvement in the disease is relevant and fulfils an important role in the pathophysiology of chorea, as treatment of Sydenham chorea has in the past relied on the use of antidopaminergic drugs (Shannon & Fenichel 1990, Sokol 2000, Dale 2005, Demiroren *et al.* 2007, Moretti *et al.* 2008).

Dopamine is the main catecholamine neurotransmitter in the central nervous system (CNS) and is synthesized from tyrosine via tyrosine hydroxylase and stored in vesicles in axon terminals. Dopaminergic signalling is a balance between dopamine release and re-uptake by the pre-synaptic terminal. The five dopamine receptor types are divided into two families: D1-like (stimulatory) and D2-like (inhibitory), both of which act through G-coupled protein receptors (Pollack 2004, Pivonello *et al.* 2007). Dopamine plays a pivotal role in modulating the functioning of the basal ganglia brain circuitry (Gerfen *et al.* 1990, Graybiel 2000, Pivonello *et al.* 2007), and abnormalities in the functioning of this neurotransmitter have been implicated in disorders like Sydenham chorea (Harris & Singer 2006). The dopamine receptors are associated with the regulation of the second messenger cAMP through G protein-mediated signalling (Beaulieu & Gainetdinov 2011). D1 receptors typically are associated with an increase in cAMP, but D2 receptors are inhibitory, with a decrease in cAMP, as was shown for mAb 24.3.1 as well as Sydenham chorea and PANDAS sera. D2 receptors are generally more complex than D1 because they are expressed pre- and post-synaptically (Beaulieu & Gainetdinov 2011).

CAM kinase activation has been reported in association with heterodimers of dopamine receptors containing both D1 and D2 (Hasbi *et al.* 2011). Our recent report indicates that the ratio of antibodies against both D1R and D2R were correlated with the USCRS symptom scale of Sydenham chorea. We investigated further the possibility that dopamine receptors such as the D1 and D2 receptors may be involved in disease (Cox *et al.* 2013, 2015). Studies using the membrane antigen targets in an ELISA as well as dopamine receptor signalling assays in transfected cell lines and FLAG-tagged epitopes on the D2 dopamine receptor all confirmed the reaction of mAb 24.3.1, and IgG from chorea serum and CSF additionally confirmed reactivity with the D2 dopamine receptor (Cox *et al.* 2013). The D2R might be expected to be involved in Sydenham chorea as haloperidol, a D2R blocker, is used to treat symptoms of Sydenham chorea (Shannon & Fenichel 1990, Sokol 2000, Dale 2005, Demiroren *et al.* 2007, Moretti *et al.* 2008).

Sydenham chorea-derived mAb 24.3.1 and SC IgG reacted with FLAG-tagged D2R, while PANDAS IgG did not (Cox *et al.* 2013). However, human chorea mAb 24.3.1 and sera from the human SC mAb B cell donor as well as PANDAS sera all induced inhibitory signalling of D2R, which may be the consequence of antibody targeting of dopaminergic neurones (Cox *et al.* 2013). Our data support the hypothesis that in human disease, antibodies against

D2R in SC or PANDAS with fine choreiform movements may target dopaminergic neurones in the basal ganglia and cortex and alter brain function to contribute to movement and behavioural disorders. Fine choreiform movements were reported in 95% of the first 50 cases of PANDAS (Swedo *et al.* 1998). Our novel findings suggest that autoantibodies which may signal neurones in chorea and other movement and behavioural disorders may signal through dopamine receptors.

Chorea-derived antibody targets dopaminergic neurones in basal ganglia in transgenic mice

To study the *in vivo* deposition of chorea-derived human mAb 24.3.1, we created transgenic (Tg) mice expressing chorea-derived human mAb 24.3.1 heavy and light chain variable region (V_H and V_L) genes as part of a chimeric (human V gene/mouse constant region) IgG1^a antibody (Cox *et al.* 2013). Chimeric Tg antibody 24.3.1 was expressed by B cells and was present in serum of the Tg-24.3.1 mice. Tg serum Ab and IgG1^a hybridoma mAbs produced from the Tg-24.3.1 mice demonstrated the same strong reactivity with D2R as human SC-derived mAb 24.3.1. Our Tg-24.3.1-IgG1^a transgenic mice were treated with lipopolysaccharide (LPS) and streptococcal cell walls to open the blood–brain barrier (BBB), thus allowing antibody penetration of the brain. To determine where chimeric Tg 24.3.1-IgG1^a antibody (IgG1^a) was localized in the brain *in vivo*, we investigated tissue sections of brains from Tg V_H 24.3.1 mice. Anti-IgG1^a allotypic Ab detected Tg24.3.1-IgG1^a within neuronal cell bodies in the basal ganglia, most likely the substantia nigra or ventral tegmental area (Fig. 1). Antibody to tyrosine hydroxylase (stained in Fig. 1b Yellow/TRITC) was used to identify dopaminergic neurones of Tg mouse brain because tyrosine hydroxylase converts tyrosine to dopamine and is a marker for dopaminergic neurones. As shown in Figure 1, the Tg-24.3.1 chimeric IgG1^a antibody penetrated the brain and was located in dopaminergic neurones in the basal ganglia of Tg mice (green/FITC Fig. 1a), most likely in the substantia nigra or ventral tegmental area (Cox *et al.* 2013). Tg-24.3.1-IgG1^a antibody was also associated with neurones in the cortex (Cox *et al.* 2013). It is worth noting that the Tg-24.3.1-IgG1^a was found within the cytoplasm of dopaminergic neurones *in vivo* sparing the nucleus and was similar in appearance to what was observed in actual disease sections of human brain taken from Sydenham chorea (Husby *et al.* 1976). Colocalization studies gave the appearance that *in vivo* Tg-24.3.1-IgG1^a antibody was internalized within TH+ neuronal cells in cross sections of the Tg mouse basal ganglia. Internalization might be expected as previous studies have shown that Abs to neuronal receptors can be internalized (Hughes *et al.* 2010), and it is well established that dopamine receptors undergo recycling (Parton & Richards 2003, Sudhof 2004, Wolstencroft *et al.* 2007). The substantia nigra and ventral tegmental area are part of the basal ganglia and contain dopaminergic neuronal cell bodies with axons which project to the striatum. D2 receptors are expressed on the dopaminergic neurones in the substantia nigra and ventral tegmental area as well as on neurones in the striatum (Gerfen *et al.* 1995, Kawaguchi *et al.* 1995, Surmeier *et al.* 2011, Anzalone *et al.* 2012). However, dopaminergic neuronal cell bodies are only seen in the substantia nigra and ventral tegmental area and the axons of these cells project into the striatum. Requirements for BBB penetration were established previously by Kowal (DeGiorgio *et al.* 2001, Kowal *et al.* 2004, 2006). Tg24.3.1-IgG1^a antibody-producing mice

not receiving the BBB stimulants did not have Tg24.3.1-IgG1^a antibody in the brain. This evidence supports the hypothesis that infections would not only promote antibody production but also have the potential to break the BBB. Disease such as Sydenham chorea and PANDAS are associated with group A streptococcal infections and are considered sequelae of the suppurative infection.

Mere cross-reactivity by antineuronal autoantibodies alone may not lead to disease, as suggested by the results with normal sera and other mAbs which do not signal human neuronal cells even though they may react with a neuronal antigen. However, when antibody avidity increases and leads to signalling of a receptor, the antibody may lead to symptoms. The similarity in antigen specificity but differences in signalling of D2R by a variety of mAbs in our studies has illustrated this principle that cross-reactivity by itself may not necessarily lead to signalling of a receptor (Kirvan *et al.* 2003, Cox *et al.* 2013).

Animal models

Animal models have supported the hypothesis that streptococcal and potentially other infections may lead to the development of antineuronal autoantibodies which can enter the brain and alter behaviour and movement. Hoffman *et al.* immunized mice with group A streptococcal antigen and found serum antibodies that deposited in brain tissues and reacted with deep cerebellar nuclei (DCN) (Hoffman *et al.* 2004). The anti-DCN antibodies were present in group A streptococcal immunized mice that developed behaviour and movement disturbances (Hoffman *et al.* 2004). Yaddanapudi *et al.* further demonstrated that antibodies from streptococcal immunized mice passively transferred the motor and behavioural alterations to naïve mice after the blood–brain barrier (BBB) was broken with lipopolysaccharide (Yaddanapudi *et al.* 2010). Infections themselves may be effective in opening the BBB. Brimberg *et al.* pioneered the studies of behaviour changes in the male Lewis rat after immunization to group A streptococcal antigens (Brimberg *et al.* 2012). Exposure of the Lewis rat to group A streptococcal antigens led to impaired food handling and impaired narrow, but not wide, beam walking by the rats, and rats obsessively groomed significantly longer than control rats after treatment with water mist. Serum IgG from the Lewis rats activated CaMKII signalling in a human neuronal cell line SK-N-SH similar to CaMKII activation by PANDAS or Sydenham chorea serum (Brimberg *et al.* 2012). Absorption of IgG from the serum abrogated the CaMKII signalling and activation. Serum IgG from the immunized rats reacted with human D1 and D2 dopamine receptor antigen (50KD) in Western immunoblots compared with no reactivity in control rat sera (Brimberg *et al.* 2012). Impaired food manipulation and obsessive grooming in group A streptococcal immunized rats were alleviated by treatment with the D2 blocker haloperidol or the selective serotonin reuptake inhibitor paroxetine, which are used to treat motor and compulsive symptoms in SC and PANDAS respectively (Brimberg *et al.* 2012).

Further studies in the male Lewis rat model demonstrated that when serum IgG purified from group A streptococcal immunized rats was infused into the striatum of naïve rats over a 21-day period, the rats demonstrated difficulty in walking towards a narrow beam similar to the streptococcal immunized rats and demonstrated obsessive marble burying (Lotan *et al.* 2014a) when compared to adjuvant control rats and naïve control rats. Thus, infusion of

purified IgG from streptococcal exposed rats intrathecally into naïve rats led to behavioural and motor alterations similar to those seen in streptococcal immunized rats.

In the study by Lotan *et al.*, serum antibody (IgG) confirmed reactivity with the D1 and D2 dopamine receptors and demonstrated for the first time the reactivity of antibodies (IgG) from streptococcal immunized mice with serotonin receptors 5HT-2A and 5HT-2C in Western immunoblots (Lotan *et al.* 2014a). Protein sequence similarities were found between dopamine receptors and serotonin receptors (Fig. 2). Sequence identity was found between the dopamine D1 and D2 receptors and the serotonin receptors, 5HT-2A and 2C, including three extracellular regions (loops) of D2R (Fig. 2a,b). Of the three extracellular loop regions, extracellular loop 1 (10 residues) (Fig. 2b) had the highest overall per cent sequence identity (76%) among the above receptors. Serotonin receptors 5HT-2A and 2C had the highest per cent identity (80%). Extracellular loop 3 (11 residues) (Fig. 2b) had 71% sequence identity among the receptors, with 5HT-2A and 2C again sharing the greatest per cent identity (73%) among the receptors. Overall per cent sequence identity of extracellular loop 2 (10 residues) (Fig. 2b) among all receptors analysed was 44% (60% between 5HT-2A and 2C). The finding of high sequence identity between the dopamine receptors and serotonin receptors in three extracellular regions of the receptors suggests that the same antibodies could bind to the four receptors. It is therefore possible that the antibody reactivity against D2 dopamine receptors found in GAS-related neuropsychiatric disorders might also react with other receptors sharing similar structures, thus altering not only the functioning of the dopaminergic system but also the functioning of the serotonergic system.

Most importantly in the infused rat animal model, IgG deposited *in vivo* in striatum of infused rats and colocalized with dopamine receptors, the serotonin transporter and other neuronal proteins. The rat animal model suggested that the antibodies in streptococcal immunized rats target neuronal cells in the brain, colocalize with dopamine receptors and potentially cause behavioural or movement alterations (Lotan *et al.* 2014a).

Evidence is accumulating which reinforces the link between exposure to group A streptococcal antigen and alteration of central dopamine pathways and associated movement and behavioural disorders.

Anti-dopamine receptor autoantibodies in Sydenham chorea, PANDAS and related disorders

Several studies provide supportive evidence for antineuronal autoantibodies in PANDAS, Sydenham chorea and related disorders such as Tourette syndrome (Husby *et al.* 1976, Kiessling *et al.* 1993, 1994, Singer *et al.* 1998). Successful immunosuppressive treatments including plasmapheresis, intravenous immunoglobulin (IVIG) and prednisone, which have been reported to result in suppression of acute childhood OCD and Tourette syndrome, support the autoantibody hypothesis (Kondo & Kabasawa 1978, Matarazzo 1992, Swedo 1994, Allen *et al.* 1995, Perlmutter *et al.* 1999). The search for neuronal antibody targets has been ongoing for some time and is reported in the literature (Husby *et al.* 1976, Church *et al.* 2002, 2003, Kirvan *et al.* 2003, 2006b, Singer *et al.* 2005, Dale *et al.* 2006, 2012, Pavone *et al.* 2006, Brilot *et al.* 2011, Mohammad *et al.* 2013, Pathmanandavel *et al.* 2013,

Ramanathan *et al.* 2013). Most recently, the dopamine D1 and D2 receptors were reported in Sydenham chorea and PANDAS (Brimberg *et al.* 2012, Cox *et al.* 2013) and by Ben Pazi *et al.* where the ratio of anti-D1/anti-D2 receptor antibodies correlated with USCRS symptoms (Ben-Pazi *et al.* 2013). These antibody-targeted antigens namely, dopamine receptors D1R and D2R, are key components in the regulation of the dopaminergic pathways which are considered to be the source of the chorea and behavioural symptoms. Consequently, both are successfully targeted with antidopaminergic drugs (Shannon & Fenichel 1990, Sokol 2000, Dale 2005, Demiroren *et al.* 2007, Moretti *et al.* 2008).

Children with PANDAS-like conditions can be categorized into at least 2 groups: those with abnormally elevated antibodies against the D2L receptor (Brimberg *et al.* 2012, Cox *et al.* 2013), which include Sydenham chorea and the first 50 cases of reported PANDAS (Swedo *et al.* 1998), and a second group without abnormally elevated autoantibodies against the D2L receptor. In the second group with chronic tics and/or OCD, antibodies may be abnormally elevated against D1R and/or lysoganglioside antigens (Cox *et al.* 2015, Singer *et al.* 2015). Both groups have markedly elevated activity in serum which signals the CaMKII in human neuronal cells. In our study of over 260 children, sera IgG from chronic OCD, tics or both reacted significantly with human D1 receptor or lysoganglioside antigens and had significantly elevated CaMKII activation compared with normal controls (Cox *et al.* 2015). Chronic tics and OCD did not react above normal levels with D2L receptor (Cox *et al.* 2015, Singer *et al.* 2015). This is in contrast to cases of PANDAS with fine piano playing choreiform movements (Swedo *et al.* 1998) which demonstrate abnormally elevated anti-D2R antibodies. In our group of over 260 youth and young adults, chronic OCD and/or tics was positively associated with streptococcal infection status (Cox *et al.* 2015) and with autoantibodies against neuronal antigens, D1R and lysoganglioside. The antibody-mediated neuronal cell signalling of CaMKII activation in human neuronal cells in these diseases suggests that the antibodies may target the receptors and alter dopamine neurotransmission leading to neuropsychiatric symptoms.

Finally, as Sydenham chorea is the established neurologic manifestation of group A streptococcal induced rheumatic fever, it may be a prototype for other group A streptococcal or infection-related movement and neuropsychiatric conditions. Evidence suggests that human anti-D2R titres in Sydenham chorea correlated with antistreptolysin O titres, a common serologic test for diagnosis of prior group A streptococcal infection, which strengthens the hypothesis that the streptococcal infection may lead to anti-D2R antibody production ultimately causing clinical symptoms (Ben-Pazi *et al.* 2013). Furthermore, evidence from animal models supports the hypothesis that streptococcal infections are associated with the induction of antibodies against the dopamine receptors in human disease.

Figure 3 summarizes the evidence for the role of streptococcal infections in the induction of antibodies that may lead to movement or neuropsychiatric disorders. The pathogenesis of Sydenham chorea and PANDAS begins with an infection with group A streptococci in the throat or soft tissues or skin. The susceptible host may produce antibodies which react with brain tissues and in particular the dopamine receptors or neural antigens such as tubulin or lysoganglioside (Kirvan *et al.* 2003, 2006a,b, 2007)(Ben-Pazi *et al.* 2013, Cox *et al.* 2013). Antibodies target primarily the basal ganglia, in particular the ventral tegmental area or the

substantia nigra which project to the striatum. The mechanism of the antibodies as shown would signal the neuronal cell through activation of CaMKII and lead to excess dopamine release. Binding of high-avidity cross-reactive antistreptococcal/antineuronal antibodies to lysoganglioside or dopamine receptors on the surface of neurones may lead to alterations in dopamine transmission. Previous studies suggest that intrathecal passive transfer of antibodies leads to increased tyrosine hydroxylase activity in neurones which is in the CaMKII pathway as shown in Figure 3 and also leads to neuropsychiatric symptoms in the animals receiving the passive transfer of antibody (Kirvan *et al.* 2006a).

Future and ongoing studies will investigate the possible receptors and mechanisms which may directly affect antibody-mediated CaMKII activation in human neuronal cells and the role of other types of receptors such as the serotonin receptor which may play a role in disease as well as the dopamine receptors and the autoantibodies that signal them. Studies already in progress suggest that antibody binding to the dopamine D1 receptor in transfected cells leads to sensitization of the receptor to the neurotransmitter dopamine (J. Zuccolo, E.V. Edwards, A.J. Zuccolo, A.I. Mascaro-Blanco, H. Ben-Pazi, S.E. Swedo & M.W. Cunningham, Manuscript in preparation). In addition, further studies of the role and effects of cytokines and T cells and their responses on the brain will all be important areas for future studies. Affects of the immune system on the brain may not be limited to the neurones directly, but may also affect microcirculation and blood vessels in the brain and lead to damage to the brain due to vasculitis and fibrosis with blockage of the blood supply to certain regions of the brain.

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Expressed 24.3.1 IgG1^a Ab binds dopaminergic neurons *in vivo*
Co-localization of anti-mouse IgG1^a(FITC-labeled) and Tyrosine Hydroxylase
(TH) Ab (TRITC-labeled) in Tg mouse brain

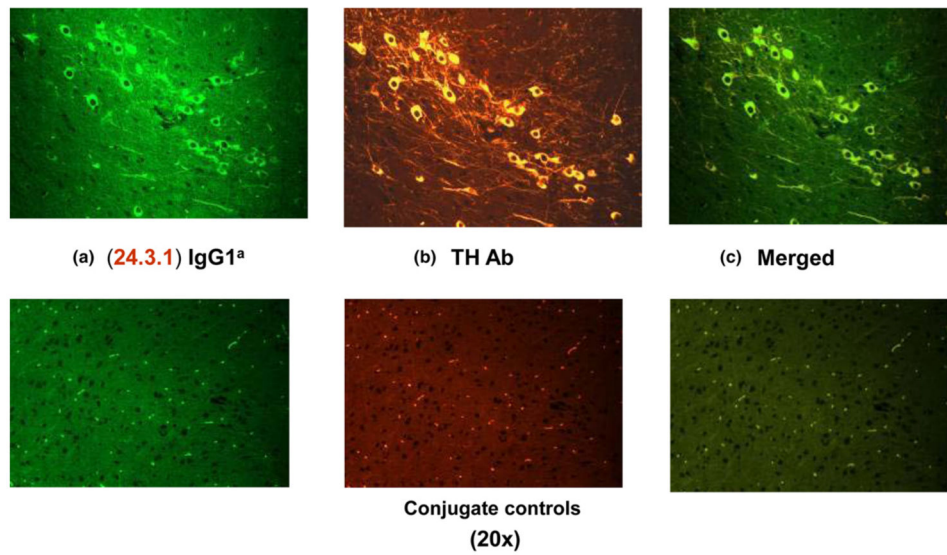
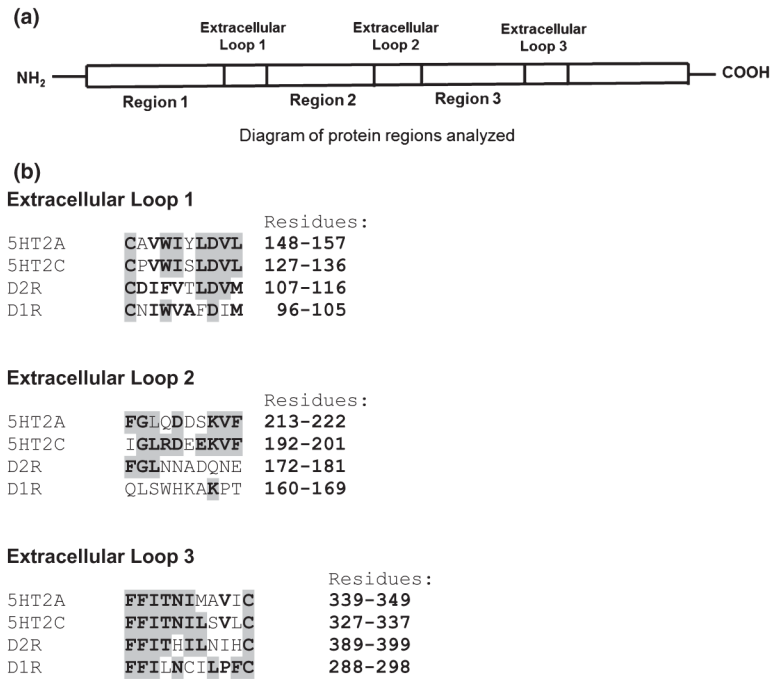


Figure 1.

Human Sydenham chorea 24.3.1 V gene expressed as human V gene-mouse. IgG1a constant region in transgenic (Tg) mice targets dopaminergic neurons. Chimeric Tg24.3.1 VH IgG1a Ab expressed in Tg mouse sera penetrated dopaminergic neurons in Tg mouse brain *in vivo*. Colocalization of Tg 24.3.1 IgG1a (anti-IgG1a Ab, green, left panel) and tyrosine hydroxylase antibody (anti-TH Ab, yellow, middle panel). TH is a marker for dopaminergic neurons. Left panel shows IgG1a (FITC-labelled), centre panel shows TH Ab (TRITC-labelled), and right panel is merged image (FITC-TRITC). Brain sections (basal ganglia) of VH24.3.1 Tg mouse (original magnification 320), showing FITC-labeled anti-mouse IgG1a (a), TRITC-labelled anti-TH Ab (b) and merged image (c). Colocalization by double immunostaining used Abs conjugated to FITC and TRITC. For localization of chimeric Tg24.3.1 VH IgG1a, primary Ab was biotin-conjugated mouse anti-mouse IgG1a (BD Pharmingen) which was used in combination with FITC-conjugated streptavidin (Invitrogen). For immunostaining of dopaminergic neurons, rabbit polyclonal Ab (Abcam) was used as primary Ab with TRITC-conjugated sheep anti-rabbit Ab as the secondary Ab (Sigma). Conjugate controls, secondary Ab controls (secondary Ab, no primary Ab) shown: FITC-conjugated streptavidin (1 : 20) (left panel) and TRITC-conjugated sheep anti-rabbit Ab (1 : 100) (middle panel); right panel is merged image (FITC-TRITC) (Cox *et al.* 2013).

**Figure 2.**

Protein sequence similarities between dopamine D1 and D2 receptors and serotonin receptors. Protein sequence alignments were performed between 5HT-2A and 2C serotonin receptors and dopamine receptors D1 and D2. (a) is a diagram of protein regions analyzed, including three extracellular regions (extracellular loop 1, extracellular loop 2, extracellular loop 3) of D2R. (b) shows residues analysed in each extracellular loop (1, 2 and 3). Identical residues are indicated in bold and highlighted (grey). Protein sequence alignments were performed using the basic local alignment search tool analysis program (BLAST) (National Center for Biotechnology Information) and WUR MUSCLE multiple alignment analysis program (www.bioinformatics.nl/tools/muscle.html).

Pathogenic Mechanisms In Sydenham Chorea and PANDAS

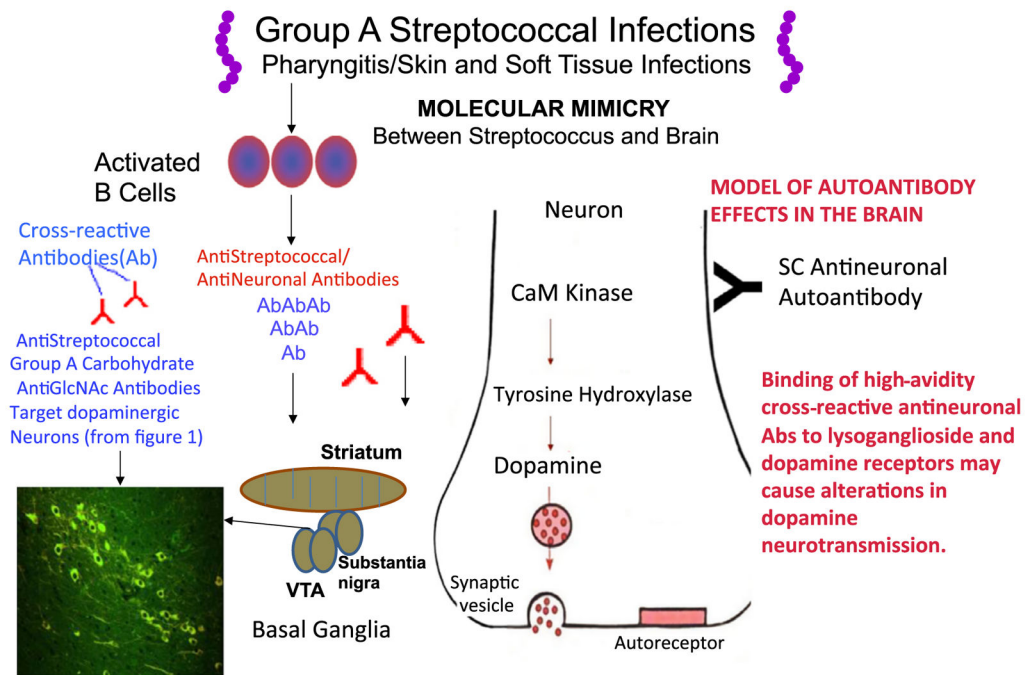


Figure 3.

Summary of evidence for the role of streptococcal infections in the induction of antibodies that may lead to movement or neuropsychiatric disorders. The pathogenesis of Sydenham chorea and PANDAS begins with an infection with group A streptococci in the throat or soft tissues or skin. The susceptible host may produce antibodies which react with brain tissues and in particular the dopamine receptors or neural antigens such as tubulin or lysoganglioside (Kirvan *et al.* 2003, 2006a,b, 2007, Ben-Pazi *et al.* 2013, Cox *et al.* 2013). Antibodies target primarily the basal ganglia, in particular the ventral tegmental area or the substantia nigra which project to the striatum. The mechanism of the antibodies as shown would signal the neuronal cell through activation of CaMKII and lead to excess dopamine release. Binding of high-avidity cross-reactive antistreptococcal/antineuronal antibodies to lysoganglioside or dopamine receptors on the surface of neurons may lead to alterations in dopamine transmission.