Review

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The diagnostic utility of folate receptor autoantibodies in blood

Abstract

Folate supplementation reduces the risk of neural tube defect (NTD) pregnancy, and folinic acid has been used to correct cerebral folate deficiency (CFD) in children with developmental disorders. In the absence of systemic folate deficiency, the discovery of autoantibodies (AuAbs) to folate receptor α (FRα) that block the uptake of folate offers one mechanism to explain the response to folate in these disorders. The association of FRα AuAbs with pregnancy-related complications, CFD syndrome, and autism spectrum disorders and response to folate therapy is highly suggestive of the involvement of these AuAbs in the disruption of brain development and function *via* folate pathways. The two types of antibodies identified in the serum of patients are blocking antibody and binding antibody. The two antibodies can be measured by the specific assays described and exert their pathological effects either by functional blocking of folate transport as previously shown or hypothetically by disrupting the FR by an antigen-antibody-mediated inflammatory response. We have identified both IgG and IgM AuAbs in these conditions. The predominant antibodies in women with NTD pregnancy belong to the IgG1 and IgG2 isotype and in CFD children, the IgG1 and IgG4 isotype. This review describes the methods used to measure these AuAbs, their binding characteristics, affinity, cross-reactivity, and potential mechanisms by which folate therapy could work. Because these AuAbs are associated with various pathologies during fetal and neonatal development, early detection and intervention could prevent or reverse the consequences of exposure to these AuAbs.

Keywords: autism; autoantibodies; brain development; cerebral folate deficiency; folate receptor; pregnancy.

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Introduction

Folate, in its various reduced forms, participates in numerous single-carbon exchange reactions that are essential for purine/DNA/pyrimidine synthesis and amino acid metabolism [1]. The absorption of folate in the gut is primarily mediated by a proton-coupled folate transporter in the upper ileum [2], and the cellular uptake of folate is mediated by folate receptor α (FRα) [3]. This receptor is highly expressed in reproductive tissues and plays the all-important role of providing folate to the embryo during the critical stages of development [4]. Folate deficiency in the mother can lead to pregnancy-related complications including neural tube defects (NTDs) in the fetus. Numerous studies have now established the benefits of folate supplementation in reducing the incidence of NTD pregnancy [5–7]. The benefits of folate supplementation in the absence of systemic folate deficiency or genetic abnormalities of folate pathways have not been adequately explained. The identification of FR autoantibodies (AuAbs) in women with a history of NTD pregnancy provides a mechanism by which the developing embryo could be deprived of folate [8]. Although the cause of this autoimmune disorder in mothers remains unknown, the AuAb directed against the FRα could disrupt folate transport to the fetus. This first report has been confirmed by two independent studies [9, 10]. However, our own larger study in an Irish population failed to establish a statistically significant correlation of NTD pregnancy with the presence of FRα antibodies [11]. Nevertheless, the prevalence of the FRα AuAb and NTD pregnancy in Ireland remains higher than in the rest of Europe and the USA. These FRα AuAbs are also associated with numerous developmental disorders in children such as cerebral folate deficiency (CFD) syndrome [12], Rett syndrome [13], low functioning autism with neurological

deficits [14], and autism spectrum disorders (ASD) [15, 16]. The AuAbs could exert their effect either by blocking folate transport or potentially by an antibody-mediated immune reaction. In the absence of gross fetal abnormalities, exposure to these AuAbs during fetal development or in early infancy could disrupt the structural refinement of the brain and cause functional deficits in later life. These observations further attest to the importance of folate status during early brain development and to the identification of factors that could disrupt this essential need. Early detection and prompt treatment to correct the deficits could prevent or reverse the neurodevelopmental disorders due to FRα autoimmunity and folate deficiency.

Discovery of FRα AuAbs

Folate deficiency in humans leads to hyperhomocysteinemia and megaloblastic anemia [1]. The rapid onset of the deficiency and the severity of the hematological abnormality present clinically as anemia and the condition is promptly diagnosed and treated. In adults, neurological deficits such as that seen in B12 deficiency are normally not associated with acquired folate deficiency other than some reports of neurological deficits and dementia in the elderly population [17, 18]. Maternal folate deficiency has been associated with pregnancy-related complications including miscarriages and birth defects [19]. Chemotherapeutic drugs that interfere with folate metabolism [20] and many autoimmune disorders [21] are also implicated in pregnancy-related complications. Reports of the effects of folate deficiency on embryonic development and functional deficits have been gathered primarily from animal models of dietary folate deficiency [22, 23] and, more recently, from mouse gene knockout models [24, 25]. Antibodies to various tissue proteins can be teratogenic to the developing embryo, as amply demonstrated in rat models [26]. We hypothesized that perhaps an autoimmune mechanism involving the primary transporter of folate to the fetus could play a role in fetal brain abnormalities, and therefore, a systematic analysis of FRα expression and the effects of an antibody to this protein was investigated in a rat model [4]. Immunohistochemical localization of FRα in the rat with a polyclonal rabbit antiserum to rat placental FRα demonstrated high expression in reproductive tissues and in the developing embryo. Subsequent studies with pregnant dams showed that the antiserum induced embryonic resorptions at higher doses and malformations or no gross structural defects at lower doses when administered on gestation day 8 [4]. These effects were preventable with the use of pharmacologic doses of folinic acid, suggesting

that the antibodies may be blocking the folate uptake *via* the FRα, leading to folate deficiency in the embryo and to the ensuing pathology. The rescue with folinic acid, which is transported *via* the reduced folate carrier (RFC), suggested that the deleterious effects of the antibodies could be prevented by treating with adequate amounts of folinic acid. This suggested that if the AuAbs to the FR in women with NTD pregnancy are contributing to the fetal abnormalities, these could be rescued by folinic acid treatment. Subsequent analysis of human serum samples demonstrated the presence of FRα AuAbs in women with a history of an NTD pregnancy [8]. The properties of these antibodies were confirmed by the high-affinity binding to FRα (K_a=10^9–10^{10} L/mol) and by their ability to block folate uptake in FRα-expressing KB cells in culture [8].

The observation of severely reduced cerebral spinal fluid (CSF) folate levels in children with the CFD syndrome prompted the analysis of serum from these children for the presence of FRα AuAbs, which were found in 89% of the patients with this disorder. Intervention with pharmacologic doses of folinic acid normalized the CSF folate levels with clinical improvement [12]. Because a number of the patients with CFD showed symptoms that were similar to those associated with ASD, we evaluated patients with low functioning autism and identified FRα-blocking antibodies in 76% of these patients [14]. This analysis was subsequently extended to children with ASD. In the American ASD population, more than 75% of the children had FR AuAbs, and there was a significant correlation between those with blocking AuAbs and those with lower CSF folate concentrations [15]. Treatment with folinic acid over 4 months showed a significant improvement in verbal communication, receptive and expressive language, attention, and stereotypical behavior. An independent study conducted in Belgium provided similar results for the FRα-blocking antibody and, in addition, showed that parental FRα AuAbs were associated with an increased risk for ASD [16]. This review provides data on the detailed characterization of FRα AuAbs and methods for the measurement of these antibodies in serum to diagnose FRα autoimmune disorders along with a discussion of published reports.

Methods

Identification of AuAb isotypes

Apo-FRα (FRα without bound folate), purified from human milk using a protocol previously described for human placental FR [8, 12], was covalently attached in

96-well maleic anhydride-coated plates (Pierce™). Unreacted sites were blocked with goat serum, and an aliquot of test serum or plasma was added and incubated overnight at 4°C. The presence of bound human AuAbs was detected by complexing with a secondary peroxidase-conjugated goat antibody against human IgG or human IgM (Vector Laboratories™), followed by a colorimetric reaction with tetramethylbenzidine. The determination of the IgG isotypes was performed in a similar assay using biotin-conjugated secondary monoclonal antibodies against human IgG1, IgG2, IgG3, or IgG4 (Sigma™). After incubation with an avidin-peroxidase complex (Vector Laboratories™), a colorimetric reaction with tetramethylbenzidine identified an AuAb isotype bound to the FRα.

Immunological cross-reactivity of FRα antigen derived from animal sources

FRα from human, bovine, goat, and camel milk was used as the antigen to measure the immunoreactivity of FR antigen with the human AuAb in a functional blocking assay whereby the binding of Ab to FRα prevents the subsequent binding of [³H] folic acid to FRα. In this assay, 0.3 pmol of each FRα antigen was incubated with acid/charcoal-treated serum (*vide infra*) that was titrated to block approximately 0.1 pmol of human milk FRα (used as reference). After overnight incubation at 4°C, [³H] folic acid was added for 20 min at 25°C. Unbound folic acid was adsorbed to dextran-coated charcoal (*vide infra*), and the amount of bound [³H] folic acid FRα in the supernatant was determined. The decrease in bound [³H] folic acid represents the amount of FRα blocked by the AuAb.

Immunoreactivity of AuAb with native and denatured FRα

Purified human milk FRα was denatured using urea and DTT, with a temperature of 50°C, followed by alkylation with *n*-ethylmaleimide to prevent refolding. Urea and DTT were removed by dialysis, and the denaturation of FRα was confirmed by a complete loss of [³H] folic acid binding to the protein. The denatured FRα was then tested for immunoreactivity against the native receptor in the blocking FRα AuAb assay (*vide infra*). Immunological cross-reactivity was also tested in an ELISA-based assay for binding AuAbs. Acid/charcoal-treated serum samples (*vide infra*) to remove endogenous folate were incubated with 100-fold excess native FRα or with FRα denatured overnight at 4°C and added to maleic anhydride-coated ELISA plates containing covalently bound native FRα.

The same serum samples without preincubation with excess native, or denatured FRα were used as positive controls, and AuAb-negative serum samples were used to correct for any non-specific reactivity in the assay.

Determination of optimum binding of AuAb to FRα and affinity constant

Acid/charcoal-treated serum from subjects positive for blocking AuAb were incubated with 0.3 pmol of purified apo-FRα from human milk. At various periods (1–24 h), an aliquot was removed and tested for the quantity of the remaining apo-FRα by incubating it with [³H] folic acid for 20 min followed by the measurement of bound [³H] folic acid. The decrease in apo-FRα fraction compared with the FRα sample lacking AuAb indicated the inhibition of [³H] folic acid binding by the blocking AuAb.

For the determination of the affinity of the AuAb, increasing amounts of apo-FRα purified from human milk were incubated overnight at 4°C with a constant amount of serum containing AuAbs. [³H] Folic acid was added, and the bound folate was subtracted from the total folate binding capacity of the receptor to determine the quantity of receptors blocked (in picomoles) by the antibody. The ratio of the blocked receptor to the free apo-receptor (B/F) was used for the Scatchard analysis of the binding data.

Displacement of bound AuAbs from FR by folate

To determine the form and concentration of folate needed to displace the bound AuAb from FRα, varying concentrations of folic acid or 5-methyl folate (20–800 nM) were used to determine the concentration required to displace the FRα-bound AuAb. Acid/charcoal-treated serum samples containing blocking IgG AuAb were added to a maleic anhydride ELISA plate with covalently attached FRα. After overnight incubation at 4°C, various concentrations of either folic acid or 5-methyl folate were added to the wells, and the reference (control) wells contained no added folate. After incubation for 2 h at 25°C, the wells were washed, and the quantity of the blocking IgG AuAb remaining bound to the FRα in each well was determined by incubating with a second peroxidase-conjugated anti-human IgG antibody for 1 h at 25°C and a colorimetric reaction with tetramethylbenzidine. Any reduction in IgG bound, compared with the reference wells lacking added folate, represented the displacement of bound AuAb by the specific folate form used.

Current methodology of binding and blocking AuAb assays

Although the initial identification of the FRα AuAbs was done by isolating IgG in the patient's serum that bound [3H] folic acid-labeled FRα, technical refinements were deemed necessary to analyze larger number of samples, and therefore, a functional blocking assay was developed that specifically identifies antibodies that, by virtue of their binding location, prevent the binding of folate and therefore prevent the transport of folate *via* the FRα. A second assay developed for the detection of binding antibodies utilized an ELISA format whereby apo-FRα immobilized in an ELISA plate assay would capture any IgG that would bind to the protein and could be quantitatively identified using a peroxidase enzyme-linked second antibody and a colorimetric substrate.

Assay for blocking AuAbs to FRα

The testing for blocking AuAbs against FRα was performed by measuring the blocking of radiolabeled folic acid binding to a known amount of purified FRα from human milk as previously described [8]. Briefly, 200 μL of serum was acidified with 300 μL of 0.1 M glycine/HCl, pH 2.5/0.5% Triton X-100/10 mM EDTA and was added to 12.5 mg of dextran-coated charcoal pellet to remove the free folate in the sample. After centrifugation, the supernatant was collected and the pH was neutralized with 40 μL of 1 M dibasic sodium phosphate. This sample was incubated overnight at 4°C with 0.34 pmol of apo-FRα purified from human milk. [3H] Folic acid was added, and the mixture was incubated for 20 min at room temperature. Free [3H] folic acid is then adsorbed to dextran-coated charcoal (5% activated charcoal, 1% dextran in 0.1 M sodium phosphate buffer, pH 7.4), and the receptor-bound radioactivity in the supernatant fraction was determined. Blocking AuAbs prevent the binding of [3H] folic acid to FRα, and the AuAb titer is expressed as picomoles of FRα blocked per milliliter of serum. The blocking Ab could be either IgG or IgM, and this method does not identify any specific antibody type.

Assay for binding AuAbs to FRα

An ELISA-based measurement was used to determine the presence of IgG immunoglobulins that bind to epitopes on purified apo-FRα. In this assay, apo-FRα purified from human milk was immobilized in 96-well maleic anhydride plates (Pierce). Unreacted sites were blocked with goat serum. Aliquots of plasma or serum were added and incubated overnight at 4°C. The presence of AuAbs was detected by complexing with a second peroxidase-conjugated goat antibody against human IgG, followed by a colorimetric reaction with tetramethylbenzidine. The AuAb titer (IgG) was quantified from a standard curve using known amounts of human IgG captured in a protein A-coated ELISA plate and color development as described above. Non-specific binding was determined using negative control serum samples. Data are presented as 'binding AuAbs' and expressed as picomoles of FRα binding IgG per milliliter of serum.

Results and discussion

Properties of FRα AuAbs

Stability of AuAbs upon storage

The first study identifying FRα AuAbs was done with fresh serum samples [8]. However, subsequent studies in both NTD pregnancy and CFD syndromes were done with serum samples kept frozen for various periods. Because the stability of proteins in a biological sample is a valid concern, we performed both blocking and binding assays on samples stored under two different conditions. Serum samples containing varying titers of AuAbs to FRα were aliquoted and stored at either 4°C or –20°C. At various time intervals (1, 85, 155, 266, 337, and 407 days), an aliquot was assayed for blocking and binding AuAb to FRα (Figure 1). About half the samples stored at 4°C showed some decrease in blocking Ab titer over time. However, the decrease amounted to <45% of the initial mean value after more that 10 months. This decrease was much less for samples stored at –20°C, in that only 30% of the samples showed a decrease in blocking, with the decrease amounting to <25% of the initial mean value. A similar analysis of binding Ab showed a decrease in titer in 42% of the samples, with the mean decrease amounting to 33% of the initial value in samples stored at 4°C. As with the blocking Ab, the binding Ab was more stable when stored at –20°C, with only 8% of the samples showing any decrease in titer. This decrease amounted to 24% of the initial mean value. In no case did the Ab titer change from positive to negative. From these data, it was clear that the FRα antibody in serum was relatively stable under long-term storage conditions and could be used for future studies.
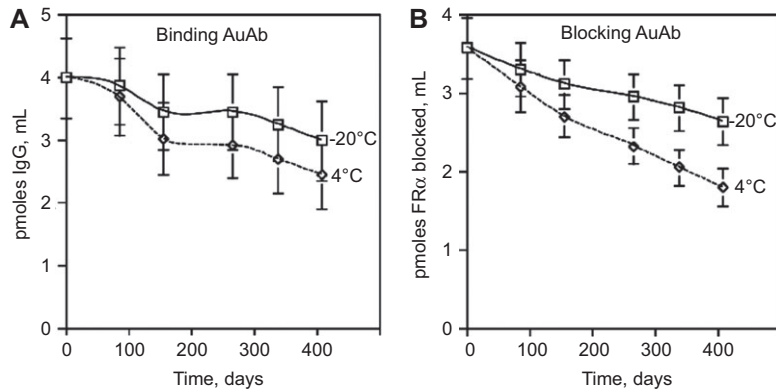
Figure 1 Effect of storage condition on stability of AuAb titer in serum.
Samples were assayed for binding (n=11) and blocking (n=20) antibody at various times following storage at 4°C or −20°C, which showed greater stability when stored at −20°C.

Specific AuAb types in FR autoimmune disorders

The initial trigger for FRα AuAb production in these disorders is not known, and therefore, to better understand the immune response, the specific type of immunoglobulin produced was identified using isotype-specific monoclonal antibodies. As shown in Table 1, all subjects with this autoimmune condition had IgG antibodies, with IgG1 as the predominant isotype. Mothers with NTD pregnancy (40%) and ASD subjects (14%) also contained IgG2; CFD (21%) and ASD (7%) subjects also had IgG3 isotype. Although the occurrence of IgG4 is rare, 79% of the CFD subjects and 14% of the ASD subjects had this isotype. The conversion to IgG4 is believed to occur as a result of repeated and frequent exposure to an antigen. A number of patients (26%–28%) also had IgM antibodies with blocking activity, and it is likely to be the only antibody type in patients who are negative for binding Ab because binding Ab assay only detects IgG. In CFD subjects, one factor contributing to the increase in antibody titer is the daily consumption of animal milk, which contains substantial amounts of FRα (Figure 2A). Bovine milk contains 1–3 μg/mL FRα,

and a compromised immune barrier in the gut either due to infection and/or inflammation appears to present the antigen to the immune system to mount a response. The substantial similarity in the primary structure of FRα from animal origin with that of human FRα provides the epitopes for molecular mimicry to produce antibodies that cross-react. This is evident from the cross-reactivity of FR AuAbs with milk-derived FRα of animal origin (Figure 2B). This hypothesis is further supported by the dramatic decrease in antibody titer in CFD patients maintained on a dairy-free diet [27]. However, patients with ASD who have been kept off milk for various reasons still show antibody titers that are considered elevated. Non-compliance to a strict dairy-free diet may partially contribute to the persistent Ab titer in ASD. Camel milk has been touted as beneficial in ASD. Unfortunately, the FRα from camel milk is highly reactive with the AuAb (Figure 2B) and is likely to increase the antibody titer, as we have observed in cases when camel milk was introduced (unpublished data).

Binding characteristics of the AuAbs

In developing assays for the detection of blocking and binding antibodies, the time of incubation for optimum binding has to be considered. As shown in Figure 3A, optimum binding required overnight incubation at 4°C, and the long incubation time required may be a function of the antibody and antigen concentration in the incubation reaction. However, the binding is a high-affinity interaction in the nanomolar range, as shown in Figure 3B. This is consistent with the affinity constants of 10^9–10^{10} L/mol that we have previously reported [8, 12].

Another property of the antibodies relevant to the assay development was the inability of the antibodies to react with

Condition	IgG	Percent IgG isotypes				IgM
		IgG1	IgG2	IgG3	IgG4	
NTD (n=7)	100	100	40	0	0	28
CFD (n=19)	100	68	0	21	79	5
ASD (n=78)	74	100	14	7	14	26

Table 1 Identification of antibody type and IgG isotype in subjects positive for the folate receptor autoantibody.
NTD, mothers with a history of neural tube defect pregnancy; CFD, children with cerebral folate deficiency syndrome; ASD, children with autism spectrum disorder.
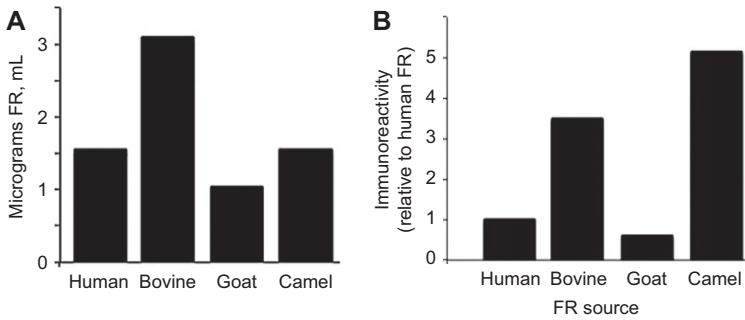
Figure 2 FRα concentration in milk from different species and cross-reactivity of blocking FR AuAb from human serum with these antigens: (A) concentration of FRα from different species and (B) immunological cross-reactivity of FRα AuAb against each of the FRα antigens. The data are plotted as cross-reactivity of antigens relative to human FRα. The AuAb shows higher cross-reactivity with bovine and camel milk FRα, which are sources of milk for human consumption.

denatured and linearized polypeptide of FR in the ELISA assay, which supports the notion that the AuAbs are conformation specific and can bind to the native protein with or without the folate attached (Figure 4A). Similarly, the denatured FRα protein does not react in the blocking assay, indicating the need for a fully functional native protein in this assay as well (Figure 4B). Therefore, synthetic peptides or truncated protein fragments are unlikely to be suitable antigens in future assay developments for FRα AuAbs.

In addition to decreasing the antibody titer, the treatment of choice in these autoimmune disorders has been to administer daily pharmacologic doses of folinic acid or methylfolate orally to restore the CSF folate and correct the CFD. These two reduced forms of folates can be transported by alternate routes such as the high-capacity/low-affinity RFC, which can transport these folates when the local concentration is increased. An empirical dose of 0.5–2 mg of folinic acid or 0.1–0.25 mg of methylfolate per kilogram of body weight for the two reduced form of folates has been reached by trial and error. The advantage

of administering 5-methylfolate is that, in addition to being transported *via* the RFC, a high local concentration of methylfolate, which also binds to the FRα with relatively high affinity, could effectively dissociate the AuAb from the FRα and prevent AuAb from binding to the FRα while it is transporting the methylfolate *via* this receptor. However, this form of folate has to be processed *via* the methionine synthase pathway for folate-dependent reactions. The advantage of using folinic acid is that it is an approved drug tested for use at high doses in other conditions and is readily available for folate-dependent reactions. Because a fraction of the folinic acid can be converted to methylfolate during absorption in the gut, it can increase the local concentration of folinic acid as well as methylfolate. The ability of methylfolate to displace the bound antibody from FRα, tested using Ab-positive serums, showed that 50 nM 5-methylfolate was able to displace more than 80% of the bound Ab (Figure 5A). Under identical conditions, folic acid was not as effective and needed an 8-fold higher concentration even though it has
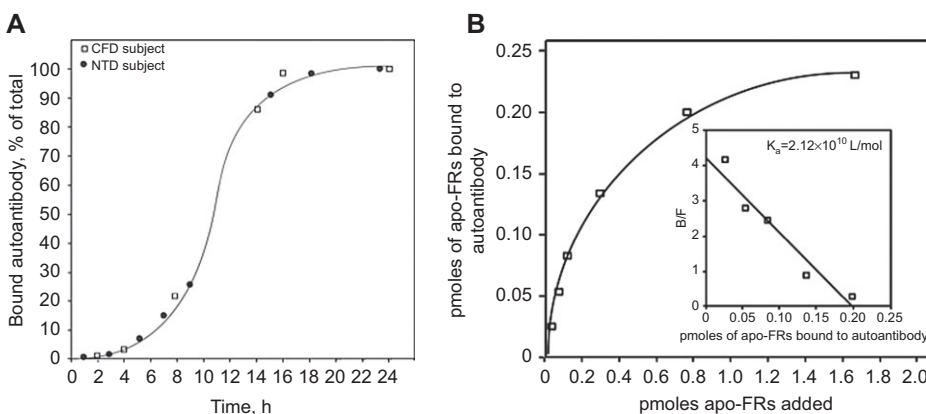
Figure 3 Time course of blocking AuAb binding to FRα from human milk and binding affinity. Square symbols represent data with AuAb from a CFD subject, and the circles represent data with AuAb from a mother with a history of an NTD pregnancy. Optimum complex formation required overnight incubation (A). However, a Scatchard analysis of the binding data indicated high-affinity binding (B).
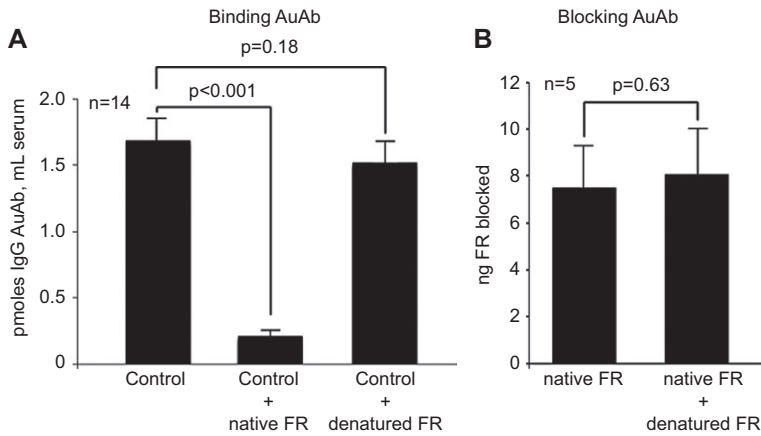
Figure 4 Binding of AuAb to native and denatured FRα.
The lack of neutralization of the antigen-antibody complex formation by denatured FRα and the effective neutralization by native FRα indicate that conformation of the native protein is necessary for epitope recognition by the AuAb.

a high affinity for FRα (Figure 5B). As previously reported [3], folinic acid binds weakly to FRα and therefore cannot displace the antibody. Based on the K_t of approximately 5 nM for transport of methylfolate *via* the FRα [28] and a concentration of 50 nM required to displace the AuAb, an oral dose sufficient to increase the folate concentration in the circulation to 50–100 nM would be sufficient to normalize the CSF folate status. Because of the K_t of approximately 5 μM for folinic acid transport *via* the RFC [29], the folinic acid dose would have to be considerably higher to increase the concentration of this folate to the 10- to 50-μM range. The conversion of a fraction of this during intestinal absorption would also provide some methylfolate to displace the AuAb and be transported *via* the FRα.

Using the current assays described in the Methods section, we have analyzed serum samples from disorders linked to pregnancy and fetal abnormalities to

neurodevelopmental disorders in children, and the results are summarized in Table 2.

The significant association of FRα AuAbs with NTD pregnancy has been confirmed by three independent studies [8–10]. The lack of a significant association of FRα antibodies with NTD pregnancy in the Irish population [11], despite the higher prevalence of the antibody and the higher incidence of NTD pregnancy than in the US population, points to other compounding factors. What additional genetic and epigenetic factors contribute to the NTD outcome in different populations? Whether FRα AuAbs contribute to the pathology in the Irish population is yet to be determined. However, the association of FRα AuAbs with neurodevelopmental disorders including CFD syndromes and ASD is very strong, and treatments to reduce the antibody titer and inflammation along with pharmacologic doses of folinic acid to restore the CSF and
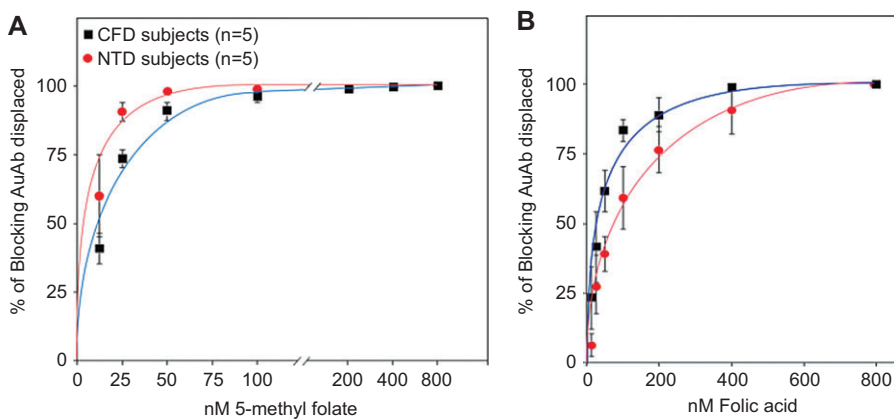
Figure 5 Displacement of blocking AuAb from FRα by 5-methyl folate (A) and folic acid (B).
A preformed FR-AuAb complex was incubated with increasing amounts of folate to determine the lowest concentration of folate needed to displace most of the AuAb. Square symbols represent data obtained with AuAb from CFD subjects, and the circles represent data obtained with AuAb from mothers with a history of an NTD pregnancy.

Condition	Blocking AuAb	Binding AuAb	Percent prevalence Control		Reference
			Blocking	Binding	
NTD (n=12)	–	75	–	10	8
NTD (n=103)	17	30	13	33	11
CFD (n=28)	89	–	0	–	12
RS (n=33)	24	–	–	–	13
LFA (n=25)	76	–	0	–	14
ASD (n=93)	60	44	–	–	15
ASD (n=75)	46	–	3	–	16

**Table 2** Prevalence of FR AuAbs in various conditions.
NTD, mothers with a history of neural tube defect pregnancy; CFD, children with cerebral folate deficiency syndrome; LFA, children with low-functioning autism; ASD, children with autism spectrum disorder; RS, children with Rett syndrome.

cerebral folate levels have been extremely beneficial in correcting and preventing neurological deficits.

As we age, the immune system also changes, leading to immunosenescence, and therefore, AuAbs to many antigens are prevalent in the elderly population [30, 31]. This trend is also seen for blocking FRα AuAbs, whose prevalence increases from <2% in those younger-than-16-years age group to approximately 10% in the 18-to-35-years age group, and with approximately 25% of the older-than-65-years age group testing positive for the antibody (Figure 6). The significance of the increase in antibody titer in the elderly population, and if this contributes to CFD and dementia or decrease in brain function and if this population would benefit from folate supplementation, needs to be evaluated.

Utility of FR AuAb testing

The identification of FR AuAbs and its association with pregnancy-related complications as well as neuro-
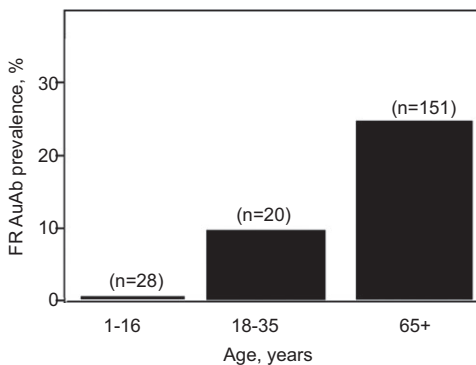
Figure 6 Prevalence of AuAb to FRα with aging.
Serum samples from subjects of different age groups with no known disease condition were analyzed for the presence of blocking AuAb to FRα.

developmental disorders adds a whole new dimension to evaluating and treating folate deficiency-related disorders. It highlights the role of folate in embryonic and neural development. The association of FRα AuAbs in a majority of ASD children and one or both parents and significant improvement in the core clinical symptoms of ASD with folinic acid therapy further highlights the role of folate in structural and functional integration of the developing brain. Even though we do not understand the molecular basis for the FR autoimmune disorder, identifying women and children at risk could be accomplished by a simple blood test. Considering the risk to fetal as well as neonatal brain development, early detection and intervention is likely to yield the best outcome. Population studies involving children, women, and men of all ages should be initiated to evaluate the prevalence, association, and risk of FRα AuAbs.

Conflict of interest statement

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