IMMEDIATE COMMUNICATION

Brain-reactive IgG correlates with autoimmunity in mothers of a child with an autism spectrum disorder

L Brimberg1, A Sadiq1, PK Gregersen2 and B Diamond1

It is believed that in utero environmental factors contribute to autism spectrum disorder (ASD). The goal of this study was to demonstrate, using the largest cohort reported so far, that mothers of an ASD child have an elevated frequency of anti-brain antibodies and to assess whether brain reactivity is associated with an autoimmune diathesis of the mother. We screened plasma of 2431 mothers of an ASD child from Simon Simplex Collection and plasma of 653 unselected women of child-bearing age for anti-brain antibodies using immunohistology on mouse brain. Positive and negative plasma from mothers with an ASD child were analyzed for anti-nuclear antibodies and for autoimmune disorders. Mothers of an ASD child were four times more likely to harbor anti-brain antibodies than unselected women of child-bearing age (10.5 vs 2.6%). A second cohort from The Autism Genetic Resource Exchange with multiplex families displayed an 8.8% prevalence of anti-brain antibodies in the mothers of these families. Fifty-three percent of these mothers with anti-brain antibodies also exhibited anti-nuclear autoantibodies compared with 13.4% of mothers of an ASD child without anti-brain antibodies and 15% of control women of child-bearing age. The analysis of ASD mothers with brain-reactive antibodies also revealed an increased prevalence of autoimmune diseases, especially rheumatoid arthritis and systemic lupus erythematosus. This study provides robust evidence that brain-reactive antibodies are increased in mothers of an ASD child and may be associated with autoimmunity. The current study serves as a benchmark and justification for studying the potential pathogenicity of these antibodies on the developing brain. The detailed characterization of the specificity of these antibodies will provide practical benefits for the management and prevention of this disorder.

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Keywords: anti-brain antibodies; ASD; autoimmunity; maternal

INTRODUCTION

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder with poorly defined and complex etiology, characterized by a broad range of symptoms in the domains of socialization and communication and by restricted behavioral patterns.1 Over the past decades, a substantial increase in the prevalence of ASD has been reported with a current estimation of over 1% incidence in the general population.2 The need for early diagnosis and prevention is pressing. Although the rapid increase in incidence may be in part attributable to better diagnosis and public awareness, environmental causes may also contribute. It has become apparent that brain-reactive antibodies can contribute to human pathobiology. Some well-characterized examples of antibody-mediated neurological disease are neuro-myelitis optica, paraneoplastic syndromes and neuropsychiatric lupus for which pathogenic antibodies that target antigens in the brain have been identified (for a review see the study by Diamond et al.3). Although much less well-characterized, anti-brain antibodies have also been implicated in the pathogenesis of a variety of childhood neuropsychiatric syndromes such as obsessive compulsive disorder,4 Tourette’s syndrome5 and ASD.6 Several studies have linked maternal infections or inflammation during pregnancy to the development of ASD in the offspring (reviewed by Patterson7), suggesting that activation of the maternal immune system might lead to an increased risk of a child with ASD. Other studies have addressed the potential risk of exposure to maternal antibody in utero. Specifically, several investigators have identified the presence of antibodies that bind to human fetal brain tissue in mothers with an ASD offspring.8,9 When these antibodies were administered to gestating mice or monkeys, the offspring exhibited abnormal behavior (reviewed by Enstrom et al.5). In an early study, serum-containing antibodies reactive with neuronal antigens from a mother of an ASD child was passively administered to pregnant mice. The offspring were observed to have deficits in social behavior and motor skills and cerebellar abnormalities on histopathology.10 In a subsequent study, IgG isolated from pooled sera of mothers of a child with ASD were administered to pregnant mice. The in utero exposed offspring had increased activity at a young age as well as anxiety-like behavior, alterations in sociability and increased startle following acoustic stimuli in adulthood.11 In addition to these studies in rodents, IgG pooled from sera of mothers of a child with ASD were administrated to pregnant rhesus monkeys. The offspring were observed to have social deficits, increased motor activity and increased stereotypic behaviors compared with monkeys born of mothers given control IgG pooled from mothers of a normally developing child.12 The results from these studies suggest that maternal antibodies targeting brain antigens can alter neurodevelopment in the fetus.

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A number of epidemiological studies have found an association between a maternal history of autoimmune disease and increased risk of having a child with ASD. Increased risk of ASD in the child was found if the mother was diagnosed with psoriasis,\textsuperscript{13} rheumatoid arthritis or celiac disease.\textsuperscript{14} With a substantial increase in the prevalence of ASD, as has also been observed for most autoimmune diseases over the past four decades,\textsuperscript{15} we hypothesize that production of brain-reactive antibodies may be associated with features of autoimmunity.

The goal of this study was to provide evidence, using the largest cohort reported so far, that mothers of an ASD child have an elevated frequency of anti-brain antibodies and to assess whether brain reactivity is associated with an autoimmune diathesis of the mother.

The implication of this work could lead, in the future, to prevention of some proportion of ASD.

**METHODS**

**Research subjects**

Plasma from mothers with an ASD child was obtained from the Simons Simplex Collection (SSC, http://sfari.org/resources/simons-simplex-collection)\textsuperscript{16} and the Autism Genetic Resource Exchange (AGRE, http://agre.autismspeaks.org). Details on recruitment of families, consent and protection of the privacy of participants, evaluation and basic description of the cohort can be found for the SSC by Fischbach and Lord\textsuperscript{16} and for AGRE by Geschwind et al.\textsuperscript{17}

Control plasma from women of child-bearing age were obtained from the North-Shore-LIJ health system clinical laboratory, participants in a registry at the Feinstein Institute for Medical Research (http://www.gapregistry.com) and a previously characterized adult cohort enrolled in the North-Shore-LIJ health system clinical laboratory, participants in a longitudinal cohort of rheumatoid arthritis patients recruited across the New York cohorts,\textsuperscript{18} the Feinstein Institute (www.gapregistry.com) and the North-Shore University Hospital laboratories. Table 1 describes, by each source of subjects, race, ethnicity and the age of the subject when blood was drawn.

Plasma of women with rheumatoid arthritis were derived from subjects enrolled in the the National Data Bank for Rheumatic Diseases, a longitudinal cohort of rheumatoid arthritis patients recruited across the United States\textsuperscript{19} that has been included in previous genetic studies of rheumatoid arthritis.\textsuperscript{20} A full description of this cohort including demographic and clinical variables can be found in the study by Wolfe et al.\textsuperscript{21}

Individuals provided informed consent through the appropriate institutional review board.

**Immunohistology**

Preparation of adult mouse tissue sections. Twelve-week-old unmanipulated C57BL/6 mice (Jackson Laboratories, Bar Harbor, MA, USA) were anesthetized with isoflurane before perfusion. Brains were perfused with 4% paraformaldehyde, following replacement of circulation with heparanized preperfusion buffer. Brains were removed, postfixed over-night and infiltrated with 30% sucrose 48 h at 4 °C. Brains were next frozen in freezing medium (O.C.T. compound, Sakura, San Francisco, CA, USA) on dry ice and mounted on gelatin-coated slides and stored at −80 °C until use. To assess IgG reactivity to the brain, 12-μm sections were air dried, rinsed twice for 5 min with phosphate buffered saline (PBS) containing 0.1% Tween-20. Following blocking for 1 h with PBS containing 10% heat-inactivated normal goat serum in 0.2% Triton X100 at room temperature, sections were incubated for 1 h with 1:500 human plasma diluted in PBS with 3% heat-inactivated normal serum in 0.2% Triton X100. After washing in PBS/Tween, binding of plasma to the brain was detected using Alexa 488 goat anti-human IgG (Invitrogen, Grand Island, NY, USA) and visualized with OpenLab software on an Axio-Plan2 microscope (Zeiss, Thornwood, NY, USA).

Intensity and localization of reactivity was determined by two independent observers. One observer was blind to the status of the subjects (that is, mother of an ASD child; control). A third reviewer, also blind to the status of the subjects, assessed random samples (~10%).

**RESULTS**

To test whether mothers of an ASD child are more likely to harbor anti-brain antibodies than unselected women of child-bearing age, we assessed plasma reactivity to mouse brain tissue.

Intensity was determined to be an absolute negative when no staining was visualized, positive when strong staining was visualized and indeterminate for all other cases (Supplementary Figure 1). There was no disagreement between the observers on positive and negative plasma.

The study was performed on 2431 mothers of an ASD child obtained from the SSC and 653 controls obtained from local New York cohorts,\textsuperscript{18} the Feinstein Institute (www.gapregistry.com) and the North-Shore University Hospital laboratories. Table 1 describes, by each source of subjects, race, ethnicity and the age of the subject when blood was drawn.

Mothers of an ASD child are nearly four times more likely to harbor anti-brain antibodies than unselected women of child-bearing age ($P < 0.00001$). In total, 10.7% of the plasma of mothers of an ASD child (260/2431) displayed strong reactivity to mouse brain antigens compared with 2.6% plasma of unselected women of child-bearing age (17/653). Only 28% plasma of mothers of an ASD child (682/2431) showed no binding compared with 64.7% plasma of unselected women of child-bearing age (423/653). The remaining women showed indeterminate binding.

**Statistical analysis**

Two-sample Student's t-tests were used to compare means of two independent groups. To analyze categorical data, a $\chi^2$-test for independence was used. When expected sample size was smaller than 5, the Fisher's exact test was used. Values were considered significant for $P < 0.05$. For multiple comparisons, the Keppel multiple comparisons correction was used.
data are described in Supplementary Table 5). In all, 8.8% of the plasma of mothers of an ASD child (28/318) displayed strong reactivity to mouse brain antigens. Only 22.6% plasma of mothers of an ASD child (72/318) showed no binding. The remaining women showed indeterminate binding. \( \chi^2 \)-Analysis yielded no difference between sources (AGRE and SSC, \( P > 0.5 \)).

Staining patterns from antibody-positive woman localized to distinct regions of the adult mouse brain (Figure 1). Generally, binding to neurons in the frontal cortex, hippocampus and the cerebellum was evident. In only 5 of 288 cases was there clear binding to neurons in the frontal cortex, hippocampus and the cerebellum. In only 5 of 288 cases was there clear binding to neurons in the frontal cortex, hippocampus and the cerebellum.

Immunohistology of adult mouse brain was corroborated by immunohistology of mouse fetal brain (data not shown) and western blots of human fetal brain lysates. Distinct bands were commonly bound by IgG from positive plasma, although there was also binding in western blot by some plasma that were negative by immunohistology (Supplementary Figure 2).

We hypothesized that brain reactivity would be associated with an autoimmune predisposition; we, therefore, tested for the presence of anti-nuclear antibodies, which are commonly present in individuals with many autoimmune diseases. Reactivity was assessed as positive, negative or indeterminate (Supplementary Figure 3).

As can be seen in Table 3, mothers of an ASD child who were positive for anti-brain antibodies were significantly more likely to harbor anti-nuclear autoantibodies than mothers of an ASD child or unselected women of child-bearing age who lacked anti-brain antibodies (\( \chi^2 \)-test, \( P < 0.0001 \)). There was no significant difference between mothers of an ASD child with no anti-brain antibodies and controls. In total, 53% (152/284) of mothers of an ASD child with anti-brain antibodies also exhibited anti-nuclear autoantibodies compared with 13.4% (99/738) of mothers of an ASD child without anti-brain antibodies and 15% (52/345) of unselected women of child-bearing age. Only 23.2% (66/284) of mothers of an ASD child with anti-brain antibodies showed no binding compared with 54.6% (403/738) of mothers of an ASD child without anti-brain antibodies and 62.3% (215/345) of unselected women of child-bearing age. The remaining subjects showed indeterminate binding. These data are consistent with a predisposition to more generalized autoimmunity in some mothers with anti-brain antibodies who have a child with ASD.

Next, we used the self-reported data available from the SSC to analyze whether autoimmune diseases were more common in mothers of an ASD child with anti-brain antibodies compared with mothers of an ASD child who lack anti-brain antibodies. Data were available only for subjects from the SSC and included the following diseases: juvenile inflammatory arthritis, rheumatoid arthritis, systemic lupus erythematosus, Hashimoto’s thyroiditis, celiac disease, type 1 diabetes, psoriasis, multiple sclerosis and adrenal deficiency. The number and percentage of cases for each disease are presented in Supplementary Table 6. The analysis of ASD mothers with anti-brain antibodies revealed an increased incidence of autoimmune diseases especially rheumatoid arthritis, and systemic lupus erythematosus (Table 4).

Finally, to ask whether autoimmunity predisposes to production of anti-brain antibodies, we determined whether women with

<table>
<thead>
<tr>
<th>Race (%)</th>
<th>White</th>
<th>African-American</th>
<th>Asian</th>
<th>Other</th>
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</thead>
<tbody>
<tr>
<td>SSC</td>
<td>80</td>
<td>3.9</td>
<td>5</td>
<td>11.1</td>
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<tr>
<td>NYC population cohort</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GAP</td>
<td>76</td>
<td>3.3</td>
<td>8.9</td>
<td>11.8</td>
</tr>
<tr>
<td>North-Shore-LIJ laboratories</td>
<td>70</td>
<td>13</td>
<td>6</td>
<td>11</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Ethnicity (%)</th>
<th>Hispanic</th>
<th>Non-Hispanic</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSC</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>NYC population cohort</td>
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<td>100</td>
</tr>
<tr>
<td>GAP</td>
<td>7</td>
<td>93</td>
</tr>
<tr>
<td>North-Shore-LIJ laboratories</td>
<td>5</td>
<td>95</td>
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<table>
<thead>
<tr>
<th>Age at the time blood was drawn</th>
<th>Mean</th>
<th>s.d.</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSC</td>
<td>40.28</td>
<td>5.73</td>
<td>22</td>
<td>58</td>
</tr>
<tr>
<td>NYC population cohort</td>
<td>43</td>
<td>3.42</td>
<td>37</td>
<td>49</td>
</tr>
<tr>
<td>GAP</td>
<td>38.8</td>
<td>9.6</td>
<td>19</td>
<td>50</td>
</tr>
<tr>
<td>North-Shore-LIJ laboratories</td>
<td>36.17</td>
<td>8.14</td>
<td>18</td>
<td>50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anti-brain antibodies negative (N = 644)</th>
<th>Anti-brain antibodies positive (N = 238)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at the time the blood was drawn</td>
<td>Mean</td>
<td>s.d.</td>
</tr>
<tr>
<td>SSC</td>
<td>39.9</td>
<td>5.74</td>
</tr>
<tr>
<td>Years elapsed between birth of the child and the time blood was drawn</td>
<td>Mean</td>
<td>s.d.</td>
</tr>
<tr>
<td>SSC</td>
<td>8.87</td>
<td>3.45</td>
</tr>
<tr>
<td>NYC population cohort</td>
<td>31.01</td>
<td>5.021</td>
</tr>
<tr>
<td>GAP</td>
<td>31.01</td>
<td>5.021</td>
</tr>
<tr>
<td>Has the subject had pregnancies that did not end in live birth? (%)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>SSC</td>
<td>31</td>
<td>69</td>
</tr>
</tbody>
</table>

Abbreviations: ASD, autism spectrum disorder; GAP, genotype and phenotype; LIJ, Long Island Jewish; NYC, New York City; SSC, Simons Simplex Collection.

Abbreviations: ASD, autism spectrum disorder; NS, not significant.

*Student’s t-test or \( \chi^2 \)-test for categorical data, all \( P \)-values > 0.12.
rheumatoid arthritis ($n = 363$) were more likely to have anti-brain antibodies compared with the control women. Interestingly, we found that women with rheumatoid arthritis are as likely to have anti-brain antibodies as mothers of an ASD child (rheumatoid arthritis positive, 13.5% ($n = 49/362$); negative 32.3% ($n = 117/362$); indeterminate 54.1% ($n = 196/362$)). Supplementary Table 7 describes race and ethnicity, age of disease onset and the age when blood was drawn of women with rheumatoid arthritis. None of these variables could account for the difference in the presence of anti-brain antibodies.

**DISCUSSION**

The passage of maternal antibodies across the placenta is a well-known mechanism for fetal immune protection. In the fetus, the blood–brain barrier is not fully formed, making the developing brain vulnerable to blood-borne substances. In utero exposure to maternal anti-brain antibodies has been posited as an important potential trigger for abnormal brain development. Growing evidence suggests that maternal antibodies can target the fetal brain, as maternal antibody can penetrate fetal brain tissue, which is not protected by a fully functional blood–brain barrier.

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Table 3. Mothers with anti-brain antibodies and a child with ASD are more likely harbor for anti-nuclear antibodies

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Source</th>
<th>Number of subjects</th>
<th>Anti-nuclear antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Mothers of an ASD child anti-brain IgG positive</td>
<td>SSC</td>
<td>256</td>
<td>22.65% ($n = 56$)</td>
</tr>
<tr>
<td></td>
<td>AGRE</td>
<td>28</td>
<td>35% ($n = 10$)</td>
</tr>
<tr>
<td>Mothers of an ASD child anti-brain IgG negative</td>
<td>SSC</td>
<td>667</td>
<td>55.5% ($n = 357$)</td>
</tr>
<tr>
<td></td>
<td>AGRE</td>
<td>71</td>
<td>64.7% ($n = 46$)</td>
</tr>
<tr>
<td>Unselected women of child-bearing age anti-brain IgG negative</td>
<td><em>Combined</em></td>
<td>345</td>
<td>62.3% ($n = 215$)</td>
</tr>
</tbody>
</table>

Abbreviations: AGRE, Autism Genetic Resource Exchange; ASD, autism spectrum disorder; SSC, Simons Simplex Collection. \( \chi^2 \)-Analysis yielded no significant difference between the two cohorts for mothers of an ASD child positive for anti-brain antibodies or mothers of an ASD child negative for anti-brain antibodies, or controls. The remaining subjects in each cohort showed indeterminate binding (see Supplementary Figure 3). *Control includes subjects for the NYC population study, GAP and North-Shore-llj laboratories.

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Figure 1. Representative plasma showing reactivity of plasma of an unselected woman of child-bearing age (first panel) and mother of an autism spectrum disorder (ASD) child to mouse adult brain (second and third panel). (a–c) Transverse tissue sections were incubated with plasma of mother of an ASD child or control. Anti-brain IgG was detected using Alexa 488 anti-human IgG. As can be seen, plasma of mother of an ASD child primarily labeled the (a) hippocampus, (b) cerebellum and (c) frontal cortex. \( \times 5, \times 40 \) state the magnification.
barrier. For example, anti-DNA, anti-N-methyl-D aspartate recep-
tor, cross-reactive antibodies specifically present in women with
systemic lupus erythematosus, have been shown in mouse models
to be neurotoxic to the developing brain. More recently, it has
been shown that these antibodies have differential effects on
male and female fetuses, causing cognitive impairments in the
former and fetal death in the latter. These antibodies affect the
adult mouse if, and only if, there is a compromise to blood–brain
barrier integrity.24

western blotting using commercially available human fetal brain
performed immunohistology on fetal mouse brain sections as
the brain regions targeted by antibody. In order to confirm the
samples reported in this study as well as to broadly identify
allowed us to screen relatively quickly the large number of
antibodies. Their specificity and functional role remain to be
determined.

The utilization of immunohistology of adult mouse brain
showed that women with either rheumatoid arthritis or celiac
disease and ASD. We have shown that mothers of an ASD child
found a more frequent incidence of autoimmune disease and ASD. We hypothesize that the in utero experience may contribute to ASD in the offspring.

We demonstrated that anti-nuclear antibodies and autoimmune
disease are increased in mothers with anti-brain antibodies and
with an ASD child. The possibility of autoimmune mechanisms
being a contributing factor in ASD has been entertained as early
studies suggested that individuals with ASD have a family history
of autoimmmune disease.14,31 A recent large study examined
autoimmune disorders in women with over 600,000 births and
showed that women with either rheumatoid arthritis or celiac
disease have an increased risk of having a child with ASD.14
Interestingly, there was no increased incidence of rheumatoid
arthritis in fathers of a child with ASD, consistent with the
hypothesis that the in utero experience may contribute to ASD in the
offspring.

Our findings of increased autoimmune disease and specifici-
cally rheumatoid arthritis and systemic lupus erythematosus
are consistent with the hypothesis that autoimmune diseases
may confer risk for ASD in the offspring of mothers with these
disorders.

We have shown that an autoimmune diathesis such as is
present in women with rheumatoid arthritis predisposes to the
production of anti-brain antibodies in a similar proportion to what
we reported for mothers with an ASD child. Plasma of women with
rheumatoid arthritis showed similar staining patterns as plasma of
mothers with an ASD child. Future studies will need to determine
whether common antigens are involved. It is interesting to
postulate that production of anti-brain antibodies may be the
mechanism for the reported associations between autoimmune
disease and ASD. We have shown that mothers of an ASD child
who were positive for anti-brain antibodies had more self-
reported autoimmune conditions than mothers of an ASD child
with no anti-brain antibodies. We also found that anti-nuclear
antibodies, a common marker of both subclinical autoimmunity
as well as clinical autoimmune disease, are more frequent in mothers
of an ASD child who harbor anti-brain antibodies.

A large number of genetic risk variants have been identified
with autoimmune disorders,12 and we have considered the
possibility that these variants may also predispose to the deve-
lopment of anti-brain antibodies in mothers of an ASD child. Our
preliminary studies of mothers in the SSC for whom genetic data

The majority of previous reports have used western blotting of
brain antigens as a primary screening tool. The specific brain
antigens detected in these studies have not yet been identified,
although some studies have identified 39 and 73 kDa bands that
are often seen on western blots of brain lysates. In this
study, we have screened for anti-brain antibodies by histology of
adult mouse brain sections. We, as others, have not identified a
specific antigenic target; yet, the use of an immunohistological
assay allowed us to identify the brain regions exhibiting
the highest reactivity with maternal IgG, including the frontal
cortex, hippocampus and cerebellum. These regions have been
implicated in ASD through imaging and post mortem studies;27

cyt架构tural organizational abnormalities of the cerebral
cortex, limbic area, cerebellum, as well as other subcortical
structures have been documented. In particular, studies have
demonstrated neuronal abnormalities such as loss and atrophy
of Purkinje cells, abnormal development of neurons in the
hippocampus and changes in neuronal size and number in the
cerebellum and midbrain.27,28 The immunostaining patterns we
observed were almost exclusively neuronal and confined to
regions that have consistently been shown to be involved in
ASD, thus strongly supporting the physiological relevance of
the antibodies. Their specificity and functional role remain to be
determined.

The utilization of immunohistology of adult mouse brain
allowed us to screen relatively quickly the large number of samples
reported in this study as well as to broadly identify
the brain regions targeted by antibody. In order to confirm the
relevance of the findings to mouse fetal brain and human, we
performed immunohistology on fetal mouse brain sections as
western blotting using commercially available human fetal brain
lysates. Without expectation, plasma binding to adult brain bound
fetal brain as well, but it was not possible to localize regions of
immunoreactivity in fetal brain. Although we were also able to
demonstrate bands on western blot, the bands identified were
variable using a limited number of seropositive and seronegative
plasma making it difficult to characterize plasma as positive or
negative. These data, did however, show that binding to tissue
antigens was not species-specific. Reactivity across species is also
consistent with the studies mentioned above showing that anti-
brain serum or IgG from mothers of an ASD child given to
pregnant mice or monkeys cause cognitive and behavioral
abnormalities in the offspring.11,12 It has also been shown that
many human autoantibodies target highly conserved region of
the autoantigen and therefore reacts with antigen present in
multiple species.29,30

We demonstrated that anti-nuclear antibodies and autoimmune
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of an ASD child who harbor anti-brain antibodies.

A large number of genetic risk variants have been identified
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possibility that these variants may also predispose to the deve-
lopment of anti-brain antibodies in mothers of an ASD child. Our
preliminary studies of mothers in the SSC for whom genetic data

Table 4. Autoimmune diseases are more common in mothers with anti-brain antibodies and a child with ASD

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Mothers of an ASD child</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-brain antibody negative (n = 622)</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>n = 9</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>n = 1</td>
</tr>
<tr>
<td>Total number of autoimmune diseases</td>
<td>n = 55</td>
</tr>
</tbody>
</table>

Abbreviation: ASD, autism spectrum disorder.

Table 4 presents the significant results from the data that was available for subjects from SSC. For each disease the number of positive cases and the percentage that these cases represent is shown. The full analysis (Supplementary Table 4) includes the following diseases: Juvenile inflammatory arthritis, rheumatoid arthritis, systemic lupus erythematosus (SLE), Hashimoto’s thyroiditis, celiac disease, type 1 diabetes, psoriasis, multiple sclerosis, adrenal insufficiency. *Keppel multiple comparison correction, \( P < 0.035 \).
were available have not revealed strong evidence of associations with established autoimmune loci. However, our sample size is small and large cohorts will be required to perform a definitive analysis of autoimmune risk alleles in mothers of a child with ASD.

In a preliminary analysis on a subset of ASD subjects, we did not find an association between the presence of maternal anti-brain antibodies and the age of diagnosis of the child, severity of disease, IQ or distinct behavioral patterns in the child (data not shown). We believe that this may reflect the heterogeneity of the antigenic specificity of anti-brain antibodies. One previous report, however, did demonstrate a correlation between maternal antibody reactivity with specific bands on a western blot of brain lysates and irritability and communication impairments in the affected offspring.33 Once distinct antibody specificities are identified, we will repeat the analysis if phenotypes of children with ASD relate to target antigens.

We evaluated antibodies from blood that was obtained from the mother years after pregnancy. Our assumption is that these antibodies reflect a chronic immune state of the mother, in which the abnormal serology persists for years, and thus was present at the time of pregnancy. Autoantibodies have been shown to be present years before the clinical diagnosis of disease.34 We, however, cannot rule out that at least in some cases the anti-brain antibodies appeared after pregnancy. Only one study has assessed mid-pregnancy antibodies to fetal brain antigens in early marker for ASD.8 This study suggested that reactivity to 39 and 73 kDa proteins tended to be more common in mothers of an ASD child compared with mothers of a normally developing child. Of note, the same antigenic reactivities have been identified in blood collected after pregnancy from mothers of an ASD child.33

Control samples were obtained from various different sources, enhancing the reliability and validity of the data. No differences were observed among the sources despite some variability in ethnicity and race.

We should further note that child-bearing age was the only criteria applied for selecting the control group of women. We have, therefore, no data whether those women have had a child or have an autoimmune disease. Yet, if indeed our control group is representative of overall female population, 80% have children (according to the Census Bureau) and around 6% have an autoimmune disease.35 This latter frequency of autoimmune disorders is similar to what we report in the current paper for the mothers of an ASD child without anti-brain antibodies.

We did not control for sex of the mice used to assess anti-brain reactivity. As the male to female ratio is skewed toward males in ASD, there might be a specific immune reaction to male brain antigens. We are, however, not aware of sex specific brain antigens.

Several ASD susceptibility genes have been identified in the past decade; collectively may account for 10–20% of ASD cases.36 Once pathogenicity of anti-brain antibodies is determined, our study by has the potential to explain up to 10% of additional cases of ASD.

Our data suggest that anti-brain antibodies are associated with autoimmunity and are increased in mothers of an ASD child. Work is currently under way to assess the etiological role of these antibodies in autism. The detailed characterization of the antigenic specificity of these antibodies is likely to shed light on the neurobiology of autism as well as provide practical benefits to the management and prevention of this disorder.

NOTE ADDED IN PROOF
Since the submission of this work, two studies have been published providing further evidence that maternal anti-brain antibodies may lead to autism in the offspring. Bauman et al.37 demonstrated the pathogenic potential of antibodies directed against specific fetal brain proteins in rhesus monkeys. Braunischweig et al.38 described a panel of six proteins that might be recognized by those anti-brain antibodies.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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Reconstitution of the human biome as the most reasonable solution for epidemics of allergic and autoimmune diseases

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A wide range of hyperimmune-associated diseases plague post-industrial society, with a prevalence and impact that is staggering. Strong evidence points towards a loss of helminths from the ecosystem of the human body (the human biome) as the most important factor in this epidemic. Helminths, intestinal worms which are largely eradicated by elements of post-industrial culture including toilets and water treatment facilities, have an otherwise ubiquitous presence in vertebrates, and have co-evolved with the immune system. Not only do helminths discourage allergic and autoimmune reactions by diverting the immune system away from these pathologic processes and stimulating host regulatory networks, helminths release a variety of factors which down-modulate the immune system. A comprehensive view of hyperimmune-related disease based on studies in immunology, parasitology, evolutionary biology, epidemiology, and neurobiology indicates that the effects of biome depletion may not yet be fully realized, and may have an unexpectedly broad impact on many areas of human biology, including cognition. Fortunately, colonization with helminths results in a cure of numerous autoimmune and allergic diseases in laboratory rodents, and clinical studies in humans have indicated their utility for treatment of both multiple sclerosis and inflammatory bowel disease. Based on these considerations, commitment of considerable resources toward understanding the effects of “biome depletion” and systematically evaluating the most effective approach toward biome reconstitution is strongly encouraged.

Introduction

The field of clinical immunology is faced with epidemics of a wide range of allergic, autoimmune, and other inflammation-associated diseases with an impact that is staggering. These epidemics, which appear to be products of post-industrial society, have strongly resisted ever-increasing efforts focused on the development of pharmaceuticals to combat the problem [1]. In this paper, we examine evidence from the fields of evolutionary biology, ecology, immunology, parasitology, neurobiology, and medicine for the hypothesis that the only reasonable solution to these epidemics is to reconstitute the ecosystem of the human body, the “human biome”, with factors, especially helminths, that have been depleted in post-industrial society. A range of arguments which might be mounted against this hypothesis are addressed, as well as the implications for biomedical research and the field of clinical immunology.

Epidemiology of hyperimmune-associated disease

Epidemics of diseases associated with a hyperactive immune system occur much more frequently in countries that enjoy the widespread use of modern medical care, sanitation practices, and water treatment technology [2-4]. These diseases can be associated with an increased propensity for inflammation, and often involve pathologic immune reactivity directed against self and non-self antigens, producing autoimmune and allergic diseases, respectively. The wide range of these non-infectious conditions is striking, and includes lupus, multiple sclerosis, inflammatory bowel disease, hay fever, appendicitis, eczema, Graves’ disease, asthma, and dangerously allergic reactions against antigens in common food substances including nuts, milk and grains [5,6]. The potential that some neurological disorders such as autism may also be hyperimmune-associated [7] adds further to the milieu. The prevalence of these diseases in post-industrial societies is staggering [6,8]. For example, more than one person in 1000 acquires lupus [9], approximately one in 600 acquires multiple sclerosis in some areas of the world [10], another one in 600 acquires type 1 diabetes [11], one in 40 suffers from food allergies [12], approximately one in 20 acquires autoimmune thyroid disease [13], about one in 18
The human biome and the immune system: a single, functional unit

The Biome Depletion Theory is based on the intimate connection between the human immune system and those organisms that live in and on the human body. Studies in which animals are completely separated from all microorganisms have been instrumental in our understanding of the connection between the immune system and the rest of the human biome. Decades of experiments have reproducibly demonstrated that microbe-free animals, known as gnotobiotic or germ free animals, have an immune system which is grossly undeveloped relative to the immune system of animals that are colonized with a normal bacterial flora [18–21]. The addition of bacteria to previously germ free animals results in restoration of the immune system [21], and demonstrates the dependence of the immune system on the microbial flora. Ongoing work on the relationship between the microbial flora and the immune system has led to the understanding that the immune system is better described as an interface with the environment more so than as a defensive wall against the environment. The immune system’s role as an environmental interface is illustrated by its support of mutualistic microorganisms that deter pathogenic microorganisms, a process which potentially provides a more effective barrier to contagious disease than does anti-microbial measures carried out directly by the immune system [22].

Understanding the connection between the microorganisms normally associated with our bodies and the immune system has garnered great interest, and knowledge in this field is expanding quickly. These human-associated microorganisms, collectively known as the “microbiome”, comprise an important part of the human biome. The importance of this part of the human biome extends far beyond the immune system, affecting metabolism and potentially the development of other parts of the biome, including worms, or helminths, that inhabit the gut of all vertebrate species [23]. Modern sanitation and water treatment practices, the widespread use of antibiotics, modern practices in obstetrics (e.g., delivery by caesarian section and the use of antisepsics), and the use of substitutes for mother’s milk, have all profoundly affected the microbiome. These changes almost certainly contribute to hyper-immune-associated disease [24,25], but it is profound changes in colonization of the gut by helminths rather than changes in colonization of the gut by microorganisms that has been identified as the single most important factor impacting epidemics of hyper-immune-associated disease [26–29]. Helminths interact in a complex manner with the immune system of the host as well as with the microorganisms of the human biome, forming a key component of the highly interdependent network normally associated with the human biome (Fig. 1). This key component of the biome has largely been eradicated in post-industrial society, and a wide range of evidence suggests that this depletion leaves the remaining components of the biome in an unbalanced, relatively unstable, and disease-prone state.

Coevolution of vertebrates with helminths

The ancient coevolutionary history of the immune system with helminths provides insight into the entrenched nature of the immune system-helminth connection. The evolutionary origin of jawed fishes more than 400 million years ago marked not only the appearance of immune systems containing all of the major components found in humans, but also presumably provided suitable vertebrate hosts for flatworm parasites [30]. Although it remains unknown when in evolutionary history helminths took up residence in the vertebrate gut, several lines of evidence point toward helminths residing in the guts of vertebrates more than 100 million years ago. For example, the observation that several clades (groups of related species) of invertebrates are composed entirely of species that are obligate associates of vertebrate guts suggests that gut colonization has been present for as long as these clades have been in existence [31]. Thus, gut colonization probably dates back to the origin of tapeworms (cestodes, with more than 1000 species), spiny-headed worms (acanthocephalans, with more than 1100 species), and several clades of flukes (trematodes) and roundworms (nematodes) [32]. Additional evidence for long-term associations between helminths and vertebrates comes from phylogenetic analyses that have uncovered cases where host and helminth groups show similar patterns of speciation [31,33], suggesting that helminths and hosts have co-evolved. Hoberg et al., for example, considering the coevolutionary history of tapeworms and vertebrates, estimated that these helminths have co-evolved with their vertebrate hosts for a minimum of 350–420 million years [34]. Additional assessments of helminth–vertebrate gut associations by molecular clocks or by the geographic distribution of living species relative to tectonic events indicates that helminth–vertebrate associations are at least 100–200 million years old [32,35,36].

“Typical” association of helminths with humans

An understanding of “typical” helminth colonization in humans living in a Neolithic culture might provide some insight into the environment for which our immune systems are evolved to function. For several reasons, however, defining such colonization remains problematic. First, epidemiological data on helminth associations is often geographically patchy and gathered using
different methods, making generalization difficult. Second, most of the data in humans are not derived from populations living in a Neolithic culture, but rather are based on populations living in towns and cities, where sanitation and anti-helminthic alter the incidence of colonization and may affect which species are present. Third, colonization status differs enormously among human populations. Climates and socioeconomic status are two of the largest determinants of these differences, but other factors can have a strong influence [37,38]. Because many helminths have complex life cycles involving intermediate hosts such as snails or insects, the local ecology of these other species has a major impact. Activities such as farming and hunting increase the likelihood of contact with vectors of early life history stages of some helminths, and can also influence colonization rates and species composition. As a consequence, the epidemiological data we have are likely representative primarily of the past tens to hundreds of years of human history; they are almost certainly not characteristic of the vast sweep of time humans have been living in association with helminths.

Despite these uncertainties, it seems clear that colonization by multiple helminth species would have been nearly universal throughout human evolutionary history [39]. The most ancient direct evidence comes from two frozen humans dating to the fifth century BCE that contain embryos of hookworms [40]. However, physical evidence of this kind is rare, and the most extensive evidence comes from epidemiological studies. Over 340 species of helminths colonizing humans have been recorded [41], with about 30 of those species regarded as medically relevant. Rates of colonization are very high for some helminth species even today [41,42]. For example, more than one-sixth of the world’s population is inhabited by just two soil-transmitted helminths, *Ascaris lumbricoides* and *Trichuris trichiura*, and several other helminths each inhabit more than 100 million humans [41,43]. In some parts of the world, colonization by multiple helminths is the norm rather than the exception [37,42]. Perhaps the most useful information regarding normal colonization status comes from indigenous populations, where the influence of confounding factors such as sanitation, anti-helminthics, and exotic food sources are minimal. For example, a study of people living in a pre-industrial culture in Rwanda revealed serological evidence of helminth colonization (average IgE levels in serum above normal for post-industrial society, despite the absence of allergy), even in those individuals which did not test positive for helminths based on a fecal analysis [44,45]. Collectively, the epidemiological evidence strongly suggests that humans have been living with multiple helminths throughout their evolutionary history, and that these associations are a normal part of our ecology.

Although the typical colonization of humans by helminths might be difficult to access with certainty, the number and diversity of helminth species has been assessed in a variety of wild (i.e., not laboratory bred or domesticated) animals, and may provide insight into what is typical of a mammalian species. Most individual animals in natural environments are colonized by both a wide diversity and a larger number and intensity of helminth species. Although geographic and ecological variations exist in the patterns of colonization, what is clear is that helminth colonization is the norm. From a broad dataset consisting of 56 mammalian species, in any given population, the number of helminth species was found to range from 2 to 39 species [46,47]. Further, rates of colonization by helminths are typically high in mammalian species closely related to humans, including the great apes [48]. For example, it is clear that our closest living relatives, common chimpanzees and bonobos, are both host to a diverse set of helminths in their natural habitats [31,48]. Each helminth species may be present in up to one-third of the individuals in these great ape populations, although sampling is exclusively based on feces and therefore may underestimate true colonization rates [49,50]. Thus, studies evaluating colonization of non-human mammals by helminths suggests strongly that colonization by helminths is the norm for mammals, despite the fact that humans living in post-industrial societies have come to regard the absence of resident helminths as “normal”.

Well-studied ecological biomes provide parallels that might help understand the dynamics of the human biome and the effects of biome depletion. Ecological biomes such as grasslands and tropical forests reach new, unstable equilibria as a consequence of rapid and prolonged loss of co-evolved components [51]. These biomes often contain “keystone species” that, relative to their biomass or density, have a disproportionately heavy impact on the ecosystem when lost [52]. We propose that the human biome follows the same properties and has been rendered unstable by the recent loss of a critical, co-evolved component: helminths. This instability is apparent in the widespread, aberrant function of the immune system in the absence of ‘typical’ helminth colonization, and suggests that helminths might collectively be considered as the equivalent of a keystone species of the human biome.

**Modulation of the immune system by helminths**

Helminths have evolved to secrete dozens if not hundreds of molecules that down regulate their host’s immune system [53,54]. The range of immunoregulatory molecules includes a variety of protease inhibitors, including metalloprotease inhibitors, toll-like receptor signaling inhibitors, a variety of antioxidant enzymes, cytokine homologs, and a wide range of other molecules that inhibit or regulate the function of T-cells, macrophages, and dendritic cells [53]. Each species of helminth secretes different proteins that often exhibit strong host specificity, and many helminths secrete different proteins at different stages of their life cycle. Helminths also produce non-protein molecules such as phosphorylcholine, glycans, and lipids which down-regulate the immune system. It seems inevitable that the host has adapted to the presence of these immunosuppressive “drugs” [28].

Immunosuppression of the immune system by helminth-derived factors is only one means by which helminths might avert allergic and autoimmune reactions. Another very important property of helminth colonization that seems almost at odds with the immunosuppressive effect is that it causes a substantial and chronic immune response, which probably has several effects on the host. For example, by inducing chronic commitment of the immune system toward helminth-derived factors, helminths leave the immune system with fewer resources available for allergic or autoimmune responses. Further, chronic stimulation of the immune system by helminths results in the induction of a substantial feedback inhibition system [55,56]. In laboratory rodents, this is readily observed as an increased production of T-regulatory cells following colonization by helminths [53,57]. With these observations in mind, it seems evident that the ubiquitous presence of helminths and their omnipresence during the evolution of the immune system have profoundly shaped the biology of both helminth and immune system [58], although many of the effects of this coevolution probably remain to be discovered.

**Biome depletion as the cause of epidemics of hyperimmunee-associated disease, but not the sole cause of hyperimmunee-associated disease**

Almost paradoxically, The Depleted Biome Theory does not imply that biome depletion is the primary cause of allergies or autoimmune diseases. In addition to biome depletion, two other equally important factors are often or perhaps even usually found to contribute to hyperimmune-associated diseases (Fig. 2). First,
hyperimmune-associated diseases are often associated with a degree of heritability, having genetic and possibly epigenetic factors that predispose people to disease. Second, hyperimmune-associated diseases are often triggered by environmental factors that include viral infections, chemical pollutants, or common antigens produced by a wide range of organisms, ranging from ragweed to cockroaches to cows and dust mites. Thus, the Biome Depletion Theory involves a “three-hit paradigm” that is gaining increasing support. Hay fever, for example, is associated with genetic factors [59], and is, of course, triggered by exposure to antigens such as those produced by ragweed. Further, chemical pollutants probably affect the incidence and the severity of hay fever [60]. Thus, biome depletion by itself does not actually cause hay fever. However, hay fever is a disease only associated with post-industrial society [61], and ongoing studies indicate that it is biome depletion which positions the immune system to react toward harmless (non-pathogenic) antigens [62,63]. Thus, the epidemic of hay fever, like other hyperimmune-associated diseases, is apparently caused by biome depletion, even though biome depletion is not sufficient by itself to cause hay fever.

Multiple sclerosis is an example of a hyperimmune-associated disease that can be triggered by viral infection [64], is associated with certain genetic markers [65], but yet can be effectively treated by colonization with helminths [55,56]. Similarly, type 1 diabetes is potentially associated with specific proteins found in the diet [66,67] and is associated with certain genetic factors [68]. Yet, helminth colonization is an effective treatment for type 1 diabetes in autoimmune prone mice [69], and the increase in prevalence of type 1 diabetes in post-industrial cultures has not been explained by the introduction of new proteins into the diet or by a change in the genetics of the population. Thus, epidemics of multiple sclerosis and type 1 diabetes, as well as other autoimmune diseases and a variety of allergic diseases, are explained well by The Biome Depletion Theory, despite the presence of environmental triggers and genetic and/or epigenetic factors associated with the disease. Indeed, it is counterintuitive to attribute recent epidemics of disease solely to factors that have remained relatively unchanged for hundreds or even thousands of years, such as human genetics, ragweed pollen, wheat proteins, and common viruses. Rather, acute increases in disease must be attributed to factors such as biome depletion that are very recent occurrences. Unfortunately, failure to resolve this issue has resulted in considerable confusion in the biomedical literature: The identification of neither genetic factors that predispose to disease nor environmental factors that trigger disease rules out The Biome Depletion Theory as applicable (Fig. 2). Rather, it is (a) the role of “overly aggressive” immune reactivity, and (b) the increased incidence of disease in post-industrial society relative to pre-industrial agrarian or hunter gatherer societies that are the hallmarks of diseases associated with biome depletion.

**Hygiene and the roots of The Biome Depletion Theory**

The Biome Depletion theory evolved from an idea first developed in the 1980s that has long been known as “the hygiene hypothesis”. This hypothesis was based on the understanding that certain hyperimmune diseases, allergies in particular, were associated with components of modern culture that generally increase hygiene, such as the use of modern, flushing toilets. As the understanding of hyperimmune-associated diseases evolved, it was eventually recognized that epidemics of allergies share a common cause with epidemics of autoimmune diseases. More importantly for our understanding of the nature of hyperimmune-associated diseases, changes in the microbiome and, more importantly,
depletion of helminths from the human biome were identified as the leading suspects affecting epidemics of these diseases [29,70,71]. This final revelation clarified the particular component of hygiene responsible for epidemics of hyperimmune-associated disease. It was, in fact, not hygiene, per se, that was responsible. Indeed, increased exposures to a variety of components associated with an unhygienic environment, including viral infections, mold, dust mite-derived allergens, and cockroach-derived allergens, are known to be “triggers” for the induction of allergy and/or autoimmune disease. Thus, a particular house might be considered unhygienic in some regards because of high concentrations of mold or cockroach-derived allergens, which increase the propensity for allergic disease. At the same time, in the same house, the environment might be considered relatively hygienic because a modern, flushing toilet and running water are used regularly, which, as discussed below, effectively disrupts the life cycle of almost all helminths, further increasing the propensity for allergic disease. Therefore, hygiene per se does not have a predictable effect on hyperimmune-associated disease: Hygiene in the form of a toilet increases the risk for hyperimmune-associated disease, while hygiene in the form of preventing buildup of mold and cockroach-derived allergens reduces the risk of hyperimmune-associated disease. Failure to understand this distinction has unfortunately retarded progress in the field.

The timing of biome depletion and hyperimmune-associated epidemics

The use of toilets effectively blocks the life cycle of almost all helminths, rendering humans in post-industrial societies as the only vertebrates free of association with these organisms to a large extent. The few helminths with the ability to reproduce despite the use of toilets, such as pinworms, are easily defeated by modern medicine because their ability to evolve resistance to pharmaceuticals is relatively limited by their long life cycle and small population size in comparison to bacteria and viruses that readily evolve drug resistance. Despite the fact that many individuals in post-industrial societies today take for granted the presence of toilets, the introduction of that technology is very recent, occurring, for example, in urban environments less than 100 years ago in the United States, and much more recently in rural environments. Most importantly, the impact of biome-depleting technologies such as the toilet on the immune system is expected to be delayed rather than immediate. This delay between cause and effect is anticipated for several reasons. First, many helminths have a long life span, some living for decades, and thus many individuals are expected to remain colonized for extended periods even after the transmission of the helminth has been halted. Second, some aspects of immunity may be strongly influenced by colonization with helminths early in life, and these influences may not be entirely reversed by loss of colonization at a later time. Thirdly, an individual’s immune system is strongly influenced by their mother’s immune system, and the extent to which the biome of the mother might protect the offspring from hyperimmune-associated disease remains unknown. The profound impact of epigenetics on human biology is only now beginning to be understood [72,73], and, although speculative, epigenetic effects resulting from colonization with helminths might be seen for multiple generations, thus delaying at least some of the effects of biome depletion (Fig. 2). With these factors in mind, it is not surprising that the effects of biome depletion are apparently increasing at the present time, despite the fact that for many, it was their grandparents, not themselves, which first enjoyed the convenience of biome-depleting technologies. In fact, we do not yet know if the potential effects of biome depletion have reached a climax.

Treatment of epidemics of allergy and autoimmune disease with biome reconstitution: results so far

It stands to reason that if biome depletion has caused epidemics of allergies and autoimmune diseases, then restoration of the biome should result in an end of those epidemics. Consistent with this reasoning, a number of experiments using animal models indicate that biome reconstitution does indeed prevent and even in some cases treat hyperimmune-associated disease [26–29]. For example, experimentally induced colitis, experimentally induced allergy, and type 1 diabetes can be effectively averted or treated in rodents by colonization with Heligmosomoides polygyrus, a roundworm commonly found in the small intestine of a variety of rodents [69,74,75]. Experimentally induced allergy in rodents can also be alleviated by colonization with another roundworm Nippostrongylus brasiliensis [76].

Even more encouraging than studies using animal models are two particular studies in humans. First, clinical trials demonstrate that exposure to a porcine helminth, Trichuris suis, provides an effective treatment for many patients with inflammatory bowel disease whose disease had proven untreatable with modern medicine [77]. More exciting results show that the progression of multiple sclerosis is halted by accidental helminth colonization [55].

A number of clinical trials addressing a variety of hyper-immune disorders are currently underway utilizing the porcine whipworm, T. suis, which has proven useful in treating inflammatory bowel disease, as mentioned above. Importantly, this helminth is adapted to colonize pigs, and does not colonize humans. Rather, it persists in the human intestine for a few days to weeks before being eliminated, and must be re-introduced to the human intestine on a frequent basis to maintain therapeutic levels. It is potentially difficult to place these pioneering clinical trials with T. suis within the broader perspective of biome reconstitution. It remains unknown, for example, how exactly exposure of a patient to T. suis might affect any subsequent attempts to reconstitute the biome of that patient with helminths that are adapted to colonize the human intestine. It also remains unknown how effective T. suis is in comparison to a variety of other helminths that are adapted to humans, and if T. suis might work in combination with other helminths as a therapy. Further, given the high cost of T. suis, due in large part to the need for frequent re-exposure to the organisms, it seems an unlikely candidate for use in clinical trials aimed at prophylactic reconstitution of the biome, if such trials are undertaken. Regardless of the utility or lack thereof that T. suis eventually affords modern medicine, work with T. suis represents the very first pioneering efforts in what promises to be a large and productive field of clinical medicine.

The health costs of biome depletion: how much?

Some might argue that treating hyperimmune-associated diseases such as hay fever and even food allergies is not worth the time, effort and energy that will be required to intentionally and systematically re-introduce organisms classified as “parasites” into the human population. Indeed, it can be argued that hay fever may be a worthwhile price to pay for avoiding these coevolutionary partners. Crohn’s disease and multiple sclerosis are certainly less tolerable, but therapies which usually alleviate the symptoms of these diseases at least to some extent are available. Despite the generally high costs of many pharmaceuticals, the general consensus may be that some disease can be tolerated as a side effect of being parasite free. However, the apparently higher cost of biome depletion undermines this argument.

Although hyperimmune-associated diseases are not perceived as particularly life threatening, the death toll is not trivial. Allergic reactions to food alone kill an estimated 150–200 people every
added to this number are the more than 3000 people who die from asthma each year [14,79]. In addition, if the current epidemic is not abated, more than one of every 300 people will have their lives strongly impacted if not shortened by lupus, multiple sclerosis, or type 1 diabetes. The number of deaths associated with colitis and other types of gastrointestinal inflammatory diseases adds to this number.

Another factor when considering the health costs of biome depletion is that we do not yet know the extent of pathology that may result. Diseases not obviously related to a hyperimmune response may be impacted by biome depletion. For example, bioreconstitution in mice attenuates helicobacter-induced gastric atrophy [80], suggesting that biome depletion may increase the propensity for gastric atrophy, which is a precursor to stomach cancer, the second leading cause of cancer-related deaths in humans. The overall analysis leaves us uncertain of the price we are currently paying for biome depletion, and in an uneasy “wait-and-see” position, not knowing what immune-associated epidemics are yet to come. This uneasy position is made worse by the realization that the effects of biome depletion may strongly impact human brain function.

Biome depletion and cognition

Our brain is intertwined with our immune system in ways that we are only beginning to understand [1,81]. Immune-competent cells are located throughout every organ of the body, including the brain, and sophisticated interactions occur among these cells, primarily via soluble protein messengers called cytokines [82,83]. Notably, bidirectional communication between the brain and immune system by cytokines has significant consequences for neural function and behavior, including social, cognitive, and affective abilities. For instance, sick animals exhibit several well-characterized behavioral changes, including reductions in activity, exploration, and social and sexual interactions, as well as cognitive and affective changes [84,85]. Despite their maladaptive appearance, these behaviors are organized, adaptive strategies that are critical to host survival [86,87].

However, increasing evidence has linked chronic, exaggerated cytokine expression and resulting neuroinflammation within the brain to pathologic conditions as diverse as Alzheimer’s disease, autism, and depression—notably, all conditions that share some striking similarities to sickness behaviors that have somehow become prolonged or pathological [88]. Neuroinflammation, for example, is strongly suspected as having a causal role in autism as well as a number of other developmental disorders. Further, neuroinflammation may be extremely important during the perinatal period, potentially altering the “program” of the normal course of development, with the result that adult outcomes, including behavior, are significantly and often permanently altered [89]. This idea accounts for the observation that perinatal exposure to infectious or other immune activating agents (e.g., stress, environmental toxins) have a number of enduring influences on physiology and behavior, including reactivity to stress, disease susceptibility, and notably, increased vulnerability to mental illness such as autism and schizophrenia [90–94]. Thus, to the extent that biome depletion results in an increased proinflammatory environment during the perinatal period, biome depletion is expected to cause an increase in the prevalence of mental illness. In support of this idea, a variety of indicators, including epidemiological, molecular, and genetic aspects of disease, suggest that biome depletion is responsible for the increasing incidence of autism [7].

A large number of neuroinflammatory changes have also been identified in normal brain aging, including upregulation of major histocompatibility (MHC II) expression on microglia, the resident immune cells of the brain [95], and increased expression of pro-inflammatory cytokines (e.g., interleukin [IL]-1β; [96]) which in turn may influence neural function (e.g., long-term potentiation [LTP]; [97]). Cognitive decline is one of the primary consequences of normal aging, although there is significant individual variability, as some individuals age more successfully than others, with a subset of the population eventually developing dementias such as Alzheimer’s disease [98,99]. Again, the presence of neuroinflammatory changes in normal aging suggests the possibility that hyper-immune activity leading to excessive neuroinflammatory mechanisms can initiate poor cognitive aging. The potential influence of biome depletion on age-associated dementia remains unknown, but investigation of the possibility seems warranted.

Based on the above considerations, we suggest that biome depletion is changing the immune system at a population-wide level, which will undoubtedly impact the brain, particularly during sensitive periods of development and perhaps in the aging process. Importantly, this theory does not compete with any prior hypotheses or known risk factors for neuroinflammatory conditions, but rather complements them. For instance, biome depletion may set the stage for exaggerated levels of neuroinflammation, which interacts with other known risk factors (stress, infection, or genetics) in the induction of a given disease or disorder.

The physiologic effects of biome depletion

Although some of the consequences of biome depletion for health are becoming apparent, the exact nature of the effects of biome depletion on the immune system remains unknown, even in laboratory rodents. Virtually all research on the immune system has been conducted in biome-depleted (helminth free) laboratory animals or in humans living in a post-industrial society. Because of this, immunologists are now faced with the unsettling realization that the immune system they have spent all of their effort and energy dissecting over in the past 50 years is dramatically different than any immune system in a natural state of existence. In fact, “normal” is not helminth-free, and our coevolutionary partners must be included if we want to understand and appreciate the “normal” state of immunity.

Several factors potentially complicate efforts to determine the physiologic consequences of biome depletion. First, not only are the effects of biome depletion on a particular generation undoubtedly profound, the effects of biome depletion on subsequent generations, passed down by epigenetic mechanisms, might be considerable. The fact that the immune system of the fetus is strongly influenced by the immune system of the mother particularly supports this idea. In addition, the transfer of immune components through the mother’s milk adds to the potential generational effects of biome depletion. A second factor which complicates efforts to determine the physiologic consequences of biome depletion is the fact that biome depletion is not an all-or-nothing proposition. The idea that specific components of the biome have different niches exponentially increases the complexity of efforts to evaluate the effects of biome depletion. The fact that those individual components of the biome interact with other components of the biome as well as with the host [100] adds even more complexity to the issue.

A wide range of studies in laboratory animals indicate that helminths exert a potent effect on virtually all components of the immune system [53]. Studies from our laboratory using a wild-caught rat versus laboratory rat model have provided insight into the potential extent of the impact of biome depletion on the immune system [45,101]. The differences between the immune systems of wild rats and those of laboratory rats exceed many inter-species differences in immunity. For example, wild rat derived lymphocytes...
To reconstitute the biome or to develop helminth-inspired drugs that mimic a reconstituted biome?

Biomedical researchers often envision isolation and characterization of the individual components produced by helminths that modulate the immune system, with the goal of creating new helminth-inspired drugs to treat disease. On the one hand, this type of approach with other organisms has proven instrumental in the progress of modern medicine, giving us antibiotics and some important vaccines. Further, repeated injection of helminth-derived extracts or proteins in animal models of disease has already proven useful in alleviating such conditions as collagen-induced arthritis [103] and experimentally induced colitis [104], among others [105,106]. On the other hand, several factors are likely to limit the general use of this approach as a substitute for biome reconstitution.

First, it is difficult to imagine a single pharmaceutical or even a collection of pharmaceuticals that could recapitulate the vast complexity of the interaction between helminths and the host immune system. While pharmaceuticals are generally directed at one component in the immune apparatus, a single helminth species produces dozens if not more molecules that each target specific components of host immunity. The likely possibility that more than one species may be required to effectively reconstitute the biome adds a level of complexity that seems staggering to anyone interested in replicating the natural state using pharmaceuticals. Perhaps more importantly, our understanding of helminth immunoregulation is far from complete, with many gaps in our knowledge [53]. For example, many proteins secreted by helminths in low abundance may not have been identified, but yet may have important biological activity. Further, the biological role of some proteins secreted by helminths in high abundance, such as the glycolytic enzyme triose phosphate isomerase, remains poorly understood [53]. Thus, our present level of understanding of the vastly complex interactions between helminths and their hosts potentially adds a substantial hurdle to the design of therapeutics which will mimic those interactions.

Not only is the helminth/host interface vastly complex, it requires continuous input from the helminth. Helminths actively maintain a state of immunosuppression, and removal of helminths results in a loss of that regulation [53], suggesting that any pharmaceutical substitute would also need to be maintained at a relatively constant level for maximum efficacy. Such continuous maintenance is potentially difficult and costly to maintain using traditional pharmaceuticals, and regular injections of helminth-inspired drugs, even if effective, might prove burdensome and expensive. Further, the mechanism of delivery of helminth-derived immunoregulatory molecules by living helminths may be important for their function, and it is possible that new drug delivery technologies may need to be devised which mimic the delivery of a living helminth. In other words, given the long coevolution of immune systems and helminths, it seems reasonable that the immune system may be adapted to and indeed dependent upon the nature, dose, and delivery method used by living helminths.

Probably it is the field of evolutionary biology that most strongly argues for living helminths and biome reconstitution in favor of pharmaceutical substitutes. Helminths represent a potential therapeutic that has been fine-tuned by hundreds of millions of years of natural selection not to encumber a healthy host. Consistent with this idea, several helminths are known to cause few if any symptoms in most cases [55,107,108]. The bovine tapeworm (Taenia saginata), for example, has been labeled a “commensal” rather than a parasite in humans [107]. To achieve this balance, natural selection has tested countless billions of combinations of molecular tools over millions of years, selecting those that are most effective for both helminth and host survival. Quite obviously, no pharmaceutical has ever been developed to match that record.

Another advantage that living helminths have over pharmaceuticals is their potential cost effectiveness. Helminths are typically long-term therapeutics if used in their natural host species, with a single dose lasting for years if not decades. Thus, costs will be limited by the long-term effectiveness of a single treatment. Further, the prospects for new technologies that facilitate cost effective and safe production of helminths in sufficient numbers for widespread use seem almost unlimited. Biotechnology involving in vitro culture of helminths or cultivation of human specific helminths in genetically modified mice (e.g., humanized or immunosuppressed mice) are two examples of such biotechnologies which remain untapped. The technology and clinical implementation of biome reconstitution may eventually prove no more complex than that utilized for vaccinations, with biome reconstitution and maintenance being a routine part of a typical checkup at the doctor’s office.

One potential advantage of living helminths as a therapeutic is that they might be genetically altered to enhance their beneficial nature. Currently available organisms can hypothetically be modified by selective breeding to enhance their beneficial nature, or may be modified in the laboratory to create specifically altered organisms, including transgenic helminths. In this manner, genetically modified helminths could be used as an effective drug delivery agent (vector) to treat a potentially unlimited variety of medical conditions. Helminths offer a number of advantages over viral or bacterial vectors for certain applications because of their long life span, built in resistance to destruction by the immune system, and, since they do not complete their life cycle in the host, a low propensity to mutate or evolve once introduced into the host.

One potential objection to the use of mutualistic helminths for biome reconstitution is that the introduction of organisms possessing “vastly complex and uncharacterized elements” into the human body might have unforeseen consequences. This argument is easily turned on its head, however, when we ask how it is rational to eliminate our coevolutionary partners that possess vastly complex and uncharacterized elements without expecting dire consequences. Indeed, many of the factors proven to be important for human survival, including the microbiome and even the food we eat, contain vastly complex and uncharacterized elements. Thus, complexity and a lack of understanding of that complexity do not rule out the importance of an element of the normal human biome for health. Further, many pharmaceuticals, molecules with substantially less complexity than helminths, cause exceedingly complex reactions within the human body, often with consequences that remain poorly understood. Thus, the relative simplicity of a
drug at the molecular level does not necessarily lead to a complete understanding of that drug's effects, and certainly does not impart safety for patients. Finally, the idea of biome reconstitution is not to modify human biology in a new way, but to restore the ecology of the post-industrial human biome to harmony with the way that our genes have evolved to function. In a nutshell, comfort with the idea of biome reconstitution requires only an understanding that natural selection has shaped our genes so that they are compatible with the environment. It does not require a complete and total understanding of that environment or how that environment interacts with the human body.

Another potential objection to the concept of biome reconstitution is the fact that helminths extract a horrible toll of suffering and death in developing countries [109]. Given this potential to cause harm, it could be argued that these organisms should not be reintroduced into post-industrial cultures. This argument, however, is entirely undermined by a consideration of the nature of parasitic disease in developing countries. In developing countries, at least one of the following three conditions are necessary ingredients for morbidity and mortality associated with helminth infection:

1. The widespread occurrence of starvation and malnourishment, conditions which add risk to colonization by helminths.
2. The absence of water treatment facilities and sewer systems, allowing uncontrolled infections and potentially high burdens of helminths.
3. The presence of helminths that are not well adapted to the host (e.g., parasites that are harmful to even a healthy host, such as Dracunculus medinensis, which causes dracunculiasis, Taenia solium, which causes cysticercosis, and Loa loa, which causes Loa loa filariasis).

Not one of these factors presents a difficult hurdle when considering the reintroduction of helminths into post-industrial societies, which generally enjoy over-nourishment rather than malnourishment. Given a proper selection of helminths for colonization, uncontrolled infections are impossible in the face of modern sewer systems and water treatment facilities. Further, modern medicine makes it possible to easily identify individuals with pre-existing conditions that could be contraindications for helminth therapy (e.g., anemia, malnutrition, immune deficiencies, coagulopathies, advanced aging, etc.). In addition, modern clinical research practices can readily be utilized to ensure that adverse side effects are reduced to far below acceptable levels prior to widespread therapeutic application. Thus, post-industrial cultures have the luxury of selecting controlled colonization with helminths, not pathologic infection. In essence, post-industrial society will have the option to adopt biome reconstitution, since biome reconstitution is potentially a matter easily administered to the individual to implement than does an effective diet and exercise program, since biome reconstitution is potentially a matter easily handled by a physician during a routine medical exam.

Biome reconstitution and the public consciousness

A commonly expressed concern regarding biome reconstitution regards the ability of those who would benefit from the therapy to actually accept the therapy. Some speculate that the prospect of helminth colonization will prove too repulsive for most patients to accept. However, from several perspectives, it can be argued that the view of the public toward the concept of biome reconstitution will pose no obstacle whatsoever. Most importantly, it is expected that biome reconstitution will be the subject of extensive clinical trials, and will have a proven safety record prior to widespread use. In the face of clinical studies potentially showing that biome depletion can lead to lupus, multiple sclerosis, food allergies, asthma, and perhaps even an autistic child, it seems far more likely that concern over biome depletion rather than fear of biome reconstitution will be the hallmark of public opinion in the future. It seems inconceivable that an educated public would react with paranoia toward helminths rather than with concern for their health and the health of their children. Certainly the educated public has not reacted with paranoia toward probiotics. Indeed, the public is becoming aware of the dangers to their microbiome imposed by the heavy use of antibiotics. Further, no responsible individual plans to market helminths as a can of worms any more than probiotics or vaccinations have been marketed as a can of germs. There seems to be little evidence to support the idea that revulsion of helminths will be an impediment to biome reconstitution.

Reassuringly, negative views toward helminths that might be adopted by a few individuals are not expected to influence the efficacy of biome reconstitution for the population as a whole. As with diet and exercise programs, the health benefits of biome reconstitution will be limited to those who embrace the prospect, and one patient’s health will not be influenced by another's decision to embrace or reject biome reconstitution. Fortunately, biome reconstitution should eventually prove much easier for the average individual to implement than does an effective diet and exercise program, since biome reconstitution is potentially a matter easily handled by a physician during a routine medical exam.

Approaching the solution

The approach to biome reconstitution needs to be systematic and rapidly executed. The following questions need to be addressed with the utmost urgency:

1. Which helminths or combination of helminths are safe and effective? Several species, including the bovine tapeworm (T. saginata), the human whipworm (T. trichiura), and the human hookworm (Necator americanus) are well adapted to humans, asymptomatic in low numbers, and thus seem to be obvious candidates for initial consideration. New technologies might be developed (e.g., irradiation of organisms to achieve sterility and eliminate the possibility of transmission, new culture methods, or cultivation of human-specific helminths in ultra-clean and immunodeficient rodents) which could improve on the initial answers to this question.
2. Which helminths should be utilized, at what dose, and under what conditions (patient age, disease state, gender, etc.)? Biome reconstitution for a 3-year-old patient may be quite different than that required for a 30-year-old patient, and the best approach to biome reconstitution for children may depend on the status of the mother’s biome during pregnancy and the first years of the child’s life.

3. Which hyperimmune-associated diseases can be cured or effectively treated with biome reconstitution, versus which can only be prevented by biome reconstitution? The answer to this question should drive efforts (or the lack thereof) to prophylactically restore the biome in the general population.

4. How important for children is the biome of the mother prior to and during pregnancy, and while breastfeeding?

5. What medical conditions (e.g., suppressed immune system, anemia, and coagulopathy) would be contraindications for biome reconstitution?

6. Are particular human genotypes better suited to particular helminth loads? Certainly hyperimmune-associated disease is connected strongly with genotype, and it is thus reasonable to expect that an optimized approach to biome reconstitution may need to consider the genotype of the patient.

7. Does modern medicine need to work toward reconstituting components of the biome other than helminths? For example, should intentional and controlled exposure to a normal microbiota in a manner that recapitulates natural child birth replace current practices in obstetrics?

8. Will certain components of modern medicine (e.g., vaccines and immunosuppressive drugs) need to be modified so that they are effective on individuals with a healthy (reconstituted) biome? Modern medicine has been tailored to those with a depleted biome, and may require some adjustment for those with a healthy biome.

9. What are the effects of biome reconstitution on human biology in general? For example, how does it affect reproduction, aging, cancer, infectious disease, and cognition?

Obtaining the answers to these questions is the beginning of a new chapter in modern medicine and biotechnology, and potentially closes the door on a chapter aimed at treating hyperimmune-associated diseases with pharmaceuticals. The answer to these questions will potentially drive a substantial arm of clinical medicine for the foreseeable future. Given the current mortality, morbidity, and expense inflicted by epidemics of hyperimmune-associated disease, speed seems paramount. At the same time, however, a systematic rather than a piecemeal approach to the solution is strongly encouraged.

Prognosis for modern medicine

One question in the minds of many who recognize the vast potential that biome reconstitution appears to offer is how quickly modern medicine will take advantage of this potential. From several perspectives, the prognosis for modern medicine is very good. Modern immunology has proven to be a highly flexible discipline as it struggles against widespread epidemics of immune-related disease. Within a few short years of initial discovery, new findings often become "hot" topics, accruing well-funded laboratories and hundreds of research papers. With that in mind, a tipping point in medicine can be seen fast approaching, in which colonization with helminths becomes the next hot topic of basic and clinical research. The critical events that mark this tipping point are not likely public opinion or even our capacity to solve the problem, but rather a change of paradigm within the minds of the policy makers of the biomedical community. When those who strongly influence the direction of the fields of clinical and basic immunology decide to take action, biomedical research can move with astonishing speed.

After that tipping point is reached for biomedical research, financial considerations could drive a very rapid evolution of clinical medicine. For example, if restoring the biome of a few hundred patients is shown to prevent the onset of even a few cases of such costly diseases as asthma, type 1 diabetes, or autism, strong pressures on financial resources could force the health care system to adapt quickly. With that in mind, the day when a doctor will prescribe not only a vaccination against a dreaded disease, but also colonization with a domesticated helminth, may not be so far distant in the future. The best news is that this type of treatment is expected to be both inexpensive and without major complications. If we keep open minds about the possibilities of biome reconstitution – the "tipping point" may be just around the corner.

Conclusions

It was only a very few generations ago, at most, that humans first began widespread use of indoor flushing toilets and acquired access to modern medicine. These technologies, which effectively interrupt most helminth life cycles, have, over time, resulted in a profound depletion of the human biome. Realizing the intimacy and strength of the connection between the biome and the immune system, it is possible to arrive at a remarkably simple conclusion that can account for a wide range of post-industrial disease: Biome depletion has left us with an overly reactive immune system, leading to epidemics of a wide range of allergic and autoimmune diseases.

A synthesis of epidemiology, modern medicine, evolutionary biology and immunology strongly suggests that biome reconstitution and maintenance should be a major thrust of biomedical research in the very near future, and a major component of medicine in the foreseeable future. Helminth-derived pharmaceuticals might offer an alternative to biome reconstitution, but given what is already known about the complex and multi-faceted nature of helminth-host interactions, pharmaceuticals which effectively mimic the effects of helminths might prove extremely difficult and expensive to develop and utilize. Further, the low costs, high efficacy, and very low rate of side effects that are expected to be associated with biome reconstitution make this approach extremely appealing. In addition, the potential for helminths to be used prophylactically and the potential for new helminth-supported biotechnology applications add to the attractiveness of biome reconstitution. The domestication of helminths, as with the domestication of other animals, may eventually provide a means for selection and breeding of organisms that have a greater utility for humans than those helminths found naturally.

The biology imposed by our evolution is, at present, inescapable, and medical science of the future will take that fact fully into account. To achieve this aim, intensive and systematic research should now be focused on the wide range of questions regarding the effects of biome depletion and the application of biome reconstitution. Hopefully, in the future, our biomes will be checked as regularly as our cholesterol is checked, with proactive as well as reactive biome reconstitution being routine.

Conflict of interest statement

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