



State of the art; microbiology in health and disease. Intestinal bacterial flora in autism

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ARTICLE INFO

Article history:

Received 15 February 2011

Received in revised form

29 March 2011

Accepted 30 March 2011

Available online 16 April 2011

Keywords:

Autism

Intestinal microflora

Pyrosequencing

Desulfovibrio

ABSTRACT

Autism of the regressive variety is selected as an example of the importance of intestinal bacterial microflora in disease other than classical infection. Our studies have indicated that intestinal bacteria play a role in this disease since it responds to oral vancomycin, a drug that is not absorbed from the GI tract. Pyrosequencing studies document an abnormal gut microflora in regressive autism subjects as compared to controls. Finally, we present preliminary evidence suggesting that *Desulfovibrio* may play a key role in this disease.

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To illustrate the theme of this lecture, I will discuss intestinal bacteria in relation to autism. There are probably many other diseases (e.g., Parkinson's disease, multiple sclerosis, rheumatoid arthritis, etc.) in which intestinal bacteria play a role.

Autism is a complex disorder and probably embraces several differing entities. There are no specific diagnostic tests so the disease is defined by its characteristics—cognitive defects, including impairment of spoken and/or receptive language; social, communication and behavioral problems; repetitive behaviors; unusual sensitivity to stimuli such as noises; and restricted interests [1]. There are no specific criteria that distinguish the different types of autism from one another. The Autism Research Institute collected data on autism since 1965 and noted that regressive autism, beginning around 18 months of age, was very uncommon until the mid-1980's and between 1991 and 1995 there were over twice as many with onset at 18 months as at birth [2]. Autism research to date has mainly focused on finding a genetic association but it has been recognized that, in addition to heritable predisposition to the disease [3], environmental factors are undoubtedly important [4]. There has been a striking increase in incidence of autism worldwide; approximately 1% (110/10,000) of children in multiple areas of the U.S. in 2007 had an autistic spectrum disorder, up from 66/10,000 in 2002 [5]. The financial impact of autism on U.S. society is dramatic; it costs about \$3.2 million to take care of an autistic person over his or

her lifetime. Caring for all people with autism costs an estimated \$35 billion per year according to the Harvard School of Public Health [6]. The Interagency Autism Coordinating Committee (IACC) notes the need for additional studies on the role of the environment, of epidemiology, and of specific treatments for autism [7]. Bolte first hypothesized that intestinal bacteria might be involved in autism and was the impetus for the first publication based on that hypothesis [8]. That paper (which involved a small open-label treatment trial with oral vancomycin which is virtually not absorbed), and other published data lend credence to the notion that an alteration in bowel microflora is associated with autistic symptoms [9–11]. MacFabe [12] stresses the importance of the gut-brain connection and the probable role of diet, gut function, bacteria and enzymes in autism. Essential amino acid deficiencies have been noted in autism (including the neurotransmitter precursors tryptophane and tyrosine), perhaps related to restricted diets. Food additives such as colorings, sweeteners, and preservatives may be definite problems.

Autism is a major health problem, a big emotional burden for families and a large financial burden for families and municipal governments worldwide, at least in developed countries. Gastrointestinal (GI) symptoms that may be very distressing have been noted in some children who have regressive autism and there is speculation that this may represent a specific subset of autism. Our early studies of fecal flora in such children revealed higher counts of clostridia and more different species of clostridia than are found in stools of age and sex-matched children. Recently, our studies using

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the powerful pyrosequencing technique indicates that other bacteria may be more important in inducing the disease in susceptible individuals and that still other organisms, such as *Bifidobacterium* are more prevalent in controls than in autistic children; such organisms might be protective if administered as a specially tailored probiotic. We will return to these studies, in detail, later in the manuscript.

We noted impressive improvement in behavioral, cognitive and gastrointestinal aspects of autism in a small open-label study of vancomycin administered orally, and anecdotally a number of physicians have treated autistic children with oral vancomycin. The children relapse after treatment is stopped which would be consistent with the fact that most clostridia form spores that are very resistant to antibiotics and would germinate into vegetative, infective forms after the antibiotic is discontinued. Clostridia were also attractive candidates since many of them are quite virulent and produce toxins and because the spores are quite resistant to oxygen and to drying so that they can survive in the environment for extended periods of time. This might facilitate spread to other predisposed children (genetic or environmental toxins affecting the immune system, and impact of antibiotic therapy on the bowel flora). Other intestinal bacteria that are not spore-formers have other mechanisms that permit them to essentially hibernate until the antibiotic disappears. *Desulfovibrio* has several mechanisms by which it can tolerate oxygen exposure. Its flagellum permits it to travel readily within biofilms where it is protected from peristalsis, from antibiotics, and from host defenses. It should be noted that there is reason to believe that other antimicrobials such as trimethoprim/sulfamethoxazole, fluoroquinolones (which should not be used in young children), and certain cephalosporins, may predispose to autism as they do to *Clostridium difficile* infection.

The pyrosequencing studies, using the titanium modification, which were published recently [11] were very interesting. Some of the analyses indicated over 2000 operational taxonomic units and over 1000 different species were present in certain groupings of stool specimens. With regard to autism specifically, there were important differences between the control children and the autistic children studied in terms of frequency of occurrence of different bacterial phyla especially with four phyla – *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria*. In the control children's stools, *Firmicutes* accounted for 63.6% of the total flora but only 38 = 39% of the flora of autistic children's stools. *Bacteroidetes* accounted for 30% of the stool flora in controls and for 51% in the flora of stools of autistic children. *Actinobacteria* made up 1.8% of stool flora of control children and between 0.4 and 0.7% of the flora of autistic children. *Proteobacteria* made up 0.5% of the flora of control children and between 2.3 and 3.1% of the flora of autistic children. In all cases, the flora of sibling controls was between the values of autism and control populations. In summary, the fecal flora of autistic children was statistically significantly different from the fecal flora of healthy children.

We have followed up on the data from the above-mentioned pyrosequencing paper in which there was an indication that two organisms were more commonly found in stools of autistic children than in control children's stools – *Bacteroides vulgatus* and *Desulfovibrio* species. In the case of *B. vulgatus*, real-time PCR and culture studies indicate that it is actually common in both autism and controls. In the case of *Desulfovibrio*, however, the use of both real-time PCR and culture using a semi-selective and differential medium indicated that at least three species of *Desulfovibrio* (*D. desulfuricans*, *D. fairfieldensis*, and *D. piger*) were associated with regressive autism in a significant way. Fourteen of the stool specimens from 30 autistic children were positive for *Desulfovibrio* by culture or real-time PCR (46.7%), compared to stools of 7 from siblings of autistic children (28.6%) and zero of 12 stools from healthy controls. There was also a "dose response" in that the more severe the autism the higher the percent positive by either culture or real-time PCR. Additional specimens should be studied, including some in different locations, and treatment trials with an appropriate antibiotic or combination that showed a clinical response and a microbiologic response (and, preferably, also with a physiologic response in the children) would validate the current findings.

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